Arsenic Speciation in Urine by Solvent Extraction / Graphite Furnace Atomic Absorption Spectrometry and Capillary Electrophoresis / Inductively Coupled Plasma Mass Spectrometry

Ross Wenzel

University of Technology, Sydney

Masters by Thesis – 2001

Acknowledgements

In preparing this thesis I would like to acknowledge the invaluable contribution and support of the following people and organizations: -

My project supervisors: -

Mr. Graham Hams from Pacific Laboratory Medicine Services for suggesting a suitable project topic and providing guidance throughout.

Dr. Ralph Orwell from the University of Technology, Sydney for thoroughly reading through various drafts of this thesis and identifying ways in which improvements could be made.

Pacific Laboratory Medicine Services for funding this project.

Mr. Steve Toovey from the Biomedical Engineering Department at Royal North Shore Hospital for invaluable technical assistance with the electrical systems used.

Dr. Stephen Anderson from Varian Australia for generous assistance with parts and operational advice.

Pacific Laboratory Medicine Services and Royal North Shore Hospital grants committee for providing funding so that part of this project could be presented at the 17th Australian and New Zealand Society of Mass Spectrometry conference.

Division of Analytical Laboratories, Lidcombe for donating arsenobetaine.

Abstract

A modified version of the solvent extraction method described by Buchet *et al* (1980) for partially speciating arsenic in human urine specimens was investigated. It was shown to be a valid means of isolating those arsenicals associated with toxicity and was used to screen subjects with elevated total urinary arsenic levels for toxic arsenicals. This method involves the removal of the toxic arsenicals arsenite (As^{III}), arsenate (As^{V}), methanearsonic acid (MAA) and dimethylarsinic acid (DMAA) from urine by a solvent extraction process. After the addition of concentrated hydrochloric acid and potassium iodide, toxic arsenicals are extracted from the urine into cyclohexane and mixed with a matrix modifier solution of nickel nitrate for analysis by graphite furnace atomic absorption spectrometry (GFAAS). Since, in most subjects, elevated urinary total arsenic levels are due to the presence of non-toxic arsenicals following seafood consumption, partial speciation by solvent extraction / GFAAS is used to confirm or to rule out arsenic poisoning in subjects with elevated total urinary arsenic levels.

A capillary electrophoresis (CE) technique was developed, in which CE was interfaced with an inductively coupled plasma mass spectrometer (ICPMS) to permit the isolation and estimation of major arsenic species in urine and other biological specimens. It was possible to separate 5 arsenicals by CE and to detect, by coupled ICPMS, levels of individual arsenicals corresponding to 0.1 ppm (1.3 µmol/L) arsenic in urine. This CE / ICPMS technique was used to evaluate partial speciation by solvent extraction / GFAAS in a range of artificially prepared standard mixtures of arsenicals, and in urine specimens. It was confirmed that the latter technique is a valid means of assessing subjects suspected of arsenic poisoning because of their elevated total urinary arsenic levels.

Using the CE / ICPMS technique, it was also confirmed in one leukaemic patient, that, in humans, the main urinary arsenicals excreted after acute exposure to arsenite (As^{III}) are DMAA and MAA. In other healthy subjects, it was found that, after seafood consumption, the predominant arsenical present in urine was arsenobetaine. On the basis of these studies, a protocol was defined for the stepwise investigation of urine specimens for inorganic and organic arsenic compounds.

Preliminary studies were also carried out on edible seaweeds, which are known to contain arsenosugars and arsenolipids. Partial speciation, of edible seaweed digests, gave incomplete and variable arsenic recoveries. When the same digests were analysed by CE / ICPMS, the only arsenical found was arsenobetaine, suggesting that arsenosugars and arsenolipids may have been broken down to form arsenobetaine during extraction. Although inconclusive, these results demonstrate the usefulness of CE / ICPMS for evaluating arsenic extraction methods in novel sample matrices.

Table of Contents

List of Abbreviations

AMU	Atomic mass unit
AsB	Arsenobetaine
AsC	Arsenocholine
As^{III}	Arsenite
As^{V}	Arsenate
BPI	Bias performance index
CE	Capillary electrophoresis
CSV	Comma separated variable
DIN	Direct injection nebuliser
DMAA	Dimethylarsinic acid (also known as cacodylic acid)
EDTA	Ethylenediamine tetraacetic acid
EOF	Electroosmotic flow
GFAAS	Zeeman graphite furnace atomic absorption spectrometry / spectrometer
HBA	p-hydroxybenzoic acid
HGAAS	Hydride generation atomic absorption spectrometry / spectrometer
HPLC	High performance liquid chromatography
IC	Ion chromatography
ICP	Inductively coupled plasma
ICPMS	Inductively coupled plasma mass spectrometry / spectrometer
ID	Internal diameter
MAA	Methanearsonic acid
MCN	Micro concentric glass nebuliser
m/z	Mass-to-charge ratio
OD	Outside diameter
PaLMS	Pacific Laboratory Medicine Services
PAO	Phenylarsine oxide
PTFE	Polytetrafluoroethylene
RF	Radio frequency
TMAO	Trimethylarsine oxide
TTAB	Tetradecyltrimethylammonium bromide
TTAOH	Tetradecyltrimethylammonium hydroxide
TX-100	Triton X100
UV	Ultraviolet

List of Illustrations

List of Tables

CHAPTER 1. INTRODUCTION

1.1 Background

Arsenic is a well-known toxin though not all chemical compounds of arsenic are toxic. Arsenic forms a number of compounds with inorganic arsenic considered the most toxic. Acute arsenic poisoning can cause headache, nausea, vomiting, convulsions and death. Chronic occupational exposure to inorganic arsenic compounds may lead to various diseases such as cancer of the respiratory tract, skin cancer, hyperkeratosis, hyperpigmentation, cardiovascular disease and disturbances of the peripheral vascular and nervous systems (Clarkson 1991). Exposure to arsenic arises from industrial sources, environmental sources (Peters *et al.* 1996) or deliberate poisoning.

Urine arsenic measurements are used to screen workers occupationally exposed to arsenic with decisions influencing employee health and work conditions based on these measurements. Accurate methods of measuring arsenic are therefore required. Accurate and rapid urine arsenic measurements are also necessary when a patient presents with symptoms of acute arsenic poisoning. When measuring arsenic levels, consideration needs to be given to the fact that different chemical forms of arsenic, referred to as arsenic species, show different levels of toxicity. This is well illustrated by the organoarsenical compounds commonly found in many seafoods, the ingestion of which can result in elevated levels of arsenic in urine, with little or no risk of arsenic toxicity.

1.2 Chemical Forms of Arsenic

Inorganic arsenic forms two oxides: arsenic trioxide (As_2O_3) and arsenic pentoxide (As_2O_5). These oxides are readily soluble in water where they are present as arsenite (As^{III}) and arsenate (As^V). The methylation of As^{III} produces methanearsonic acid (MAA) with further methylation forming dimethylarsinic acid (DMAA). Figure 1 shows

the chemical structures of several arsenic species found in seafood and in human urine after seafood consumption or exposure to inorganic arsenic. The arsenosugars and arsenolipids shown provide an example of just a few of these types of arsenic-containing compounds that have been isolated from marine sources. For further details on the chemical naming, structure and biosynthesis of these and other organoarsenic compounds, the reader is referred to Cullen and Reimer (1989), Francesconi and Edmonds (1994), Francesconi *et al.* (1994), Phillips (1994) and Shiomi (1994).
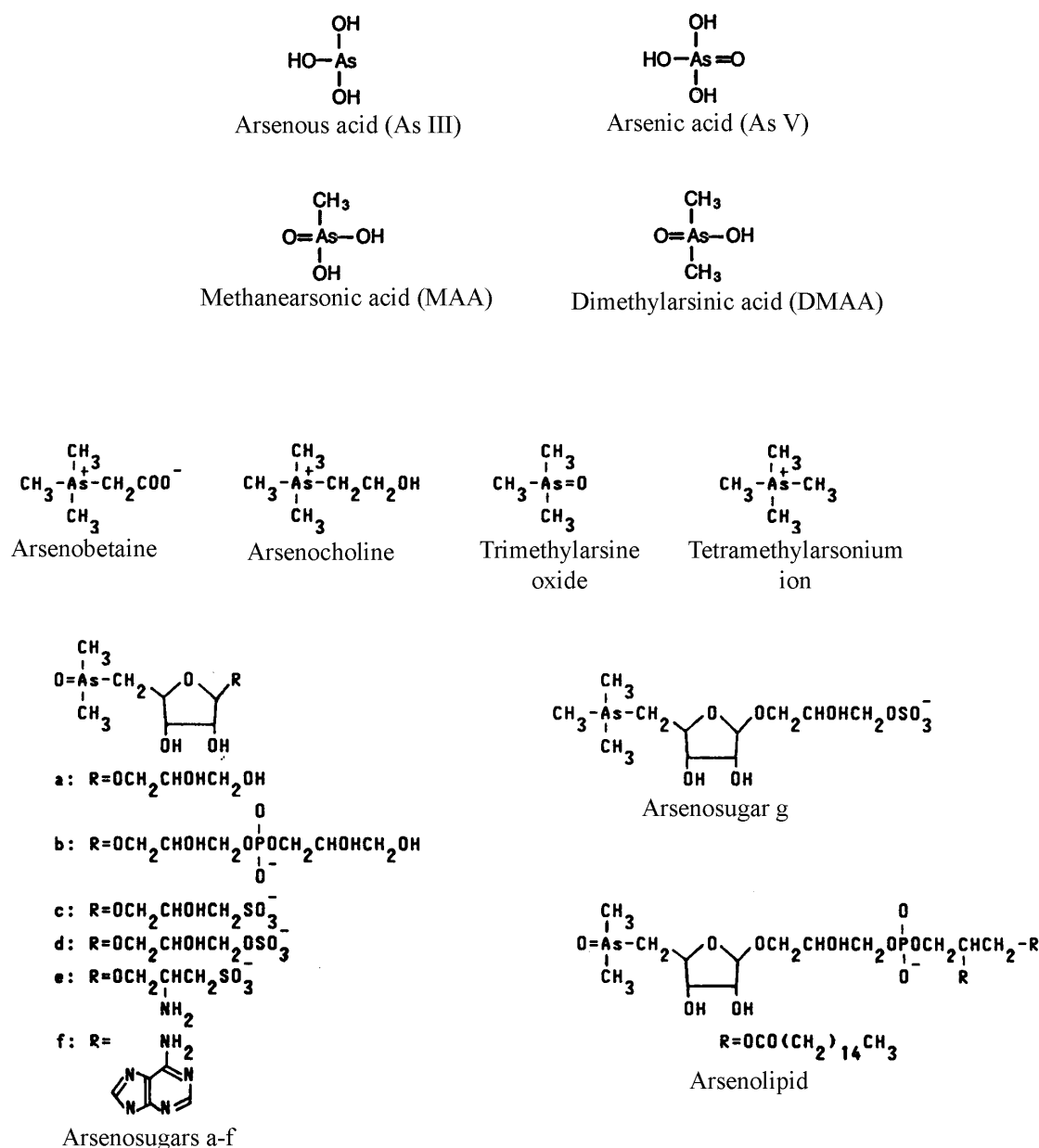
Figure 1 Chemical structures of a variety of arsenicals. Figure adapted from Shiomi (1994).

1.3 Sources of Arsenic

Human exposure to inorganic arsenic can come from a variety of sources. Inhalation or ingestion of arsenic containing airborne dusts, or a combination of both, are two of the most important sources for chronic exposure (Farmer and Johnson 1990). Acute exposure can occur with deliberate or accidental poisoning through the ingestion of arsenic containing baits and chemicals. Occupational exposure to inorganic arsenic can arise: -

> ➢ through non-ferrous smelting
> ➢ in glass making, where arsenic is used as a clarifier
> ➢ in the electronics industry, where gallium arsenide and its alloys are used in semiconductors
> ➢ from the application of arsenic as a wood preserver
> ➢ in the manufacture and use of arsenic containing pesticides and herbicides

Common sources of environmental exposure to arsenic include: -

> ➢ the consumption of drinking waters from artesian wells that have been shown to contain high levels of arsenic
> ➢ inhalation of smoke from burning wood preserved with arsenic trioxide
> ➢ consumption of foods contaminated with arsenic containing pesticides
> ➢ the consumption of marine biota (Peters *et al.* 1996).

With the exception of seafood, arsenic levels in human food products are generally low (Vahter 1988). Seafood naturally contains high concentrations of organoarsenic compounds. Arsenobetaine (AsB) is the major arsenic compound in a large range of marine animals (Table I). Tetramethylarsonium ions are the next most common organic form of arsenic. Inorganic arsenic constitutes less than 2% of the total arsenic in marine animals, while arsenocholine (AsC), trimethylarsine oxide (TMAO) and a variety of other organoarsenic compounds have been found at trace levels (Christakopoulos 1988; Francesconi and Edmonds 1994). In marine plants, arsenosugars and arsenolipids are the predominant forms of organoarsenic (Phillips 1994). Arsenate (As^V), MAA and DMAA occur in marine algae, but generally at low levels only. AsB, AsC and tetramethylarsonium ions have not been detected in marine algae (Francesconi and

Edmonds 1994). The inorganic arsenics, arsenite (As^{III}) and arsenate (As^V), and the organic arsenics, MAA and DMAA, are available commercially (Sigma Catalogue). AsB has been synthesised (Cannon *et al.* 1981).

Table I Arsenic composition of various marine organisms. Table adapted from Francesconi and Edmonds (1994).

Animal (No. of species)	Arsenic concentration (mg/kg, wet weight)	% of Arsenic Present as Arsenobetaine
Fish		
Elasmobrachs (7)	3.1–44.3	94–95
Teleosts (17)	0.1–166.0	48–95
Crustaceans		
Lobsters (4)	4.7–26.0	77–95
Prawns/Shrimps (5)	5.5–20.8	55–95
Crabs (6)	3.5–8.6	79–95
Molluscs		
Bivalves (4)	0.7–2.8	44–88
Gastropods (6)	3.1–116.5	58–95
Cephalopods (3)	49.0	72–95

1.4 Arsenic Toxicity and the Effects of Arsenic Exposure

Several studies have been performed on arsenic species to determine their toxicity to mammals. Arsenites are more soluble than arsenates, making them rapidly absorbable and more toxic than arsenates (Clarkson 1991). A lethal dose of arsenic trioxide in an adult human is 100–200 mg, though cases of subjects surviving higher doses have been reported (Benramdane *et al.* 1999). Trimethylated arsenicals are relatively non-toxic and are present in numerous marine organisms. Tetramethylated arsenicals are also found in marine organisms and can show a degree of toxicity (Shiomi 1994). The lethal doses

(LD$_{50}$) of various arsenic species in rats are shown in Table II. From this table it is evident that the determination of total arsenic alone is insufficient to assess the likely arsenic toxicity of a sample.

Table II LD$_{50}$ of arsenic compounds in rats. Table adapted from Shiomi (1994) and Le *et al.* (1994).

Arsenic Compound	LD$_{50}$ (g/kg)
Arsenite (As^{III})	0.014
Arsenate (As^{V})	0.020
Methanearsonic acid (MAA)	1.8
Dimethylarsinic acid (DMAA)	1.2
Arsenobetaine (AsB)	> 10.0
Arsenocholine (AsC)	6.5
Trimethylarsine oxide (TMAO)	10.6
Tetramethylarsonium iodide	0.89

The main sites of arsenic absorption in humans are the gastrointestinal and respiratory tracts, though some absorption may occur through the intact skin. All water-soluble arsenic compounds, including those from marine sources, are readily absorbed through the gastrointestinal tract. Inorganic arsenicals are rapidly methylated in the liver and mainly excreted in the urine (Hindmarsh and McCurdy 1986). Methylation serves to detoxify inorganic arsenic and increase its excretion rate. The plasma half-life of inorganic arsenic is 4 to 6 hours and the plasma half-life of the methylated metabolites is 20 to 30 hours. AsB is not metabolised in the body and is excreted unchanged (Clarkson 1991).

The first methylation reaction is catalysed by arsenite methyltransferase. The transfer of the second methyl group is catalysed by MAA methyltransferase, which is sensitive to inhibition by As^{III}. This explains the increased concentration of MAA in urine relative to DMAA after acute intoxication (Benramdane *et al.* 1999). As^{V} is reduced to As^{III} by arsenate reductase before its methylation (Clarkson 1991). The methylation pathway of

arsenic can be represented as: - $As^{V} \leftrightarrows As^{III} \rightarrow MAA \rightarrow DMAA$. The methylated arsenicals, MAA and DMAA, show a very weak affinity for tissue, therefore their elimination in urine is rapid. As^{III} however, is bound to the sulfhydryl groups of proteins so will persist (in spite of its shorter plasma half-life) in tissues longer than MAA and DMAA (Benramdane *et al.* 1999). Studies of workers suffering occupational (non-acute) exposure to inorganic arsenic have shown that on average, urine contains 11–14% As^{III}, 1–6% As^{V}, 14–18% MAA and 63–70% DMAA (Farmer and Johnson 1990).

The clinical effects of arsenic poisoning are a reflection of the biochemical action of arsenic. An effective antidote for treating arsenic intoxication is British Antilewisite (2,3-dimercaptopropanol; "Dimercaprol"). Dimercaprol is a sulfhydryl reducing agent suggesting that the primary mechanism of action of arsenic is related to sulfhydryl binding. Arsenate competes for P_i - binding sites on enzymes, resulting in the formation of unstable arsenate esters. Arsenite is more toxic than arsenate as it is able to form a stable complex with the sulfhydryl groups of α-lipoic acid, a necessary cofactor for pyruvate dehydrogenase. Absence of this cofactor inhibits the conversion of pyruvate to acetyl coenzyme A. The net effect is to reduce the rate of oxidative phosphorylation and hence prevent adenosine triphosphate production (Hindmarsh and McCurdy 1986; Clarkson 1991; Potter 1995). Arsenic also competes with phosphate for reaction with adenosine diphosphate, resulting in the formation of the lower energy adenosine diphosphate arsenates rather than adenosine triphosphate (Clarkson 1991).

The initial symptoms of acute arsenic poisoning frequently include a feeling of constriction of the throat followed by difficulty in swallowing and epigastric discomfort. Abdominal pain and vomiting often start within an hour of ingestion. Death may result from a severe fall in blood pressure (shock) and follows vomiting and profuse watery, painful diarrhoea. Symptoms of chronic arsenic poisoning include chronic weakness, general debility, loss of appetite, loss of energy, loss of hair, hoarseness of the voice, loss of weight and a variable degree of dementia. Chronic exposure to inorganic arsenic compounds may lead to various diseases such as cancer of the respiratory tract, skin cancer, hyperkeratosis, hyperpigmentation, cardiovascular disease and disturbances of the peripheral vascular and nervous systems (Clarkson 1991; Hindmarsh and McCurdy 1986).

1.5 Measuring Arsenic

Urine is the preferred matrix for measuring arsenic levels. Arsenic excretion in urine is rapid, so to detect arsenic exposure, measurements must be made soon after the event. Urinary arsenite (As^{III}) and arsenate (As^V) levels peak approximately 10 hours after ingestion of arsenic trioxide and return to normal 20 to 30 hours later. Urinary MAA and DMAA levels normally peak at about 40 to 60 hours and return to baseline 6 to 20 days after exposure. Blood arsenic levels are most rapid in their rise and fall with elevated arsenic levels detectable for less than 4 hours after exposure (Clarkson 1991). If reliance were placed on blood levels alone, an acute exposure could easily be missed. The measurement of arsenic in hair, nails, stomach contents and other body tissue can prove useful in certain circumstances (Hindmarsh and McCurdy 1986; Raie 1986; Vahter 1988).

Urine arsenic concentrations in subjects without known exposure to arsenic are generally in the order of 0.1 µmol/L to 0.3 µmol/L, but the intake of a single meal of fish or shellfish may increase the urinary arsenic concentration to over 13.0 µmol/L (Vahter 1994). As human fatalities from arsenic poisoning through the consumption of seafood have not been reported, anecdotal evidence suggests that these forms of arsenic are essentially non-toxic. PaLMS (Pacific Laboratory Medicine Services) procedures for identifying elevated urine arsenic levels therefore involve the measurement of total urine arsenic with a further partial speciation of those urine samples with a total arsenic concentration exceeding 2.0 µmol/L. (Elevated urinary arsenic is defined at PaLMS as > 1.5 µmol/L, while arsenic levels > 2.0 µmol/L are considered to be potentially toxic). By partially speciating urine samples, the combined total arsenic concentration of the toxic arsenic species, namely As^{III}, As^V, MAA and DMAA, can be determined separately from the non-toxic organic arsenicals. Arsenic concentrations are given throughout this thesis in µmol/L, in accordance with the international system of units preferred in Australia. Conversion from µmol/L to µg/L requires the µmol/L value to be multiplied by 75.

A variety of techniques are available for detecting arsenic (Francesconi *et al.* 1994) and include: -

> ➢ molecular absorption spectrophotometry
> ➢ neutron activation analysis
> ➢ several electrochemical techniques
> ➢ atomic absorption spectrometry
> ➢ atomic emission spectrometry
> ➢ atomic fluorescence spectrometry (He *et al.* 1997)
> ➢ mass spectrometry

Of these techniques, atomic absorption spectrometry has been most widely used in clinical laboratories. Methods that measure total arsenic can prove an unreliable means of assessing exposure to inorganic arsenic unless the subject under study refrains completely from eating seafood before urine sampling. This is often difficult as seafood products are widely consumed and are the predominant source of arsenic exposure (Section 1.3).

A more reliable approach for assessing exposure to inorganic arsenic is through the partial speciation of urine arsenicals. The term "partial speciation" is understood by most workers undertaking clinical arsenic estimations to indicate techniques which exclude excretory products derived from ingested organic arsenicals and measure only those produced by exposure to inorganic arsenic. This definition of "partial speciation" will be adhered to throughout this report. Two techniques commonly used for the partial speciation of arsenicals in urine samples are hydride generation atomic absorption spectrometry (HGAAS) and organic solvent extraction (Francesconi *et al.* 1994). Ion exchange chromatography has also been investigated as a means of achieving partial speciation (Nixon and Moyer 1992). To resolve all arsenic species, high performance liquid chromatography (HPLC), ion chromatography and capillary electrophoresis (CE) have been used (Nixon and Moyer 1992; Spall *et al.* 1986). These techniques are enhanced through the use of mass selective detectors.

1.5.1 Hydride Generation Atomic Absorption Spectrometry

Urine is hydrolysed in acid to protonate arsenite (As^{III}), arsenate (As^{V}), MAA and DMAA. Each arsenic species is reduced to its respective arsine gas at a pH dependent upon its pKa value as only the protonated acids are reduced to arsine species. Protonated arsenic species can then be reduced with sodium borohydride to produce arsine gas. A stream of inert gas is used to carry the arsine gas into a heated quartz cell for analysis. The ability to detect the arsenic species depends upon volatility of the arsine. Each species produces a hydride of varying volatility that results in disproportionate signal intensities (Hanna *et al*. 1993). As a single arsenic species is usually used as a calibrator, not all hydride-forming arsenicals measured will be correct for this calibration. HGAAS is therefore a relatively inaccurate means of measuring arsenicals in urine (Hanna *et al*. 1993; Subramanian 1988). Varying arsenic species volatility also means there is little advantage in using hydride generation with ICPMS detection.

1.5.2 Organic Solvent Extraction of As^{III}, As^{V}, MAA and DMAA

The determination of total arsenic in urine by graphite furnace atomic absorption spectrometry (GFAAS) requires an aggressive sample digestion process, followed by a sample cleanup using a solvent extraction step with potassium iodide. By limiting the aggressiveness of this digestion procedure and modifying the solvent extraction / potassium iodide stage, methods for partially speciating arsenicals by GFAAS have been described (Buchet *et al*. 1980; Subramanian 1989). Urine is acidified with concentrated hydrochloric acid. Saturated potassium iodide is then added. The iodide forms complexes with arsenite (As^{III}), arsenate (As^{V}), MAA and DMAA. This enables the extraction of these arsenicals into an organic phase, separating them from compounds that interfere with GFAAS. After mixing in a nickel nitrate / nitric acid solution to reduce the volatility of arsenic, the extract is ready for analysis by GFAAS.

1.5.3 Speciation by Capillary Electrophoresis

Capillary electrophoresis (CE) has traditionally been used for protein and peptide separations. More recently, CE has been used to separate inorganic ions and low molecular weight ionic species. Analytical techniques of similar capability for the measurement of anionic species include ion chromatography (IC), currently the most widely used technique for anionic analysis, and HPLC. CE is superior to these techniques as it offers separation in less than 5 to 10 minutes, separation efficiencies of 200,000 to 1,000,000 theoretical plates and detection limits in the parts per trillion concentration range (Jandik *et al.* 1991). Usage of consumables and reagents are considerably less in CE than in similar techniques, making it very cost effective once the equipment has been purchased. Unlike the extraction method evaluated in this project or arsenic speciation by HPLC, arsenic speciation by CE consumes no organic solvents. The very small sample volumes used in CE, around 10 nL, make CE a valuable technique where sample size is a limiting factor.

To date, the complete separation of the arsenic species commonly found in urine have been mainly carried out by HPLC (Goessler 1997). In this project, CE was preferred for this task because it offers advantages over HPLC because not only are run times shorter, as already mentioned, but it has the potential to separate a larger number of arsenic species. As a separation technique, CE is also very versatile, creating new avenues for research beyond this project. Speciation by IC was also excluded because the large volumes of eluant required are unsuitable for nebulisation, making it less suitable for interfacing to ICPMS.

In developing a CE method for arsenic speciation several parameters needed to be optimised. Numerous methods had been published on anion analysis by CE and provided a good starting point for this investigation (Holmes 1995; Jackson and Haddad 1993; Jandik and Bonn 1993a; Kuban and Karlberg 1998; Lin *et al.* 1995; Oehrle 1996). These methods rely on the use of an osmotic flow modifier to enhance separation. Chromate is the most frequently used background absorbing species for indirect ultraviolet (UV) detection of anions. Lin *et al.* (1995) used CE to separate arsenicals in coal fly ash obtaining absolute detection limits of 3.5, 5.2, 15.6 and 15.6 pg for arsenite

(As^{III}), arsenate (As^{V}), MAA and DMAA, respectively. Using similar CE conditions, Holmes (1995) was able to apply CE to urine samples to separate the anions oxalate and citrate. By adapting features from both these papers, arsenic speciation in urine by CE was made possible.

1.5.4 Speciation and Mass Spectrometric Detection

The coupling of separation techniques with ICPMS detection has been reviewed by Sutton *et al.* (1997). Researchers using these techniques have been able to demonstrate the usefulness of elemental detection after analyte separation in a variety of sample matrices. Ranges of elements with varying detection limits were reported for different separation and sample nebulisation methods. A review of some of these techniques and their reported detection limits for various elements follows.

Systems using HPLC coupled to ICPMS have traditionally been used in the separation and detection of different chemical forms of trace elements in biological samples. For the speciation of arsenicals, Larsen *et al.* (1993) was able to separate arsenic in seafood samples by HPLC coupled with ICPMS detection. Extracts of 11 samples from marine biota were found to contain, relative to the total arsenic content, arsenite (As^{III}) and arsenate (As^{V}) 0–1.4%, MAA 0.3%, DMAA 8.2–29%, AsB 19–98%, AsC and TMAO 0–0.6%, tetramethylarsonium ion 0–2.2% and two unknown arsenic species at 3.1–18% and 0.2–6.4%. Dry mass limits of detection were in the range of 10–50 ng/g with the exception of AsB for which the limit of detection was 360 ng/g. Using a micellar HPLC mobile phase, Ding *et al.* (1995) reported separation of As^{III}, As^{V}, MAA and DMAA to a detection limit of 300 pg with the exception of DMAA for which the limit of detection was 90 pg. They also found this technique suitable for measuring arsenic in urine.

Lu *et al.* (1995) utilised the high-resolution separation of CE for very small sample volumes when investigating metal binding proteins. By creating a CE / ICPMS interface they were able to separate metallothionein isoforms and ferritin and identify their metal contents. Detection limits achieved for ^{57}Fe and ^{114}Cd in ferritin were 184 and 4 fg,

respectively, for 74 nL injections. A modified concentric glass nebuliser and impact bead spray chamber was used.

To further improve method sensitivity, Liu *et al.* (1995) used an interface with the CE capillary placed concentrically inside a fused silica direct injection nebuliser (DIN). This system was used to evaluate test samples containing selected alkali, alkaline earth and heavy metal ions, as well as selenium (IV), selenium (VI) and various inorganic and organic arsenic species. Metal species at parts per billion and parts per trillion levels were detected. Separation of As^{III} and As^{V}, each at a concentration of 20 pg/mL, were reported.

Lu and Barnes (1996) compared the use of an ultrasonic nebuliser with a concentric glass nebuliser as a CE / ICPMS interface. A 3 to 8 fold improvement in signal sensitivity was reported for the ultrasonic nebuliser interface. This nebuliser gave absolute detection limits for ^{64}Zn, ^{66}Zn, ^{111}Cd and ^{114}Cd of 37, 36 1.2 and 2.5 fg, respectively, in metallothioneins.

Tangen *et al.* (1997) evaluated the effectiveness of a concentric nebuliser designed to deliver less than 10 μL/min of liquid chromatography effluent directly into an ICPMS argon plasma. By connecting a micro liquid chromatography column to the nebuliser the separation of tetramethyllead and tetraethyllead was demonstrated. A detection limit of 0.3 pg for a 60 nL injection of a solution containing ^{208}Pb was found when using this system.

More recently, and closer to the approach employed in this project for interface construction, Michalke and Schramel (1998) used CE / ICPMS for arsenic speciation with a laboratory designed interface connecting a 50 μm internal diameter (ID) capillary to the ICPMS nebuliser. Detection limits for As^{III}, As^{V}, MAA and DMAA were reported as 0.2 μmol/L and detection limits for AsB and AsC were reported as 0.9 μmol/L.

1.6 Laboratory Constraints and Synergies

All experiments were performed at PaLMS in a purpose-built trace elements laboratory located within the Royal North Shore Hospital, Sydney. Determinations at this laboratory encompass both trace nutritional and toxic elements. Sample matrices tested include blood, urine and tissue biopsies as well as environmental analyses in samples ranging from contaminated soils to drinking water. Nutritional elements analysed include copper, zinc, selenium, chromium and manganese. Toxic elements are monitored for occupational health and safety purposes and in cases of suspected poisoning or in response to clinical symptoms of heavy metal exposure. Arsenic, mercury, lead, cadmium, thallium and antimony are typically assayed in urine, with the exception of lead, for which blood levels are more appropriate.

Constraints placed on this project were such that any techniques developed for arsenic speciation had to be compatible with the large range of elements and sample matrices tested. Any procedures, which could compromise result accuracy or delay reporting of results for other elements, were avoided. A limited project budget also meant that major equipment purchases to alleviate some of the problems identified were beyond the funding of this project. The small range of arsenic containing species held by this laboratory and lack of commercial availability of many other arsenic species also placed some constraints on the range of experiments performed.

In developing a CE / ICPMS interface, a technique with potential synergies within the laboratory was also created. In addition to the ability to speciate arsenicals, CE / ICPMS could also be applied to the speciation of other elements and compounds of clinical significance. For example, the ability to separate vanadium (V), vanadium (IV), chromium (III), chromium (VI) and various organotin compounds would provide a useful addition to the range of tests already performed at this laboratory.

1.7 Project Scope

Methods have been published which are claimed to be capable of at least partial speciation of arsenic in biological samples. One such procedure is the solvent extraction / GFAAS method of Buchet *et al.* (1980). The evaluation and validation of a modified version of this solvent extraction method follows. In recent years, CE has also been shown to be capable of rapidly separating various arsenical compounds derived from biological samples, but the detection methods normally employed in CE systems (UV / visible absorptiometry, laser induced fluorescence, electrical conductivity) are relatively insensitive and non-specific. In this report, a method of interfacing a CE apparatus with an ICPMS instrument is described. In this way, the high-resolution separation of arsenical species achievable in CE can be combined with the high sensitivity and element specificity of ICPMS to produce an excellent arsenic speciation technique.

Details of CE method development and CE / ICPMS interface construction are described along with instrument operating parameters and analytical results. Results from the partial speciation of arsenic by solvent extraction and the limitations of this analytical technique are presented. These results include case studies from two patients exposed to arsenic and the results of extracting organoarsenic compounds from edible seaweeds. Potential uses of CE / ICPMS beyond this project are also discussed.

1.8 Aims

The aims of this project were as follows: -

1. To evaluate and validate a modified version of the solvent extraction method described by Buchet *et al*. (1980) as currently used by PaLMS for the partial speciation of arsenic compounds in human urine.

2. As a means to achieving the first objective, to develop a CE method for separating the full range of arsenical compounds likely to be encountered in human urine.

3. To assess the feasibility of enhancing the sensitivity and specificity of the CE method by coupling the CE system to an ICPMS detection system. This required the design and development of a suitable CE / ICPMS interface.

4. To undertake a preliminary study of arsenic speciation in the urine of a subject known to have undergone exposure to inorganic arsenic.

CHAPTER 2. MATERIALS AND METHODS

2.1 Instrumentation

2.1.1 Capillary Electrophoresis

A Beckman P/ACE 2100 CE system was used which was equipped with a UV detector and 254 nm filter and controlled by Beckman System Gold Version 8.0 software. Coolant solution held at $(22 \pm 1)^{o}C$ was pumped through the capillary cartridge to prevent overheating. The inlet carousel held 25 sample vials designated positions 11–35. Positions 31–35 were reserved for inlet electrolyte solutions used during high-voltage separations. The outlet carousel held 10 vials designated positions 1–10. These vials were used to hold terminal electrolyte solutions. The principles and techniques of CE that follow have been described in the instrument manufacturer's application notes (Beckman, 1987).

When a sample mixture is introduced into a capillary tube filled with a uniform electrolyte solution, separation of ionic species occurs due to the combined actions of electroosmotic flow (EOF) and electrophoretic migration (Jandik and Bonn 1993b). In CE, the capillaries are made from fused silica tubing with IDs of 25–100 µm and are coated externally with polyimide to an external diameter of 400 µm for mechanical strength. EOF refers to the bulk electrolyte flow caused by the charged inner capillary wall when an electrical potential is applied across terminal ends of a capillary. When in contact with an electrolyte solution, silanol groups along the inner wall of the capillary ionise, creating a negatively charged region at the capillary wall / electrolyte interface (Jackson and Haddad 1993). When voltage is applied, cations in the electrolyte near the capillary wall migrate toward the cathode, pulling electrolyte solution with them to create a net EOF toward the cathode. Electrophoretic migration is the movement of charged molecules in the bulk of the solution towards an electrode of opposite charge.

Due to electrophoretic migration, positive, neutral and relatively immobile ions such as proteins and peptides migrate at different rates but are all generally drawn toward the cathode by electroosmosis. Positively charged molecules reach the cathode first because

both EOF and electrophoretic migration act in the same direction on these ions. The electrophoretic mobilities of fast-moving anionic species such as chloride, sulphate, and arsenic species towards the anode exceed EOF towards the cathode, so that these ions do not reach the detector. This problem is solved by reversing the EOF by introducing the surface active chemical tetradecyltrimethylammonium hydroxide (TTAOH) to the electrolyte. TTA^+ cations neutralise the charged silanol groups on the capillary wall to enable the remaining OH^- ions to migrate towards the anode, pulling solvent water with them. The instrument electrode polarity is also reversed so that the cathode is at the inlet end of the capillary and the anode is at the outlet end of the capillary. A combined EOF and electrophoretic migration towards the capillary outlet is therefore achieved resulting in rapid, high efficiency separation of anionic molecules (Beckman, 1987).

As anions migrate through the capillary they form into bands of similar mobility based on molecular size and charge. These bands of molecules displace molecules of the electrolyte filling the capillary. By using an electrolyte that absorbs UV light strongly at 254 nm it is possible to detect separated anions as they migrate through the capillary. Provided the sample anions do not absorb strongly in the UV, a UV detector will observe a decrease in absorbance of the background electrolyte as each band of anionic molecules displaces it. In this process, known as "indirect UV detection", the decrease in UV absorbance is proportional to the concentration of anion present. The sensitivity and detection limit of this technique will vary for different elements and is also dependent upon the choice of background electrolyte and numerous other variables. Jandik and Bonn (1993b) have given typical detection limits for a variety of elements using different electrolytes and run conditions.

2.1.2 Inductively Coupled Plasma Mass Spectrometer

A Varian (Mulgrave, Victoria) UltraMass inductively coupled plasma mass spectrometer with Varian SPS-5 autosampler was used. Cooling water held at $(18 \pm 2)^{o}$C was recirculated from a Neslab chiller. High purity argon was supplied from high-pressure cylinders and a cryogenic system reticulated to the ICPMS. The exhaust system was fitted with a back-draft damper and operated continuously to prevent environmental air from reaching the instrument. Samples were nebulised with a micro concentric glass nebuliser (MCN) model number MCN100 supplied by Cetac. Nebulised samples passed from the nebuliser into a Sturman-Masters high-density polypropylene spray chamber as supplied by the instrument manufacturer. The spray chamber baffle was removed to ensure maximum sample transmission to the inductively coupled plasma (ICP) ion source. A quartz ICP torch and nickel sampler and skimmer cones were used. The principles and techniques of ICPMS that follow have been described in the instrument manufacturer's application notes (Varian, 1997).

Figure 2 provides a schematic diagram of components of a quadrupole ICPMS. Interlaced induction coils are used to generate the argon plasma that is used as an ion source (Date 1983; Houk 1986). The argon plasma temperature ranges from (6000 to 10000 K) and is maintained by 1.2 kW of radio frequency power from a radio frequency generator. In the initial stage of the sampling process, ions are removed from the argon plasma through a small orifice in the tip of a pair of coaxial water-cooled nickel cones. The UltraMass vacuum is maintained by differential pumping to reduce the pressure in three stages: -

1. The space between the coaxial cones is kept at a pressure of about 2 torr to produce a beam of particles in a high vacuum from an atmospheric pressure source. This allows most of the gas entering through the orifice in the sampler cone to be removed while passing the beam of ions from the argon plasma into the second stage of the vacuum system. The argon plasma entering the interface undergoes extremely rapid expansion and its temperature falls very quickly to limit the recombination of ions and electrons. Consequently the composition of the sampled beam is representative of the argon plasma.

2.   The vacuum is reduced to a pressure of around 5 x 10^{-5} torr by a turbo-molecular pump. At this pressure electrostatic ion lenses can guide ions. Neutral gases diffuse from the beam and are removed by the vacuum pump.

3.   The vacuum is further reduced in the section containing the quadrupole mass analyser and the detector. This is achieved with a smaller turbo-molecular pump that maintains a pressure of around 5 x 10^{-6} torr.

Ions emerging from the back of the skimmer cone are captured and focused into the mass analyser using an ion lens assembly. The first ion optics element is the extraction lens. Ions captured by the extraction lens are directed through the hole in the gate valve that separates the first and second stage of the vacuum system. They are then focused into the mass analyser by the main lens stack that is mounted directly in front of the quadrupole. A photon stop suspended in the centre of the electrode bias supplies (lens stack) prevents the transmission of high-energy on-axis ions and high-energy neutral atoms and molecules. It also prevents light from the plasma entering the mass filter.
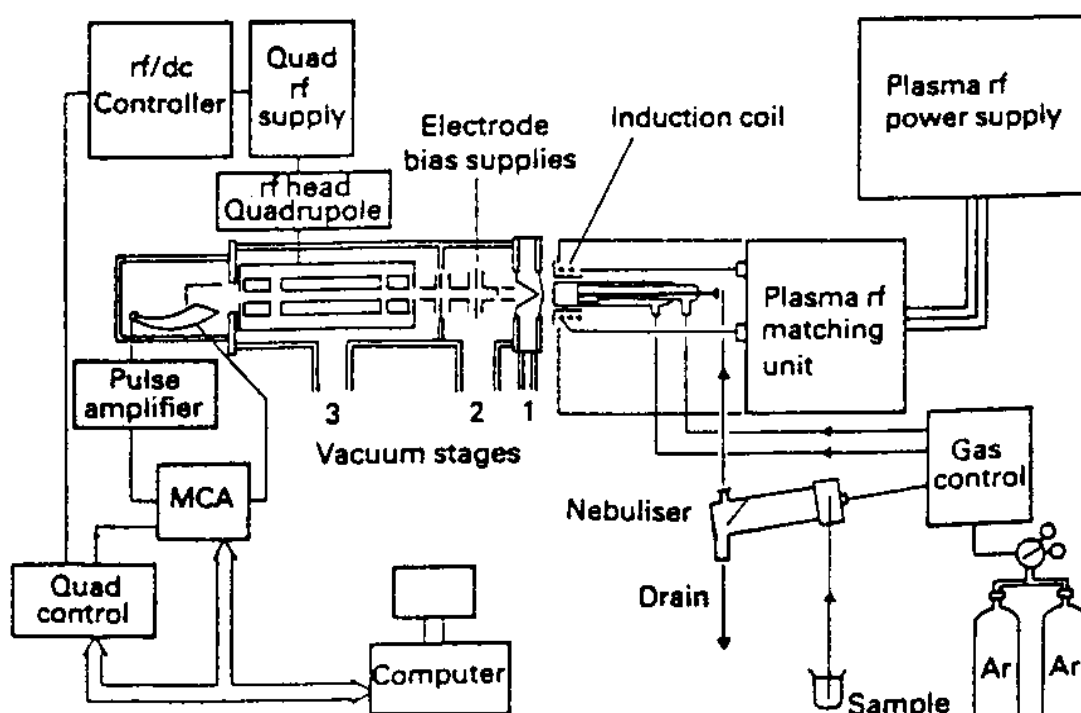
Figure 2 Schematic diagram of ICPMS components (MCA = Mass/charge analyser). Figure adapted from Delves (1988).

The quadrupole mass filter separates ions according to their mass-to-charge ratio (m/z). It consists of a set of four conductive rods mounted in insulating supports so that the rods are parallel and their axes lie on the corners of a square. The geometry of the assembly is correct to within a few millionths of a metre. Opposite pairs of rods are electrically connected. Radio frequency and DC potentials are applied to each pair of rods with opposite pairs of rods given an equal potential of opposite sign. Ions to be analysed enter at one end of the array of rods and those having the selected mass-to-charge ratio emerge at the other end. Ions with other mass-to-charge ratios have paths that cause them to collide with the quadrupole rods. Only ions having a specific mass-to-charge ratio have stable paths through the quadrupole enabling them to pass through to the detector.

Detection is by a discrete dynode electron multiplier. Each ion arriving at the detector collides with an electrode maintained at a high negative potential causing the electrode to emit a pulse of electrons. These electrons are then accelerated towards another surface that they hit and more electrons are emitted thereby amplifying the pulse. This process is repeated many times so that the arrival of one ion at the detector results in a measurable pulse of current.

2.1.3 Graphite Furnace Atomic Absorption Spectrometer

GFAAS experiments were performed on a Varian SpectrAA-800 with autosampler, controlled by Varian SpectrAA880Z Version 3.00 software. A boosted discharge hollow cathode arsenic super lamp supplied by Photron Pty. Ltd. with a Photron power supply was used. Measurements were made at the arsenic resonance line of 193.7 nm. The graphite tube atomisation power supply was a Varian GTA100 unit. The principles and techniques of GFAAS that follow are described in the instrument manufacturer's application notes (Varian, 1988).

Graphite furnaces are designed to generate a population of free, ground state atoms so that atomic absorption can be measured. This is generally achieved in three stages: -

1. A DRYING stage during which the solvent is removed from the sample in the furnace.
2. An ASHING stage which removes organic molecules or inorganic material.
3. An ATOMISATION phase in which free atoms are generated within a confined zone. The absorption signal produced in the atomisation stage is a sharp peak, the height or area of which can be related to the amount of analyte present.

These stages take place in a pyrolytically coated graphite tube held in place by graphite contact cones that are in turn held in a water-cooled copper block. The graphite tube is resistively heated by the passage of a high current through the tube. High purity argon flows through the graphite cones from each end to protect the graphite tube from rapid oxidation.

Nickel nitrate is mixed with the sample to reduce arsenic volatility. By making arsenic less volatile, higher ashing temperatures can be used to remove interfering substances prior to atomisation. This reduces simultaneous atomic and non-atomic absorption. Saturating the system with nickel produces conditions favourable for a single atomisation event. A single atomisation peak appears for the element of interest rather than a broad peak resulting from the decomposition and atomisation of a number of different molecular species.

Attenuation of the incident hollow cathode radiation due to light scattering or molecular absorption in the graphite tube can lead to erroneous results. The correction of this interference is achieved by making use of the 'Zeeman Effect'. Normal Zeeman Effect is the splitting of atomic spectral lines under the influence of a strong magnetic field. The central line (pi) remains at the original wavelength while two other lines (sigma) are produced and are symmetrically displaced about the pi line. Spectroscopic transitions involving the pi component always involve optical radiation polarised parallel to the direction of the applied magnetic field. The spectroscopic transitions involving the two sigma components always involve radiation polarised perpendicular to the magnetic field. The SpectrAA800 is operated with a magnet placed around the graphite tube to provide a modulated magnetic field. A fixed linear polariser is oriented to reject the component of the hollow cathode lamp emission polarised parallel to the magnetic field.

With the magnetic field off, the total absorbance of atomic plus background species is measured. When the magnetic field is on, only the background species are measured as they are insensitive to the presence of the magnetic field and continue to absorb. The field on and off absorbances are subtracted to yield the background corrected atomic absorbance.

2.2 Biological Specimens

2.2.1 Urine samples from human subjects

Arsenicals are rapidly metabolised from the blood and excreted in the urine making this the major pathway for the elimination of arsenic from the body (Section 1.4). Arsenic levels therefore remain higher in urine than blood making urine a good biological marker of exposure to arsenic (Le *et al.* 1994). Urine samples may be collected as random or 24 hour specimens. From our experience no preservative is required provided the urine is stored at 4–8°C for less than 1 week. Samples requiring longer storage can be frozen for up to 3 months. Details of the human urine samples used in this project follow.

2.2.1.1 Base urine

Used for matrix matched calibrations and spike additions, this urine is referred to as "Base Urine" throughout this thesis. It was collected from a healthy volunteer with no history of exposure to heavy metals and who had refrained from consuming seafood in the month prior to urine sample collection. The Base Urine was assayed by ICPMS several times in several analytical runs under reproducible conditions to establish a robust estimate of the total arsenic concentration. ICPMS analytical runs used to establish the Base Urine arsenic concentration included retained commercial quality assurance samples (2.2.1.2). Base Urine concentrations were only considered reliable when results obtained for the quality assurance samples were within 1 standard deviation of the quality control group median value (see Section 2.2.1.2). GFAAS was not used to determine total arsenic in the Base Urine, as the low concentration of arsenic in this sample was below the detection limit of this method. The Base Urine can be stored for up to 6 months at 4–8°C without the addition of preservatives.

2.2.1.2 Quality assurance urine

Quality assurance samples were obtained from Quality Control Technologies Pty Ltd. Each month this quality assurance program distributes a human urine sample, spiked to contain arsenic, to a group of 10–20 participating international laboratories. Results are statistically processed to provide subscribers with an analyte median value and distribution standard deviation as well as an index of laboratory performance within the group and laboratory bias. Typical QC median values range from 0 – 5 μmol/L arsenic.

2.2.1.3 Seafood meal urines

These urine samples were collected from subjects and referred to PaLMS Trace Elements Service for partial arsenic speciation or total arsenic determinations. They originated from various sites throughout Australia. Subjects included hospital inpatients, outpatients and mining company employees undergoing workplace testing where workers could be exposed to arsenic-containing fumes and dust from smelting and mining. Subjects identified as having high total urine arsenic levels were, upon interview, found to have consumed seafood within the days preceding urine collection. The exact nature of the seafood consumed and time between seafood consumption and urine collection were generally not pursued. Although it was highly likely that the elevated total arsenic levels in these specimens was entirely due to the ingestion of organic arsenicals from the seafood consumed, partial or full speciation (demonstrated later in this thesis) was required to exclude parallel exposure to inorganic arsenic species as a factor contributing to the high urinary arsenic levels in these subjects. With samples collected from sites throughout Australia, specimen storage conditions were out of the control of the PaLMS Trace Elements Service. Once received, samples were stored at 4–8°C. Urines were classified as "Seafood Urines" if they came from subjects who had elevated total urinary arsenic levels, and who, upon interview, admitted to consuming seafood in the days prior to urine collection.

2.2.1.4 Arsenic trioxide treated patient urine

This urine was collected from a 42-year-old female 24 hours after a single intravenous dose of arsenic trioxide (0.2 mg As_2O_3 per kilogram of body weight) had been administered as part of her chemotherapy for acute promyelocytic leukaemia. The sample was initially referred to the PaLMS Trace Elements Laboratory for a total

arsenic measurement. Due to the rarity of systemic exposure to inorganic arsenic, this sample was considered very valuable, with several aliquots frozen for use in speciation studies. This sample is referred to as "Arsenic Trioxide Urine" throughout this thesis.

2.2.2 Samples of marine biota

Numerous complex arsenic species are found in seaweeds (Shiomi 1994). These forms of marine biota were therefore chosen to provide a wide range of arsenicals to extract and challenge the arsenic speciation methods presented in this project. Three different types of edible seaweed were obtained from an Asian food store. Seaweeds are readily consumed amongst certain Asian populations where it is used in soups, is eaten with sushi or is diced and sprinkled on meals as flavouring. It is traditionally believed to protect against infection and prevent bleeding through improved clot formation. The exact identification of the seaweed species used in this project is unknown with the Korean labelling simply identifying the food as seaweed. The different types of edible seaweed investigated are differentiated on the basis of their description as follows: -

    1. Dry green seaweed – Dried green seaweed, compressed into thin layers.

    2. Moist green seaweed – Moist green seaweed, unprocessed.

    3. Kelp – Dry brown seaweed, unprocessed.

2.3 Reagents and Materials

All reagents were of analytical grade or better. Deionised water with a resistivity of greater than 18 MΩ.cm was used throughout. Solutions were stored at room temperature $(22 \pm 5)^{o}C$ unless otherwise stated. Solution storage was preferably in cleaned plasticware though storage container type was not critical unless otherwise stated. Pipettes used had a relative accuracy and precision of better than ± 2%. All glassware was cleaned by soaking in 20% nitric acid for at least 30 minutes and rinsed thoroughly with water before use. Plasticware was cleaned by soaking for at least 30 minutes in a solution of 20 g ethylenediamine tetraacetic acid (EDTA), disodium salt per litre of water and rinsed thoroughly with water before use. Glassware and plasticware were allowed to dry, covered and in a clean atmosphere, before use. Concentrations of solutions determined by ICPMS are given in units of µg/L or µg/mL as units of mass per volume bear a direct relationship to measurements made by this instrument.

2.3.1 CE Reagents and Materials

2.3.1.1   Sodium chromate concentrate, 100 mM – Prepared in a 1.000 L volumetric flask by dissolving 23.41 g of sodium chromate tetrahydrate, $Na_2CrO_4.4H_2O$ (MERCK, Product No. 6425) in 0.50 L of water then diluting to 1.000 L with water.

2.3.1.2   *p*-hydroxybenzoic acid (HBA) concentrate, 100 mM – Prepared in a 1.000 L volumetric flask by dissolving 1.38 g of HBA (Sigma, Product No. H5376) in 0.50 L of water then diluting to 1.000 L with water.

2.3.1.3   Methanol (UNILAB).

2.3.1.4   Tetradecyltrimethylammonium bromide (TTAB) concentrate, 100 mM – Prepared in a 100.0 mL volumetric flask by dissolving 3.365 g of TTAB (Sigma, Product No. T4762) in 50 mL of water then diluting to 100.0 mL with water.

2.3.1.5   Sodium hydroxide solution, 100 mM: Prepared in a 1.000 L volumetric flask by dissolving 4.00 g of NaOH (BDH AnalaR) in 0.50 L of water then diluting to 1.000 L with water. Stored in a capped plastic container.

2.3.1.6   Undeactivated fused silica capillary 75 µm internal diameter (ID) x 400 µm outside diameter (OD) - (J&W Scientific, Product No. JW160-2650).

2.3.1.7 <u>Anion exchange resin</u> (Bio-Rad AG 1-X8 Resin, 20–50 mesh hydroxide form, Product No. 140-1422).

2.3.1.8 <u>Electroosmotic Flow Modifier</u> - Tetradecyltrimethylammonium hydroxide (TTAOH). An anion exchange cartridge was prepared by adding anion exchange resin (2.3.1.7) to the 1 mL mark of a 10.0 mL mini-column. To ensure the anion exchange resin was in the hydroxide form, the cartridge was pre-rinsed with 10.0 mL of 100 mM NaOH (2.3.1.5) followed by 10.0 mL of water with the washings discarded. 4.00 mL of TTAB concentrate (2.3.1.4) was then passed through the cartridge into a 100.0 mL volumetric flask. The cartridge was rinsed with 10.0 mL of water that was also added to the flask. These steps were necessary to convert the TTAB from the bromide form into the hydroxide form (TTAOH). Three separate 100.0 mL flasks containing TTAOH and water washings (approximately 14.0 mL) were prepared and used to prepare the separate chromate, phthalate, and *p*-hydroxybenzoic acid electrolyte mixtures described immediately below.

2.3.1.9 <u>5 mM chromate, 4 mM TTAOH electrolyte</u> – 5.00 mL of sodium chromate concentrate (2.3.1.1) was added to the 100.0 mL volumetric flask prepared in step 2.3.1.8, containing approximately 14.0 mL of TTAOH and washings. The solution was then mixed with 50 mL of water and the pH adjusted to 9.0 ± 0.2 with additions of 100 mM NaOH (2.3.1.5) as required. The solution was made to a final volume of 100.0 mL with water. A 0.45 µm nylon filter (Activon Scientific Products, Product No. 9001-10) and vacuum apparatus were used to filter and degas the solution. Storage was in a capped plastic container.

2.3.1.10 <u>5 mM phthalic acid, 4 mM TTAOH electrolyte</u> – 0.17 g of phthalic acid (Sigma, Product No. P2944), 2.50 mL of methanol (2.3.1.3) and 1.00 mL of 100 mM NaOH (2.3.1.5) were added to the 100.0 mL volumetric flask prepared in step 2.3.1.8 that contained approximately 14.0 mL of TTAOH and washings. The solution was then mixed with 50 mL of water and the pH was adjusted to 9.0 ± 0.2 with additions of 100 mM NaOH (2.3.1.5) as required. The solution was made to a final volume of 100.0 mL with water. A 0.45 µm nylon filter (Activon Scientific Products, Product No. 9001-10) and vacuum apparatus were used to filter and degas the solution. Storage was in a capped plastic container.

2.3.1.11 <u>5 mM *p*-hydroxybenzoic acid, 4 mM TTAOH electrolyte</u> – 5.00 mL of HBA concentrate (2.3.1.2) and 1.00 mL of 100 mM NaOH (2.3.1.5) were added to the 100.0 mL volumetric flask prepared in step 2.3.1.8 that contained approximately 14.0 mL of TTAOH and washings. The solution was then mixed with 50 mL of water and the pH was adjusted to 9.0 ± 0.2 with additions of 100 mM NaOH (2.3.1.5) as required. The solution was made to a final volume of 100.0 mL with water. A 0.45 µm nylon filter (Activon Scientific Products, Product No. 9001-10) and vacuum apparatus were used to filter and degas the solution. Storage was in a capped plastic container.

2.3.1.12 <u>Arsenate stock, 13.3 mmol/L arsenic</u> – Prepared in a 10.0 mL volumetric flask by dissolving 0.042 g of sodium arsenate, $Na_2HAsO_4.7H_2O$ (Sigma, Product No. S9663) in 5 mL of water then diluting to 10.0 mL with water.

2.3.1.13 <u>Arsenite stock, 13.3 mmol/L arsenic</u> - Prepared in a 10.0 mL volumetric flask by dissolving 0.017 g of sodium arsenite, $NaAsO_2$ (Sigma, Product No. S7400) in 5 mL of water then diluting to 10.0 mL with water.

2.3.1.14 <u>DMAA stock, 13.3 mmol/L arsenic</u> - Prepared in a 10.0 mL volumetric flask by dissolving 0.018 g of DMAA, $C_2H_7AsO_2$ (Sigma, Product No. C0125) in 5 mL of water then diluting to 10.0 mL with water.

2.3.2 ICPMS Reagents and Materials

2.3.2.1 <u>High purity argon</u> - (BOC Gases).

2.3.2.2 <u>Triton X-100 (TX-100)</u> – (BDH Chemicals).

2.3.2.3 <u>Ethylene diamine tetraacetic acid (EDTA), diammonium salt hydrate, 97%</u> – (Aldrich Chemical Company Inc., Product No. 33,050-7).

2.3.2.4 <u>Sample diluent stock, 0.1% EDTA in 0.1% TX-100</u> - 0.50 g of EDTA (2.3.2.3) was added to 0.50 mL of TX-100 (2.3.2.2) in a 0.500 L volumetric flask with 0.25 L of water added to completely dissolve the EDTA. The resulting mixture was diluted to 0.500 L with water and stored in a polycarbonate container.

2.3.2.5 <u>Working sample diluent, 0.01% EDTA in 0.01% TX-100</u> - 50.0 mL of stock diluent (2.3.2.4) was diluted to 0.500 L with water and stored in a polycarbonate container.

2.3.2.6 <u>Tuning solution, 50 µg/L each of Ba, Be, Ce, Co, In, Pb, Mg and Th</u> - 2.50 mL of 10 µg/mL commercial stock solution (Inorganic Venture Inc.) was diluted to 0.500 L with water.

2.3.2.7 <u>Internal standard, 100 µg/L each of Bi, Ho, In, Sc, Tb and Y</u> - 0.50 mL of 100 µg/mL commercial stock solution (Alpha Resources Inc.) was diluted to 0.500 L with water.

2.3.2.8 <u>General purpose standard, 1000 µg/L each of Al, As, Ba, Be, Cd, Cr, Co, Cu, Pb, Mn, Ni, Se, Ag, Tl, V, Zn, Ca, Fe, Mg, K and Na</u> - 25.0 mL of 20 µg/mL commercial stock solution (Inorganic Venture Inc.) and 5.0 mL of SupraPur HNO_3 (MERCK) were diluted to 0.500 L with water in a volumetric flask. After mixing, the solution was immediately transferred to a clean polycarbonate storage container. The solution was stable for at least 4 months.

2.3.2.9 <u>Working standard, 50 µg/L</u> - 0.10 mL of standard (2.3.2.8) was diluted to 2.00 mL with diluent (2.3.2.5).

2.3.2.10 At least one quality control urine (2.2.1.2) was included in each analytical run.

2.3.2.11 <u>Plasticware</u> – Polyethylene tubes with a nominal volume of 5.0 mL and 10.0 mL were used to contain diluted samples and standards.

2.3.2.12 <u>Base Urine</u> – A urine sample shown to have low levels of trace elements (2.2.1.1). This urine was used as a calibration base to enable matrix matching of calibrators.

2.3.2.13 <u>Interference check solution, 0.2 M KCl</u> – Prepared in a 100.0 mL volumetric flask by dissolving 1.492 g of potassium chloride (BDH Chemicals, Product No. 10198) in 50 mL of water then diluting to 100.0 mL with water. This solution contained 200 mmol/L chloride.

2.3.3 CE / ICPMS Reagents and Materials

2.3.3.1 <u>High purity argon</u> - (BOC Gases).

2.3.3.2 <u>Arsenate stock, 1000 mg/L (13.3 mmol/L) arsenic</u> - Prepared in a 10.0 mL volumetric flask by dissolving 0.042 g of sodium arsenate, $Na_2HAsO_4.7H_2O$ (Sigma, Product No. S9663) in 5 mL of water then diluting to 10.0 mL with water.

2.3.3.3 <u>Arsenite stock, 1000 mg/L (13.3 mmol/L) arsenic</u> - Prepared in a 10.0 mL volumetric flask by dissolving 0.017 g of sodium arsenite, $NaAsO_2$ (Sigma, Product No. S7400) in 5 mL of water then diluting to 10.0 mL with water.

2.3.3.4 <u>DMAA stock, 1000 mg/L (13.3 mmol/L) arsenic</u> - Prepared in a 10.0 mL volumetric flask by dissolving 0.018 g of DMAA, $C_2H_7AsO_2$ (Sigma, Product No. C0125) in 5 mL of water then diluting to 10.0 mL with water.

2.3.3.5 <u>Arsenobetaine hydrobromide stock, 290 mg/L (3.9 mmol/L) arsenic</u> – A 1000 mg/L $(CH_3)_3AsCH_2COO.HBr$ solution in distilled water was kindly supplied by the Division of Analytical Laboratories, Lidcombe in 1990. This solution was stored in the freezer. The compound was initially synthesised and supplied by the Western Australian Marine Research Laboratories (Cannon *et al.* 1981).

2.3.3.6 <u>Tuning solution, 50 µg/L each of As, Ba, Be, Ce, Co, In, Pb, Mg and Th</u> - 2.50 mL of 10 µg/mL commercial stock solution (Inorganic Venture Inc.) and 0.025 mL of arsenate stock (2.3.3.2) were diluted to 0.500 L with water.

2.3.3.7 <u>*p*-hydroxybenzoic acid concentrate, 100 mM</u> - Prepared in a 1.000 L volumetric flask by dissolving 1.38 g of HBA (Sigma, Product No. H5376) in 0.50 L of water then diluting to 1.000 L with water.

2.3.3.8 <u>Tetradecyltrimethylammonium bromide concentrate, 100 mM</u> - Prepared in a 100.0 mL volumetric flask by dissolving 3.365 g of TTAB (Sigma, Product No. T4762) in 50 mL of water then diluting to 100.0 mL with water.

2.3.3.9 <u>Sodium hydroxide solution, 100 mM</u> - Prepared in a 1.000 L volumetric flask by dissolving 4.00 g of NaOH (MERCK, Product No. 6425) in 0.50 L of water then diluting to 1.000 L with water. This solution was stored in a capped plastic container.

2.3.3.10 <u>Undeactivated fused silica capillaries</u>, 75 µm and 100 µm ID x 400 µm OD (J&W Scientific, Product Nos., JW160-2650 and JW160-2660). Capillaries were cut to a total length of 75 cm and a window was burnt into the polyimide coating 17 cm from the inlet side of the capillary.

2.3.3.11 <u>Anion exchange resin</u> - (Bio-Rad AG 1-X8 Resin, 20–50 mesh hydroxide form, Product No. 140-1422).

2.3.3.12 <u>Electroosmotic Flow Modifier</u> - TTAOH. An anion exchange cartridge was prepared by adding anion exchange resin (2.3.3.11) to the 1 mL mark of a 10.0 mL mini-column. To ensure that the anion exchange resin was in the hydroxide form, the cartridge was pre-rinsed with 10.0 mL of 100 mM NaOH (2.3.3.9) followed by 10.0 mL of water. The rinse washings were discarded. 4.00 mL of TTAB concentrate (2.3.3.8) was slowly passed through the cartridge into a 100.0 mL volumetric flask. The cartridge was then rinsed with 10.0 mL of water that was also added to the flask. This step was necessary to convert the TTAB from the bromide form into the hydroxide form (TTAOH). The 100.0 mL flask containing TTAOH and water washings (approximately 14.0 mL) was used in the preparation the *p*-hydroxybenzoic acid electrolyte mixture described immediately below.

2.3.3.13 <u>5 mM *p*-hydroxybenzoic acid, 4 mM TTAOH electrolyte solution</u> - 5.00 mL of HBA concentrate (2.3.3.7) was added to the 100.0 mL volumetric flask prepared in step 2.3.3.12 that contained approximately 14.0 mL of TTAOH and washings. This solution was mixed and diluted to 50 mL with water. 100 mM NaOH (2.3.3.9) was added drop wise until the pH reached 11.3. The final volume was then made up to 100.0 mL with water and mixed once again. Before use, this solution was filtered and degassed through a 0.45 µm nylon filter (Activon Scientific Products, Product No. 9001-10) using a vacuum apparatus. A capped plastic container was used to store the solution.

2.3.3.14 <u>0.5 mM *p*-hydroxybenzoic acid, 0.4 mM TTAOH electrolyte solution</u> - 10.0 mL of the 5 mM HBA, 0.4 mM TTAOH electrolyte solution (2.3.3.13) was diluted to 100.0 mL with water in a 100.0 mL volumetric flask. Before use, this solution was filtered and degassed through a 0.45 µm nylon filter (Activon Scientific Products, Product No. 9001-10) using a vacuum apparatus. A capped plastic container was used to store the solution.

2.3.3.15 <u>Modified CE cartridge</u> - detailed in Chapter 3.

2.3.4 Organic Solvent Extraction / GFAAS Reagents and Materials

2.3.4.1 <u>Concentrated nitric acid, 65% w/w</u> - (MERCK).

2.3.4.2 <u>Concentrated sulphuric acid, 98% w/w sp. gr. 1.84</u> - (BDH AnalaR).

2.3.4.3 <u>Concentrated hydrochloric acid, 36% w/w sp. gr. 1.16</u> - (BDH AnalaR).

2.3.4.4 <u>Sulphuric acid, 50% w/w</u> - Equal volumes of concentrated sulphuric acid (2.3.4.2) and water were mixed.

2.3.4.5 <u>Graphite tube of notched partition type</u> - (Varian Part No. 63-100023-00).

2.3.4.6 <u>High purity argon</u> - (BOC Gases).

2.3.4.7 <u>Nickel nitrate solution, 7.6 g/L</u> – Prepared in a 1.000 L volumetric flask by dissolving 7.6 g of nickel (II) nitrate 6-hydrate (BDH AnalaR) in 50 mL of water then diluting to 1.000 L with ethanol. The solution was stabilised with the addition of 0.20 mL of nitric acid (2.3.4.1) and stored from 4–8°C.

2.3.4.8 <u>Potassium iodide solution, 830 g/L</u> – Prepared in a 0.500 L volumetric flask by dissolving 415 g of potassium iodide (UNIVAR) and 20 g of ascorbic acid in 0.25 L of water then diluting to 0.500 L with water. The solution was stored from 4–8°C.

2.3.4.9 <u>Cyclohexane</u> - (UNILAB).

2.3.4.10 <u>Stock standard arsenic solution, 13.3 mmol/L</u> - Prepared in a 10.0 mL volumetric flask by dissolving 0.017 g of sodium arsenite, $NaAsO_2$ (Sigma, Product No. S7400) in 5 mL of water then diluting to 10.0 mL with water.

2.3.4.11 <u>Working standard arsenic solution, 26.6 µmol/L</u> – Prepared in a 0.500 L volumetric flask by diluting 1.00 mL of stock standard (2.3.4.10) and 5.00 mL of nitric acid (2.3.4.1) to 0.500 L with water. This solution was stable for at least 2 years.

2.3.4.12 <u>Sampler cups</u> - (GBC Part No. 99-0022-00).

2.3.4.13 <u>Extraction tubes</u> – Polyethylene tubes with a nominal volume of 10.0 mL were used to perform the extractions. These tubes had screw cap lids and were marked with 0.5 mL graduations.

2.3.4.14 <u>DMAA stock solution, 11.4 mmol/L arsenic</u> – Prepared in a 200.0 mL volumetric flask by dissolving 0.315 g of DMAA, $C_2H_7AsO_2$ (Sigma, Product No. C0125) in 100 mL of water then diluting to 200.0 mL with water. This solution was stored from 4–8°C.

2.3.4.15 <u>DMAA working solution, 57 µmol/L arsenic</u> – Prepared in a 200.0 mL volumetric flask by diluting 1.00 mL of DMAA stock solution (2.3.4.14) to 200.0 mL with water. This solution was stored from 4–8°C.

2.3.4.16 <u>Arsenobetaine hydrobromide stock solution, 3.9 mmol/L arsenic</u> – A solution of 1000 mg/L $(CH_3)_3AsCH_2COO.HBr$ in distilled water was kindly supplied by the Division of Analytical Laboratories, Lidcombe in 1990. This solution was stored in a freezer. The compound was initially synthesised and supplied by the Western Australian Marine Research Laboratories (Cannon *et al.* 1981).

2.3.4.17 <u>Arsenobetaine hydrobromide working solution, 39 µmol/L arsenic</u> - 1.00 mL of the AsB stock solution (2.3.4.16) was diluted with 90.0 mL of water in a 100.0 mL volumetric flask. 0.20 mL of nitric acid (2.3.4.1) was added to stabilise the solution. The final solution volume was made up to 100.0 mL with water. The solution was stored from 4–8°C.

2.3.4.18 <u>Arsenate stock solution, 13.3 mmol/L arsenic</u> - Prepared in a 10.0 mL volumetric flask by dissolving 0.042 g of sodium arsenate, $Na_2HAsO_4.7H_2O$ (Sigma, Product No. S9663) in 5 mL of water then diluting to 10.0 mL with water. This solution was stored from 4–8°C.

2.3.4.19 <u>Arsenate working solution, 26.6 µmol/L arsenic</u> – Prepared in a 0.500 L volumetric flask by diluting 1.00 mL of stock standard (2.3.4.18) to 0.500 L with water. This solution was stored from 4–8°C.

2.3.5 Reagents and Materials used to Extract Arsenicals from Marine Biota

2.3.5.1 <u>Concentrated nitric acid, 65% w/w</u> - (MERCK).

2.3.5.2 <u>2 M potassium hydroxide (KOH) in methanol</u> - 1.12 g of KOH (BDH, AnalaR) was dissolved in 9.0 mL methanol (UNILAB) in a 10.0 mL volumetric flask. Once completely dissolved, further methanol was added to a final volume of 10.0 mL.

2.3.5.3 <u>Extraction tubes</u> - Polyethylene tubes with a nominal volume of 10.0 mL were used to perform the extractions. These tubes had screw cap lids and were marked with 0.5 mL graduations.

2.4 Analytical Procedure

2.4.1 CE

2.4.1.1 CE Operating Conditions

All instrument components were maintained and operated to manufacturer specifications (Beckman P/ACE 2000 Series Instrument Manual, 1987). Three electrolyte solutions containing 4 mM TTAOH and 5 mM chromate (2.3.1.9), 5 mM phthalic acid (2.3.1.10) or 5 mM HBA (2.3.1.11), were investigated for their suitability to speciate arsenicals. The Beckman P/ACE 2100 CE apparatus (Section 2.1.1) instrument parameters are shown in Appendix A. Separations were performed in an undeactivated fused silica capillary that was cut to a total length of 50 cm and had an ID of 75 µm and an OD of 400 µm (2.3.1.6).

2.4.1.2 Modifications to CE Procedure

The effect of doubling the electrolyte concentration in each solution was investigated. The volume of stock chromate (2.3.1.1) added to the chromate electrolyte solution (2.3.1.9) was increased to 10.0 mL to increase the final chromate concentration from 5 to 10 mM. The phthalic acid concentration of the phthalic acid electrolyte solution (2.3.1.10) was similarly increased from 5 to 10 mM by adding an additional 0.17 g of phthalic acid. Lastly, the HBA concentration of the HBA electrolyte solution (2.3.1.11) was increased from 5 to 10 mM by increasing the volume of stock HBA (2.3.1.2) added from 5 to 10 mL.

The effect of altering pH was also investigated with the dropwise addition of 100 mM NaOH (2.3.1.5) to the three electrolyte solutions, 5 mM chromate (2.3.1.9), 5 mM phthalic acid (2.3.1.10) and 5 mM HBA (2.3.1.11). A pH meter was used to ensure sufficient NaOH was added to cause the electrolyte solution pH to increase from 9 to 10.

2.4.1.3 CE Conditions Finally Adopted

From the experimental results presented in Section 4.1, 5 mM HBA was established as the preferred background electrolyte. 4 mM TTAOH was added as the osmotic flow modifier and the solution pH was adjusted to 11.3 with the dropwise addition of 100 mM NaOH (2.3.3.13) before making the solution to final volume. An undeactivated fused silica capillary (75 µm ID x 400 µm OD) cut to a total length of 50 cm (2.3.1.6) was found to give excellent results. The Beckman P/ACE 2100 CE apparatus (Section 2.1.1) instrument parameters were as shown in Appendix A.

2.4.2 ICPMS

The Varian UltraMass ICPMS apparatus (Section 2.1.2) was maintained to manufacturer's specifications (Varian UltraMass operation manual, 1997). A peristaltic pump was used to deliver sample to the nebuliser and to drain waste from the spray chamber. A T-piece was used to connect internal standard and sample uptake lines. Steps followed to determine total arsenic concentrations are listed below. Instrument operating parameters are shown in Appendix B. This technique also permitted the simultaneous determination of other urine trace elements including cadmium, copper, zinc, lead, aluminium, thallium, antimony and cobalt. A 'matrix matched' calibration was used to limit the effect of spectroscopic interferences. In ICPMS, spectroscopic interferences arise when an analyte peak cannot be easily resolved from an overlapping peak (Tan and Horlick 1986). These spectroscopic interferences may be isobaric interferences (overlapping peak from a singly charged ion), doubly charged ion interferences (overlapping peak from a doubly charged ion) or molecular inferences (overlapping peak from a molecular species such as argon chloride). The inclusion of scandium as an internal standard helped compensate for chemical and physical interferences (Vanhaecke *et al.* 1992).

Analysis Steps.

a) Tuning solution (2.3.2.6) was pumped so that Be, In and Pb and Ce/CeO and Ba/Ba^{2+} ratios could be monitored to optimise torch position, peak resolution, mass calibration, detector voltage, plasma parameters and ion optics. Optimum tuning was achieved when the sensitivities of Be, In and Pb were maximal while the Ce/CeO and Ba/Ba^{2+} ratios were minimal (Vaughan and Horlick 1986).

b) One uptake line was placed in the internal standard solution (2.3.2.7) while the other uptake line was connected to the autosampler.

c) Calibrators were prepared in 10.0 mL tubes (2.3.2.11) using the volumes shown in Table III. Calibrators were stable for at least 14 days when stored at 4–8°C.

Table III Protocol for the preparation of matrix matched urine calibrators.

Calibrator name	Base Urine (mL) (2.3.2.12)	Diluent (mL) (2.3.2.5)	1000 µg/L standard (mL) (2.3.2.8)	50 µg/L standard (mL) (2.3.2.9)
C1	1.000	9.000	-	-
C2	1.000	8.800	-	0.200
C3	1.000	8.950	0.050	-
C4	1.000	8.900	0.100	-

d) 0.200 mL of sample was diluted with 1.800 mL of diluent (2.3.2.5). Quality control samples (2.3.2.10) and interference check solution (2.3.2.13) were treated in the same manner.

e) The UltraMass method editor was programmed with the parameters shown in Appendix B. The UltraMass sequence editor was programmed so that calibrators, controls and samples were automatically sampled from the autosampler.

f) The software generated a calibration curve enabling the interpolation of arsenic concentrations for the unknown samples. Results were automatically corrected for internal standard variation. The %RSD of sample replicates was reported to give an indication of result reliability. Results were reported in units of µmol/L to a detection limit of 0.1 µmol/L. Samples with arsenic concentrations exceeding the calibration range were repeated after dilution in water.

2.4.3 CE / ICPMS

The development of a CE / ICPMS interface constituted a major achievement in this project. Details of its construction are discussed in Chapter 3. All CE and ICPMS components were maintained to manufacturer's specifications (Varian UltraMass operation manual, 1997; Beckman P/ACE 2000 Series Instrument Manual, 1987). Instrument operating parameters are shown in Appendix C.

The CE apparatus was positioned next to the nebuliser of the ICPMS with the CE side panel opened to allow access to the CE high-voltage cables. The peristaltic pump electronics of the ICPMS were disconnected to prevent ICPMS shutdown through voltage leakage from the CE high-voltage cable. The ICPMS nebuliser argon flow rate was set to 0.8 L per minute. At this rate self-aspiration through the MCN was at 400 μL per minute. The ICPMS was tuned for optimal sensitivity to arsenic by adding sodium arsenate (2.3.3.2) to the tuning solution (2.3.3.6) to a final concentration of 50 μg/L. Peak resolution, mass calibration and detector voltage were the same as those set in Section 2.4.2 (Appendix B). The tuning solution was self-aspirated while monitoring As and Ce/CeO and Ba/Ba^{2+} ratios to optimise torch position, plasma parameters and ion optics. Optimum tuning was achieved when the sensitivity of As was maximal while the Ce/CeO and Ba/Ba^{2+} ratios were minimal (Vaughan and Horlick 1986).

The capillary outlet from the modified CE cartridge (2.3.3.15) was connected to the nebuliser as described in Chapter 3. Steps for speciating arsenicals by CE / ICPMS were as follows: -

    a) The ICPMS software was configured to enable the data generated to be saved in a comma separated variable (CSV) format. To do this: -
- An OS/2 window was opened and the run directory C:\VARIAN\ICPMS\RUN was selected.
- The ini file editor INI C:\VARIAN\ICPMS\SYSDATA\ICPMS\INI was run.
- Section MSQ was selected.
- Tag TR_MASS_FILE was selected.

> ➤ The value in the contents text box was changed from 0 to 1.

> ➤ The Add/Change Button was selected.

> ➤ Tag TR_ISO_FILE was selected.

> ➤ The value in the contents text box was changed from 0 to 1.

> ➤ The Add/Change Button was selected.

> ➤ The ini file editor was closed and the UltraMass software restarted.

> ➤ Changing the contents text box values back to 0 disabled CSV file writing.

b) The nebuliser argon flow was set to 0.8 L/min to self-aspirate electrolyte from the electrolyte reservoir. Once sufficient electrolyte had been drawn up to cover the CE capillary outlet and stainless steel tube, the argon flow was stopped.

c) A 1 minute high-pressure injection was initiated to fill the capillary with electrolyte from the inlet vial.

d) Sample was injected and the high-voltage separation started. The inclusion of HBA in the electrolyte provided a strong UV absorbing background so that, by observing the UV detector response, the capillary patency could be checked. After 7 minutes run time, the voltage was shut down and a low-pressure injection of water from Vial 33 was applied to the inlet side of the capillary. This slowly pushed separated arsenicals through the capillary.

e) As soon as the high-voltage separation had ceased, plasma ignition was initiated and the ICPMS sequence activated. This started the nebuliser argon flow so that electrolyte was drawn past the capillary outlet. As electrolyte passed the capillary outlet, separated arsenicals that had been pushed from the capillary outlet were swept into the nebuliser.

f) Nebulised sample passed into the spray chamber then into the argon plasma. Once in the argon plasma, elements were ionised before passing through the sample and skimmer cones and then into the quadrupole mass filter. The quadrupole mass filter was tuned so that only ions at m/z 75 were allowed to pass through to the detector. The detector collected counts into a CSV file using a 1 second dwell time over 10 minutes.

g) When sample analysis was complete the argon plasma was shut down. The CSV file, located in the USERDATA sub directory, was transferred to floppy disk and deleted from the USERDATA sub directory. The system was then ready to analyse further samples.

h) CSV data files were transferred to an Excel spreadsheet for analysis as described in Appendix E. Peak identification was achieved by correlating retention times and by sample spiking using appropriate dilutions of the stock arsenic solutions (2.3.3.2–5).

2.4.4 Organic Solvent Extraction / GFAAS

The Varian SpectrAA-800 GFAAS unit was tuned and optimised to the manufacturer's specifications (Varian SpectrAA-800 Instrument Manual, 1993). Instrument operating parameters are listed in Appendix D.

<u>Extraction and Analysis Steps.</u>
a) Calibrators were prepared in a series of extraction tubes (2.3.4.13) using the volumes of arsenic working standard (2.3.4.11) shown: -

   Calibrator 1 - no addition
   Calibrator 2 - 0.05 mL
   Calibrator 3 - 0.10 mL
   Calibrator 4 - 0.15 mL

b) Reagent blank and test samples were prepared by pipetting 1.50 mL of water or test sample into labelled extraction tubes (2.3.4.13).

c) Quality control samples were prepared in labelled extraction tubes (2.4.3.13) by adding an additional 0.05 mL of DMAA working solution (2.3.4.15) or 0.05 mL of AsB working solution (2.3.4.17) to two separate 1.50 mL aliquots of the test urine. These additions were equivalent to 1.8 µmol/L and 4.3 µmol/L arsenic for the DMAA and AsB, respectively.

d) 0.30 mL of sulphuric acid (2.3.4.4), 4.00 mL of hydrochloric acid (2.3.4.3), 1.50 mL of potassium iodide (2.3.4.8) and 2.00 mL of cyclohexane (2.3.4.9) were added to all extraction tubes.

e) The tubes were then capped and inverted backwards and forwards for 1 minute. It was essential that all tubes were mixed the same way to standardise extraction efficiency. Tubes were centrifuged at 4000 rpm for 5 minutes to separate the phases. Arsenite (As^{III}), arsenate (As^{V}), MAA and DMAA formed complexes with iodine. These arsenic iodide complexes concentrated in the cyclohexane layer. The cyclohexane layer was uppermost after sample centrifugation.

f) 500 µL of the cyclohexane layer was pipetted and mixed in a sampler cup (2.3.4.12) containing 500 µL of nickel nitrate solution (2.3.4.7). The solutions were then ready for atomisation.

g) Using the instrument parameters specified in Appendix D, the software plotted a calibration curve from which the test results were interpolated and reported in units of µmol/L. Each reading was the average of duplicate measurements. The %RSD of the duplicate measurements was reported to give an indication of result reliability. The total arsenic concentration of the blank solution was subtracted from all other solution arsenic concentrations prior to reporting. Sample results outside the calibration range were diluted in water and repeated.

2.4.5 Extraction of Arsenicals from Marine Biota

Samples of marine biota (Section 2.2.2) were finely diced with a scalpel blade to decrease overall digestion time. The samples were then weighed into tared 10.0 mL digestion tubes (2.3.5.3). A greater sample mass was used when extracting arsenic from wet samples (0.5–1.2 g) than for dried samples (0.1–0.4 g). Each sample was digested in 1.00 mL nitric acid (2.3.5.1) or 1.00 mL of KOH / methanol (2.3.5.2). Digestion tubes were capped loosely and allowed to stand for between 12–18 hours before being placed in a heating mantle for 4 hours at 60°C. Samples digested in nitric acid were made up to the 10.0 mL graduation with water while samples digested in KOH / methanol were made up to the 2.0 mL graduation with water.

Determination of arsenic in marine biota extracts by ICPMS.

All components of the Varian UltraMass ICPMS were maintained to manufacturer's specifications (Varian UltraMass operation manual, 1997). A peristaltic pump was used to deliver sample to the nebuliser and drain waste from the spray chamber. A T-piece was used to connect internal standard and sample uptake lines. Steps followed to determine total arsenic concentrations are listed below. The operating parameters used were the same as those given in Appendix A except for the calibrator concentrations. These concentrations are shown in Table IV. The inclusion of scandium as an internal standard helped compensate for chemical and physical interferences. Reagents and materials were the same as those listed in Section 2.3.2.

Analysis Steps.
   a) Tuning solution (2.3.2.6) was pumped so that Be, In and Pb and Ce/CeO and Ba/Ba^{2+} ratios could be monitored to optimise torch position, peak resolution, mass calibration, detector voltage, plasma parameters and ion optics. Optimum tuning was achieved when the sensitivity of Be, In and Pb was maximal while Ce/CeO and Ba/Ba^{2+} ratios were minimal (Vaughan and Horlick 1986).
   b) One uptake line was placed in the internal standard solution (2.3.2.7) while the other uptake line was connected to the autosampler.

c) Calibration solutions were prepared in 10.0 mL tubes (2.3.2.11) using the volumes shown in Table IV. Calibrators were prepared fresh and used within 4 hours of preparation.

Table IV Protocol for the preparation of aqueous arsenic calibrators.

Calibrator name	Water (mL)	1000 µg/L standard (mL) (2.3.2.8)	Concentration of arsenic (µg/L)
Blank	10.000	0.000	0
C1	9.950	0.050	5
C2	9.900	0.100	10
C3	9.500	0.500	50
C4	9.000	1.000	100

d) 0.200 mL of sample (marine biota digest; see Section 2.4.5) was diluted in 1.800 mL of water. Quality control samples and interference check solution (2.3.2.13) were treated in the same manner.

e) The UltraMass method editor was programmed with the parameters shown in Appendix B. The UltraMass sequence editor was programmed so that calibrators, controls and samples were automatically sampled from the autosampler.

f) The software generated a calibration curve to enable the interpolation of arsenic concentrations in the unknown samples. Results were automatically corrected for internal standard variation. The %RSD was reported to give an indication of result reliability. Results were reported in units of µg/L to a detection limit of 0.1 µg/L. Samples with arsenic concentrations exceeding the calibration range were repeated after dilution in water.

Partial speciation of arsenicals in marine biota extracts by solvent extraction / GFAAS.

Digests from marine biota (see Section 2.4.5) were treated in the manner described for urine samples in Section 2.4.4.

CHAPTER 3. CE / ICPMS INTERFACE DEVELOPMENT

The major goal achieved in this project was the design and construction of a novel CE / ICPMS interface. To construct the interface, several important modifications to the available CE and ICPMS instruments were required. As both instruments were routinely operated separately, any modifications needed to be easily reversible.

Once a suitable CE method for separating arsenicals in urine had been developed (Section 2.4.1), it was necessary to create a way of redirecting the capillary effluent from the terminal end of the capillary to the ICPMS nebuliser. Initial experiments along this line focused on creating a longer terminal end of the capillary to project from the capillary cartridge to the outside of the CE instrument. A wire attached to the terminal platinum electrode could then run parallel to the extended capillary to create an external electrode. The sharp angle required to bend the capillary so that it would remain free of the CE sample carousel, the fact that the capillary emerged on the right hand side of the instrument when the ICPMS nebuliser was on the left hand side of this instrument, the difficulty in running a wire to the terminal electrode and the need to drill holes in the instrument cover to provide an exit point for the capillary and electrode made this approach undesirable.

After consultation with the Biomedical Engineering Department at Royal North Shore Hospital, it was decided that a better option would be to redirect the path of the capillary through the capillary cartridge so that it emerged from the top of the cartridge. This way it would not foul any of the other components of the CE sample carousel. The results of this modification are shown in Figure 3 where the capillary can be seen emerging from a small hole drilled in the top of the capillary cartridge. It was now possible to connect the capillary outlet to the ICPMS nebuliser. By modifying the capillary in this way, sample could still be pressure injected into the capillary at the inlet point and the CE sample carousel remained fully functional to hold samples and reagents. Sample progress through the capillary could also be monitored with the CE UV detector and much more of the capillary remained bathed in coolant within the capillary cartridge. This prevented capillary overheating that could lead to the formation of convection currents in the capillary, leading to loss of resolution between separated bands.
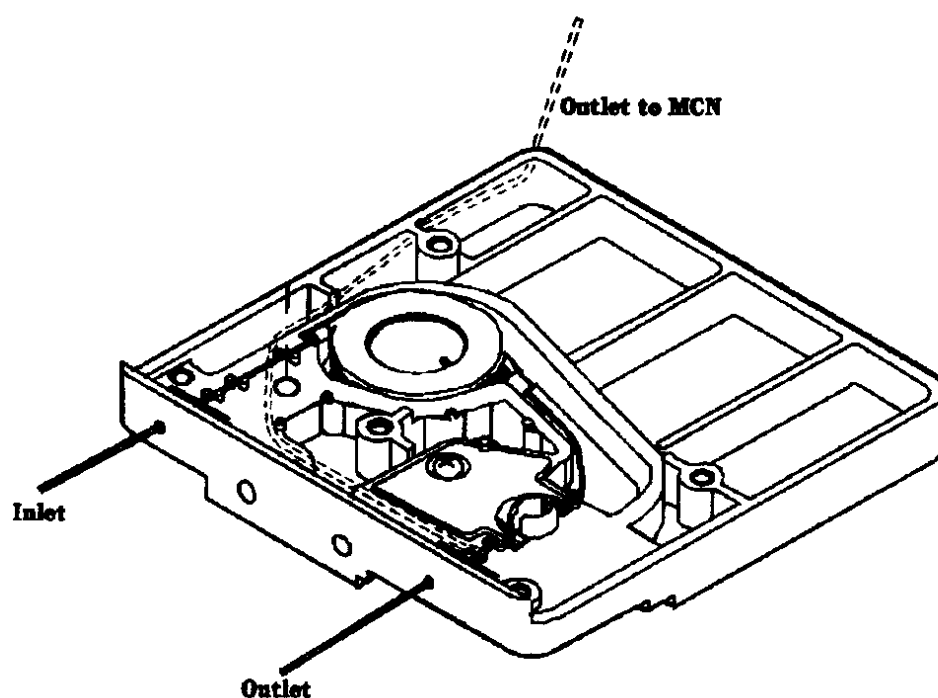
Figure 3 Diagram of capillary path through a cartridge. The broken line indicates the
modified path taken by the capillary to enable it to connect to a micro concentric
glass nebuliser (MCN).

Once the path of the capillary had been redirected, it was necessary to create a high-voltage connection external to the CE unit. The polarity of the voltage applied to the capillary can be reversed in the P/ACE 2100 CE system by reversing the order of the inlet and outlet high-voltage cables. These cables are accessed through a panel in the side of the instrument. By purchasing a spare high-voltage cable from Beckman Coulter it was possible to plug the cable into the outlet connector to run it outside the instrument. A hollow stainless steel tube 50 mm long with an internal diameter of 2 mm was soldered to this high-voltage cable and encased in Araldite epoxy resin to minimise the risk of electrocution. By passing the outlet end of the capillary through the stainless steel tube a moveable external electrode was created.

Initial attempts to see if this system would work were conducted by placing the outlet from the capillary in the modified cartridge and the external electrode into a flask containing electrolyte and performing a high-voltage separation. Each time the high-voltage was activated the instrument produced a high-voltage error message and failed

to operate. This error message is produced when circuitry in the CE instrument senses voltage leakage and shuts down the power to prevent possible operator injury. After investigating and eliminating possible sources of voltage leakage, it was found that a safety switch on the door enclosing the high-voltage cables triggered the error condition. As the creation of an external electrode required this door to be left open during operation it was necessary to disconnect this safety switch. Once this was done it was possible to run the instrument with the panel open and successfully separate arsenic species with the capillary outlet and terminal electrode outside the CE instrument.

The next task was to connect the capillary outlet and electrode to the ICPMS nebuliser in a way that would maintain an electrical contact between the capillary outlet and the electrode. After experimenting with several prototypes, the best configuration was devised with the capillary held in place by pushing it through a 20 mm section of polytetrafluoroethylene (PTFE) tubing with a 300 µm internal diameter. This section of tubing was then glued inside a glass T-piece with the opposite end of the T-piece attached to one end of the stainless steel tube. The other end of the stainless steel tube was connected to the MCN inlet. In this way the capillary could be pushed through the section of PTFE tubing and through the stainless steel tube to terminate at the nebuliser inlet. PTFE tubing was used to connect a reservoir of electrolyte to the side arm of the glass T-piece. These features are shown schematically in Figure 4 and as photographs in Figures 5 and 6. When the nebuliser argon flow was started, electrolyte was drawn from the reservoir, through the glass T-piece and stainless steel tube and into the nebuliser. In this way electrolyte coated the terminal end of the capillary and the inside of the stainless steel tube to complete the electrical contact.

The distance from the terminal end of the capillary to the nebuliser inlet was 0.5–1.0 mm. At this distance sample was drawn into the nebuliser before it had time to diffuse into the surrounding electrolyte. The small gap ensured that electrolyte was not sucked through the capillary but from the electrolyte surrounding the outside of the capillary tube. The electrolyte used to surround the capillary was a tenfold dilution of the electrolyte inside the capillary. By diluting the electrolyte, the total concentration
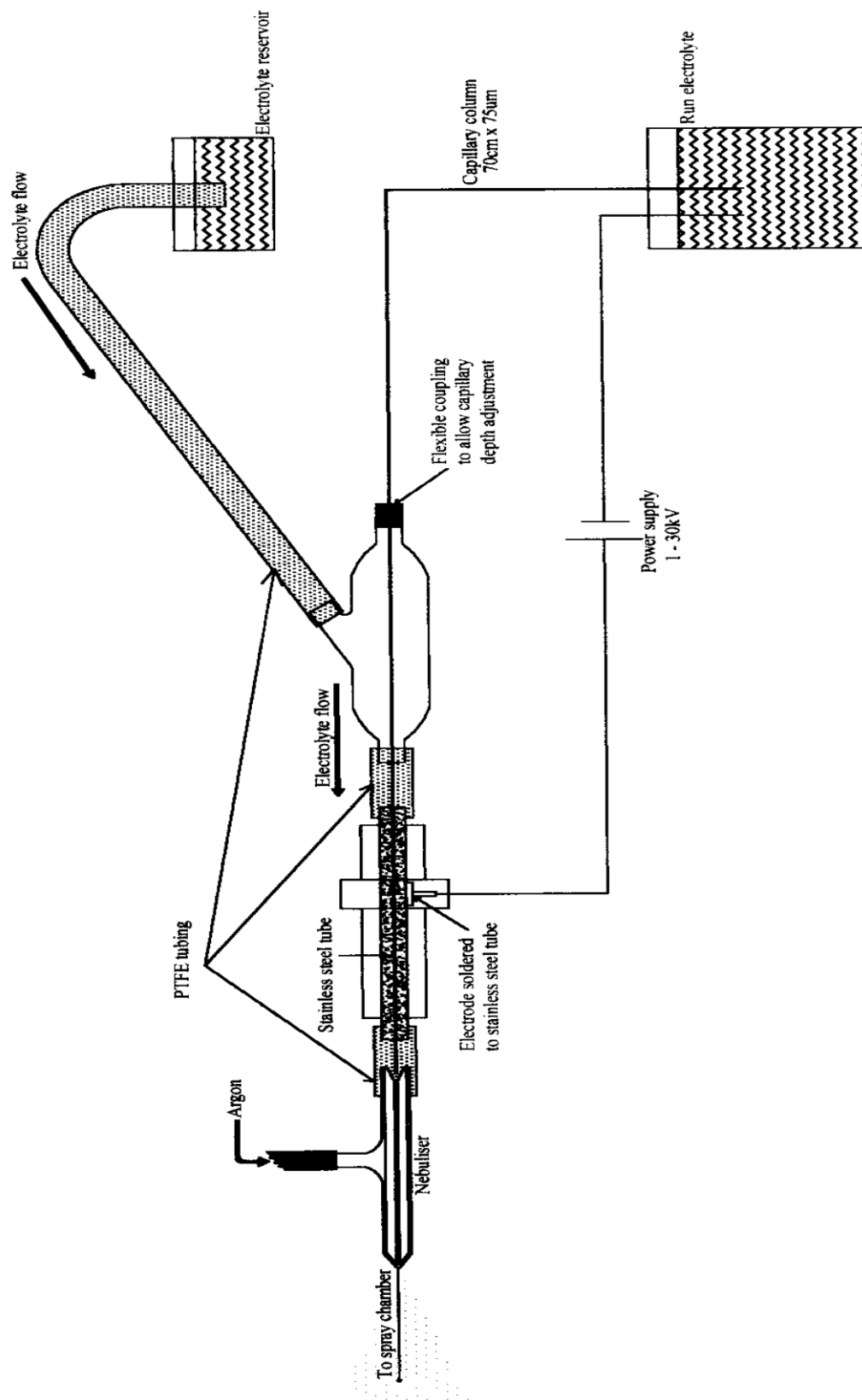
Figure 4 Schematic representation of CE / ICPMS interface.

Figure 5 CE unit (centre right) interfaced to ICPMS unit (left). The high-voltage cable can be seen extending from the CE access panel (lower centre) to the interface (centre).
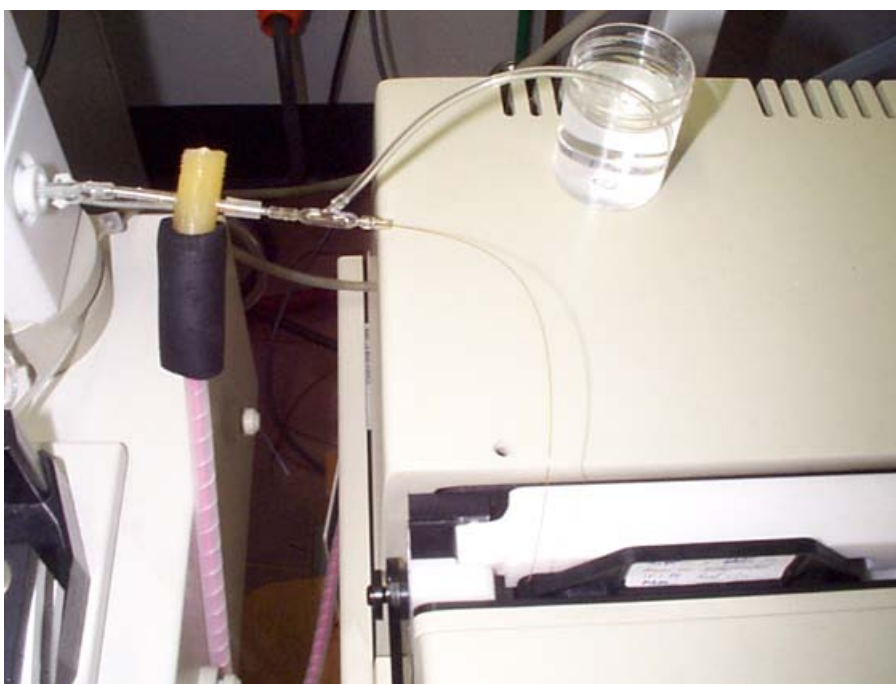
Figure 6 Close-up view of CE / ICPMS interface showing, CE capillary passing, via the glass T-piece, through the stainless steel tube connected to the high-voltage cable, then to the MCN. The electrolyte reservoir is the plastic 50 mL container seen at top right on the CE unit, while the CE cartridge is visible at bottom right.

of dissolved solid was reduced so that electrolyte could be aspirated into the argon plasma. The height of the CE unit was adjusted so that the capillary inlet was at the same height as the capillary outlet to prevent syphoning of electrolyte through the capillary.

Initial attempts to operate the CE when attached to the nebuliser were successful until the nebuliser argon flow was started. Each time the nebuliser was operated the CE would shut off its power and display a high-voltage error message. This occurred because the electrical resistance at the terminal electrode was changing because buffer was being nebulised. This was interpreted as voltage leakage by the CE unit causing a power shut down to prevent possible operator injury. This problem was overcome by performing the separation before activating the nebuliser and pushing the separated sample bands through the capillary with the application of 0.5 psi pressure at the sample end of the CE capillary. The 0.5 psi pressure was generated when argon was forced into the airspace above the inlet vial that had been made air tight with a rubber cap.

Another problem was identified during high-voltage separations with the ICPMS instrument tripping off at unpredictable points during a sample run. This caused the entire instrument to shut down, including vacuum pumps, requiring a 30 to 60 minute start-up procedure to bring the instrument back up to vacuum. This frustrating and time consuming problem also caused undue wear on the ICPMS turbo molecular pumps. The source of the problem was identified following a suggestion from Dr. Stephen Anderson of Varian Australia that small amounts of voltage leakage could affect the peristaltic pump electronics. The peristaltic pump is located directly in front of the ICPMS spray chamber. Under normal operating conditions, it pumps solutions into the nebuliser and drains waste from the spray chamber. To overcome the problem of voltage leakage through the peristaltic pump electronics, the data cable was disconnected from the peristaltic pump and the spray chamber drain tubing arranged so that waste was able to drain under gravity.

Modifications to the way the ICPMS software accumulated data were also required. Under normal operating conditions, counts collected from the ICPMS detector are processed to produce data for quantitative analysis. To collect counts at m/z 75 so that

the data could be presented in a time resolved format, the software was set-up so that data was accumulated at 1 second intervals in a comma separated variable (CSV) file. In this format, the data could be exported to an Excel spreadsheet for analysis (Appendix E).

After several trial runs, the optimum separation voltage, sample volume, capillary length, duration of high-voltage separation and time required to collect counts at m/z 75 were established. These parameters were set so that the high-voltage separation was in use for the maximum length of time before arsenicals started to emerge from the capillary outlet. This had the effect of providing maximum resolution of arsenic species as no further separation was achieved once the low-pressure water injection into the inlet side of the CE capillary (Section 2.4.3) was commenced.

Modifications to create the CE / ICPMS interface were done with the intention that they could be easily reversed in under 5 minutes so that the two instruments could be operated separately. To return the ICPMS to its normal operating state, only two steps were required. The peristaltic pump data cable was plugged in and the MCN removed and replaced with the regular concentric glass nebuliser. Changes to the CE unit were also minimal. The external high-voltage cable was unplugged and removed so that the outlet high-voltage cable inside the instrument could be plugged in. To complete the changeover, the modified CE cartridge was replaced with a standard cartridge.

Safety aspects were carefully considered and acted upon during the construction and subsequent operation of the CE / ICPMS interface. Approval was obtained from the PaLMS Trace Elements Service safety officer for the crucial changes, which included the disconnection of the safety switch on the door enclosing the CE high-voltage cables and the introduction of an external high-voltage cable. This approval of the modified apparatus was, however, subject to the maintenance of strictly controlled and limited staff access to the area where it was located. While this arrangement was adequate for an experimental CE / ICPMS set-up, it was clear that a production instrument combination, intended for routine use, would require a more thorough system of safety interlocks.

CHAPTER 4. RESULTS AND DISCUSSION

4.1 Speciation by CE

In setting up a method for arsenic speciation, several parameters were investigated. Firstly, a suitable background electrolyte had to be chosen. Three potential background electrolytes, chromate (2.3.1.9), phthalic acid (2.3.1.10) and HBA (2.3.1.11), were investigated at 5 mM and 10 mM concentrations. These electrolytes were suitable candidates because their anions absorbed strongly at 254 nm and had electrophoretic mobilities similar to the arsenic species being investigated. Chromate was the most mobile background anion (Figure 7), followed by phthalate (Figure 8) then HBA (Figure 9). This was evident when the migration times for arsenate (As^V) were compared. Thus, in Figures 7–9, it can be seen that the retention time of As^V was 4.37, 4.78 and 5.20 minutes in 5mM chromate, phthalate and HBA electrolytes, respectively. Similar migration patterns were obtained in 10 mM electrolyte solutions.

Peak shape was also used as an indicator of electrolyte mobility. Chromate migrated before all the arsenic species as all peaks (Figure 7) tail to the right. Phthalate migrated at around the same time as DMAA with a bearding (i.e., left tailing) As^V peak, a symmetrical DMAA peak and a tailing (i.e., right tailing) As^{III} peak (Figure 8). The bearding As^V peak and tailing DMAA and As^{III} peaks (Figure 9) indicate that HBA migrated between As^V and DMAA. Thus, the mid range mobilities of phthalate and HBA made these electrolytes a better choice for this application.

The lack of sensitivity of the chromate and phthalate electrolytes for detecting DMAA, even at 1.33 µmol/L, made HBA a better choice of electrolyte for separating all three arsenic species with similar sensitivity noted for arsenic species at the same molar concentration. A negative peak at the leading edge of the DMAA peak in Figures 7 and 8 was caused by the presence of an unidentified substance with a UV absorbance greater than the background electrolyte. When using HBA as an electrolyte (Figure 9) the DMAA peak was well resolved from all other peaks confirming this as the preferred

electrolyte for arsenic speciation. Chromate was also considered a less desirable choice of electrolyte as chromium contamination could compromise subsequent ICPMS chromium measurements, which are routinely conducted in the PaLMS trace elements laboratory on a variety of sample matrices (Section 1.6).
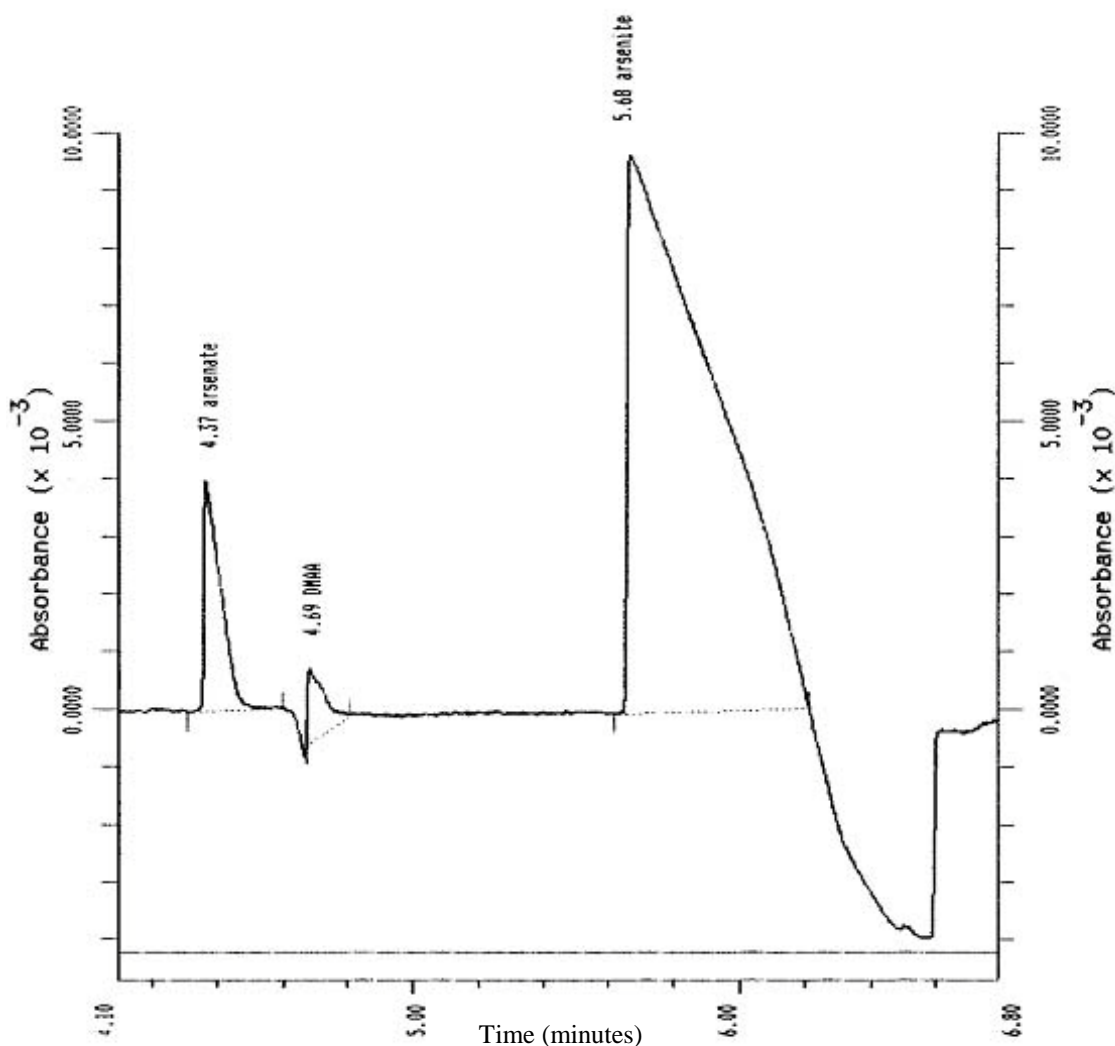
Figure 7 CE separation of an aqueous standard mixture containing 0.13 mmol/L arsenite (As^{III}), 1.33 mmol/L DMAA and 0.13 mmol/L arsenate (As^{V}). CE performed with 5 mM chromate / 4 mM TTAOH electrolyte and UV detection at 254 nm in an undeactivated fused silica capillary (75 µm ID x 400 µm OD) cut to a total length of 50 cm. Instrument operating parameters are shown in Appendix A.
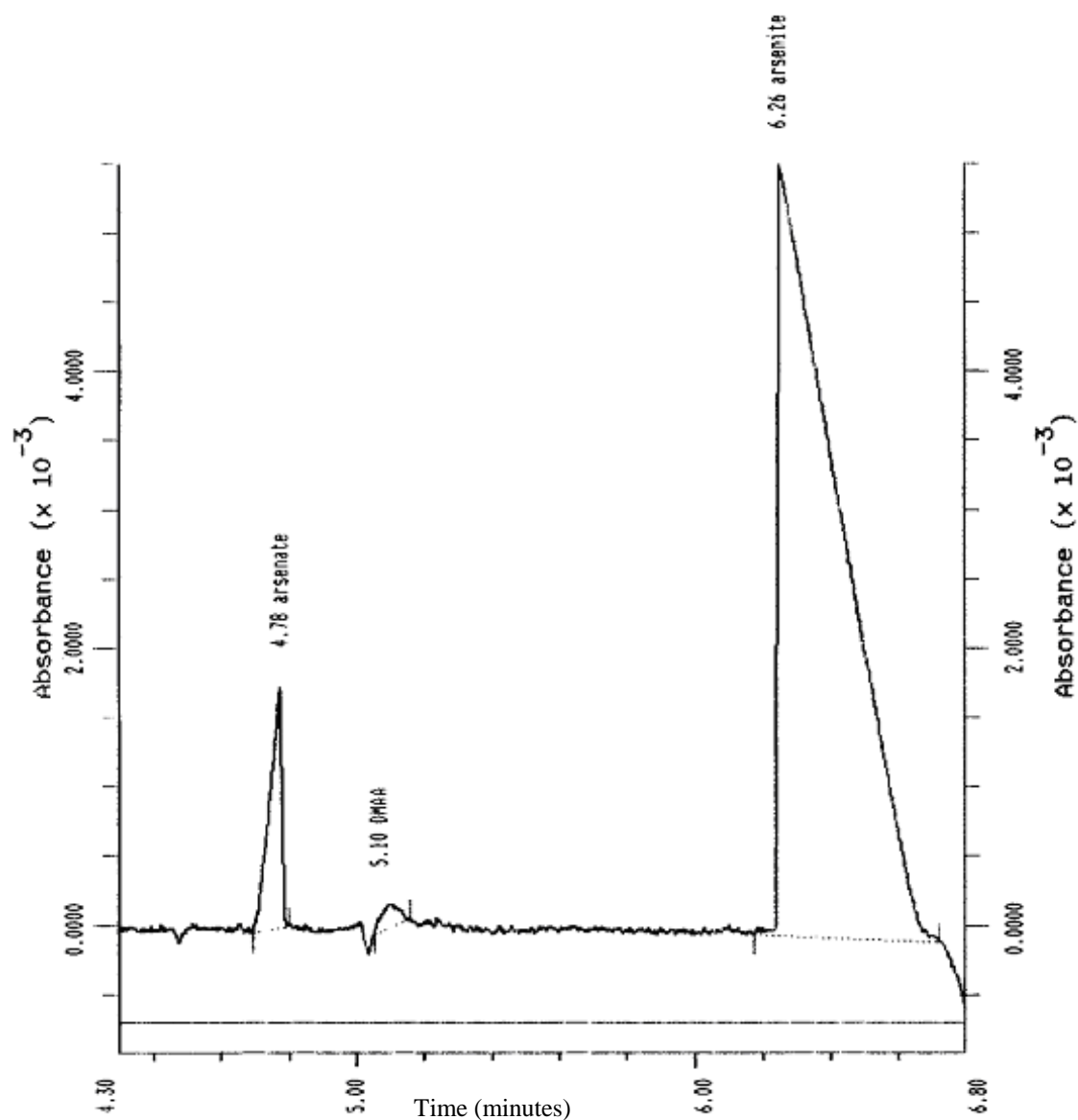
Figure 8 CE separation of an aqueous standard mixture containing 0.13 mmol/L arsenite (As^{III}), 1.33 mmol/L DMAA and 0.13 mmol/L arsenate (As^{V}). CE performed with 5 mM phthalate / 4 mM TTAOH electrolyte and UV detection at 254 nm in an undeactivated fused silica capillary (75 µm ID x 400 µm OD) cut to a total length of 50 cm. Instrument operating parameters are shown in Appendix A.
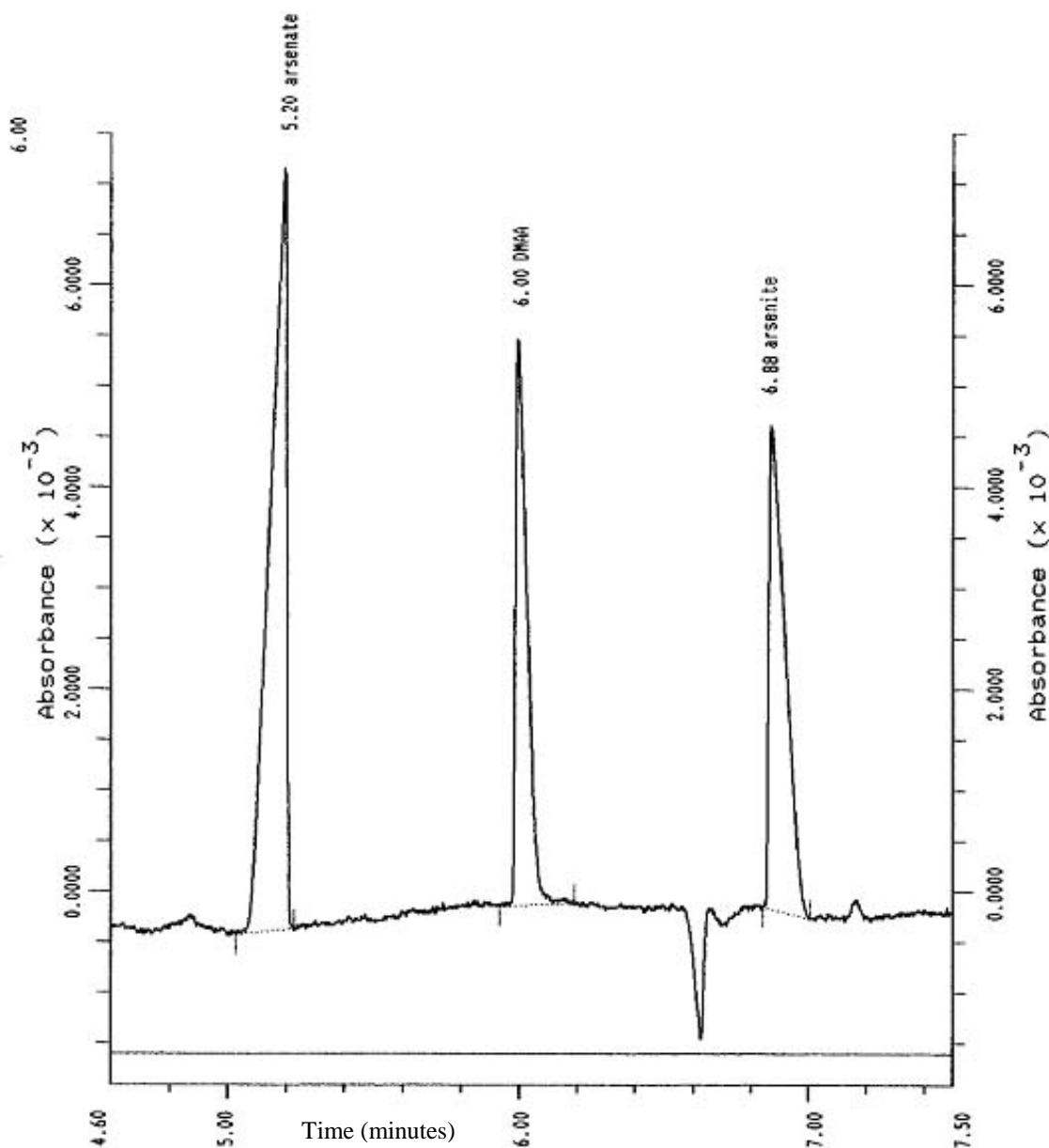
Figure 9 CE separation of an aqueous standard mixture containing 0.13 mmol/L arsenite (As^III), DMAA and arsenate (As^V). CE performed with 5 mM HBA / 4 mM TTAOH electrolyte and UV detection at 254 nm in an undeactivated fused silica capillary (75 µm ID x 400 µm OD) cut to a total length of 50 cm. Instrument operating parameters are shown in Appendix A.

The AsB peak could not be detected when AsB standards were run using indirect UV detection because the AsB was provided in the form of arsenobetaine hydrobromide. The bromide ion migrated before the AsB and displaced too much of the background

52

electrolyte to permit the recording of an AsB peak. However, when standards were analysed by CE / ICPMS (Section 4.3), the element-selective nature of this detection technique eliminated the bromine interference so that AsB could be measured.

When measuring arsenic species in urine, the presence of other anions, such as chloride and sulphate, resulted in too much of the background electrolyte being dispersed so that the slower migrating arsenicals could not be detected without diluting the sample. Urine typically contains 100 mmol/L chloride and 20 mmol/L sulphate, compared to approximately 1 µmol/L total arsenic. Chloride is of particular significance as it is often present in the highest concentration and has the shortest retention time so can compromise the detection of the later migrating arsenicals. Detection of an arsenic peak from these major anions therefore required a minimum twenty-fold dilution of the urine. This effectively meant that CE must be able to measure arsenic concentrations of 0.05 µmol/L or lower, a level 60 times less than what had been achieved here. Indirect UV detection was therefore insufficiently sensitive for measuring arsenic species in patient urine. By interfacing CE and ICPMS it was possible to gain lower levels of detection by using a system that was both element-selective and more sensitive than indirect UV detection. As chloride migrated before the arsenic species, ICPMS molecular interference through argon chloride formation was effectively removed, with chloride being expelled from the capillary before the measurement of arsenic containing capillary effluent commenced. The theory and significance behind the formation of argon chloride is described in the following section.

HBA was selected as the preferred electrolyte because it demonstrated superior resolution of the three arsenic species investigated with arsenical peaks evenly spaced while maintaining excellent shape and sensitivity. In ensuring optimum CE operating parameters were used, the effect of alterations to HBA and TTAOH concentrations, pH, column length, column internal diameter, sample volume injected and separation voltage applied were all investigated. The CE conditions consequently adopted are given in Section 2.4.1.3. Using these conditions, good separation and detection of each arsenic species were achieved down to concentrations of around 3 µmol/L.

4.2 ICPMS

ICPMS is routinely used in the PaLMS trace elements laboratory for estimating total arsenic in urine samples. When determining arsenic in urine, the potential exists for interference due to argon chloride that is formed from the major isotopes of argon and chlorine as shown in Table V. The atomic mass of this form of argon chloride is 74.9313, which differs from that of the only isotope of arsenic by 0.0097 AMU, a value less than the resolution achieved by the ICPMS. Quadrupole resolution was set using low and high mass elements with signals of around 10000 counts/second so that at 5% peak height a resolution of 0.8 AMU was achieved (Varian UltraMass operation manual, 1997).

Table V Atomic mass and isotopic abundance of some of the naturally occurring isotopes of argon, arsenic, chlorine and selenium.

Element	Atomic mass unit	Isotopic Abundance
Argon	39.9624	99.6%
Arsenic	74.9216	100.0%
Chlorine	34.9689	75.8%
Chlorine	36.9659	24.2%
Selenium	76.9199	7.6%
Selenium	77.9173	23.8%

To limit the effect that the formation of argon chloride and other interferants (Tan and Horlick 1986) might have on the determination of total arsenic in urine, a 'matrix matched' calibration (Section 2.4.2) was used. Matrix matching was achieved by adding Base Urine (2.2.1.1) to each calibration standard at the same dilution as the urine samples to be assayed. The total arsenic concentration of the Base Urine was previously determined to be 0.02 µmol/L as discussed in Section 2.2.1.1. By running replicate assays with the Base Urine as a sample, an average value of 0.01 µmol/L was obtained (Table VI). Comparing this value to that obtained from running sample blanks, where reagent grade water was added to diluent instead of urine, a difference of around 0.10 µmol/L was noted (Table VI). Negative arsenic concentrations were found for the

sample blanks because these samples did not contain some of the interferants that were present in the Base Urine used to perform the matrix matched calibration. The fact that negative sample blank arsenic concentrations were obtained supports the use of a matrix matched calibration. It was therefore concluded that interferences were present in urine at m/z 75 that could be corrected for by running a matrix matched calibration.

Table VI Results of seven separate total arsenic estimations by ICPMS on a blank sample (diluent + water), Base Urine (2.2.1.1) and interference check solution (0.2 M potassium chloride). Values were obtained over a 3 month period with each set of values from freshly prepared samples run against a fresh calibration.

Assay No.	Total Arsenic (µmol/L)		
	Blank	**Base urine**	**Interference check solution**
1	-0.11	0.04	0.24
2	-0.16	-0.04	0.17
3	-0.13	-0.01	0.16
4	-0.16	-0.04	0.12
5	-0.10	-0.01	0.18
6	-0.07	0.03	0.18
7	-0.02	0.09	0.26
Mean	**-0.11**	**0.01**	**0.19**
Std Dev.	**0.050**	**0.047**	**0.048**

An alternative method for the correction of argon chloride interference, when measuring arsenic by ICPMS, is to use a correction equation based on the assumption that argon 40 will also combine with the lesser isotope of chlorine 37 in a ratio proportional to isotopic abundance. By simultaneously measuring concentrations at m/z 75 and 77, a correction equation can be employed to subtract argon chloride formed at m/z 75. An additional measurement at m/z 78 is also required to correct for the naturally occurring isotope of selenium at AMU 77. The inclusion of a correction term for selenium in the equation is necessary as urine contains selenium at varying concentrations. This method

of correction was found to be unsuitable in urine samples as the formation of argon dimer hydrides and other argon based molecular interferants at m/z 78 and other selenium isotopes prevented the accurate measurement of selenium.

To assess the extent of interference due to argon chloride formation, an 'interference check' solution of 0.2 M potassium chloride (2.3.2.13) was prepared to approximate the level of chloride found in urine. From the results of running this solution (Table VI) it was apparent that argon chloride formation could contribute around 0.20 µmol/L to the total arsenic measurement. Some of this measurement may in fact be arsenic contamination in the potassium chloride used to prepare the interference check solution with 0.20 µmol/L of arsenic equating to 0.0001% of the compound weighed out. Variations of arsenic concentration in urine at this level are considered clinically insignificant in this laboratory. Investigation of the possibility of contamination was therefore not pursued further. To ensure argon chloride formation did not pose a problem with changing argon plasma conditions, the interference check solution was included in every batch of urine arsenic measurements.

Further evidence of analytical accuracy was obtained by repeatedly assaying monthly external quality assurance samples supplied by Quality Control Technologies Pty Ltd (QCT; 2.2.1.2). Each month, a bias performance index (BPI) is calculated to obtain a measure of bias in the results returned by one quality assurance program participant against the results of all participants. The BPI is derived by multiplying a z-score by 40. The z-score is a normalisation of the distribution of all the participant's results to the normal distribution where the mean is zero and the standard deviation is one. Thus, the z-score is the number of standard deviations a participant's result is from the consensus result. By plotting the BPI as a six month moving average, random error is reduced.

Figure 10 shows that all PaLMS BPI data points obtained over twelve months had a BPI within ± 40, which corresponds to a bias within one normalised standard deviation. This is considered to be an acceptable level of bias when compared to other laboratories performing arsenic determinations in urine samples. The minimal bias demonstrated is confirmed by the observation made on the previous page that argon chloride formation was adequately compensated.
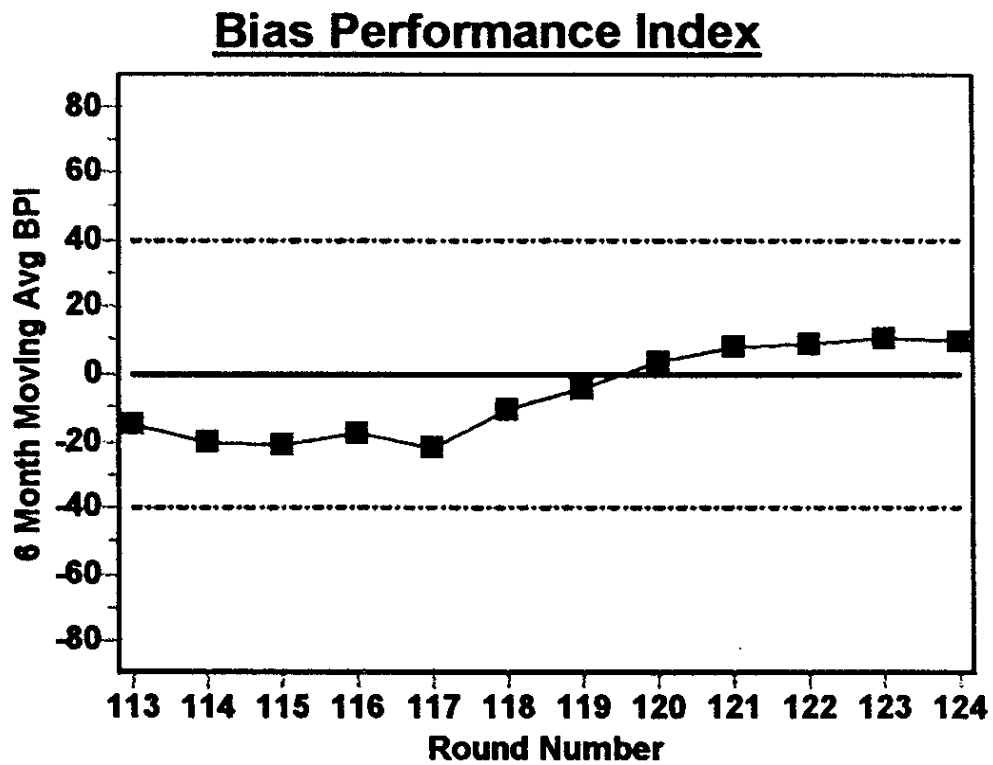
Figure 10 Bias performance indices (BPI) in the QCT external quality assurance program of PaLMS Trace Elements Laboratory for total arsenic estimations in urine over a twelve-month period. One "round" of the program comprises identical samples of one Q.C. sample being sent to and analysed by all participating laboratories, followed by collection and analysis of the data obtained.

4.3 CE / ICPMS

By using ICPMS to measure only arsenic in the CE effluent, separate arsenate (As^V), DMAA, AsB and arsenite (As^{III}) peaks could be detected (Figure 11). Peaks were identified by migration order after a series of sample spiking experiments performed previously by CE using indirect UV detection. To estimate the concentration of arsenic present, peak areas were used. Ideally, results would be downloaded into chromatography software capable of identifying peaks and calculating concentrations from peak areas. No such package capable of recognising the CSV files generated by the ICPMS was available. The results were therefore exported to an Excel worksheet where the ICPMS output of counts per second at m/z 75 could be graphically represented.

Arsenic concentrations for each arsenic species were estimated by integrating the counts under the peak corresponding to each arsenic species (Appendix E). The linearity of this technique was demonstrated by running an aqueous working standard containing four arsenicals prepared by combining aliquots of the As^V, As^{III} and DMAA stock solutions (2.3.3.2–4) with water to obtain a 100-fold dilution of each, plus a 50-fold dilution of the AsB stock solution (2.3.3.5) as shown in Figure 11a. This working standard was then diluted 10-fold in water and the analysis repeated (Figure 11b). The results of this experiment are summarised in Table VII. By dividing the counts from Figure 11a by 10, the predicted counts following a 10-fold dilution of this sample can be calculated. Comparing this value to the actual results obtained it can be seen that reasonable estimates, agreeing to within 10%, were obtained from the two dilutions of the standard.

When CE is used with UV detection, equi-molar concentrations of differing anionic species reduce the UV absorbance of the background electrolyte by different amounts, making it necessary to calibrate the response obtained from each arsenic species. In contrast, when ICPMS detection is used, the ICPMS responses (peak areas) can be expected, for all arsenic species, to be linearly related to their arsenic contents. Moreover, the sensitivity (slope) of this ICPMS response can also be expected to be the same for all arsenic species because the very high temperature of the argon plasma ensures that all the arsenic is ionised. In Table VIII, the "Original Sample" results from Table VII are shown after the counts for AsB were adjusted from 77 to 133 µmol/L, to

allow comparisons of the counts of all four arsenicals at the same concentration. Large variations, of up to 22.1%, can be seen in the ICPMS response obtained for individual arsenicals, compared to the mean response obtained for these four compounds. These results are at variance with the expectation that ICPMS peak areas will be linearly
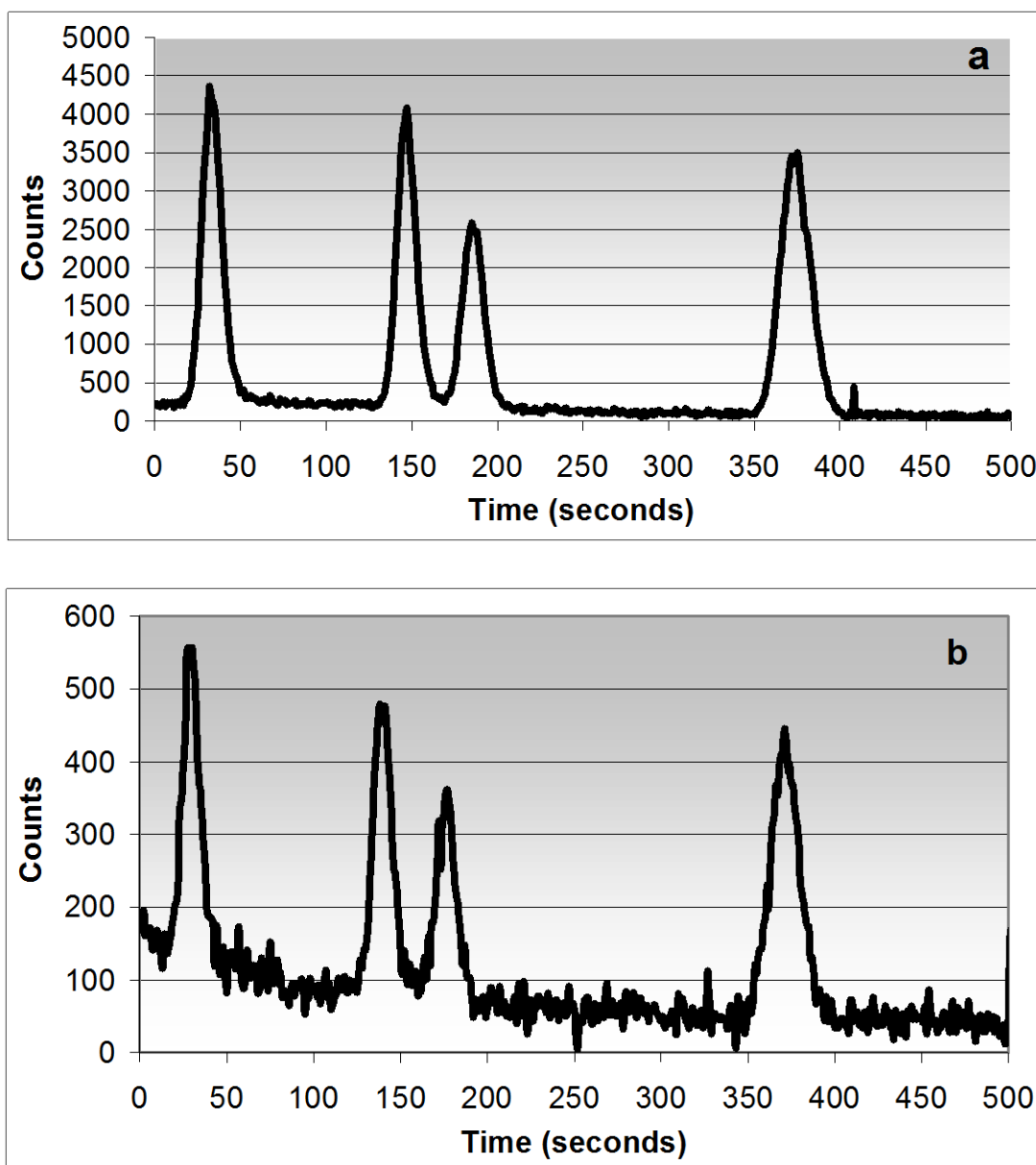
Figure 11 CE / ICPMS separation of standard containing four arsenic species. **(a)** Each species 133 μmol/L except AsB 77 μmol/L. **(b)** Same sample after tenfold dilution, each species 13.3 μmol/L except AsB 7.7 μmol//L. The migration order in both runs is (left to right): arsenate (As^V), DMAA, AsB and arsenite (As^{III}). Analysis performed by CE using 5 mM HBA / 4 mM TTAOH electrolyte coupled to ICPMS monitoring at m/z 75, as described in Section 2.4.3.

related to arsenic concentration, with the same sensitivity, for all arsenic species. The large variations between arsenicals were most likely the result of the ICPMS having insufficient time to stabilise following plasma ignition before measurements were taken as discussed towards the end of this Section. As responses are variable, the responses shown by each compound in Table VII were used to calibrate subsequent arsenic estimations. The response of either the original sample or 10x diluted sample was used depending on which was closest to the arsenic species being calculated.

Table VII ICPMS counts for the aqueous standard mixture of four arsenicals based on the separations shown in Figure 11. By dividing the counts for the original sample by 10, the expected counts for the 10x diluted sample were calculated. Differences between actual and expected values for the 10x diluted sample are listed as % error.

Analyte	Original Sample		10x Diluted Sample		
	As, µmol/L	Counts	As, µmol/L	Counts	Error, %
Arsenate (As^V)	133	57199	13.3	5208	+ 9.8%
DMAA	133	49137	13.3	5295	- 7.2%
AsB	77	33116	7.7	3039	+ 9.0%
Arsenite (As^{III})	133	71848	13.3	6809	+ 5.5%

Table VIII ICPMS counts for the aqueous standard mixture of four arsenicals based on the separation shown in Figure 11a. AsB counts adjusted to 133 µmol/L, to permit direct comparisons of the counts obtained for each species, with the mean.

Analyte	As, µmol/L	Counts	Counts, adjusted to 133 µmol/L	Difference from mean, %
Arsenate (As^V)	133	57199	57199	- 2.8%
DMAA	133	49137	49137	- 16.5%
AsB	77	33116	57200	- 2.8%
Arsenite (As^{III})	133	71848	71848	+ 22.1%
Mean	-	-	**58846**	-

Nebulisation efficiency - Some of the variations in ICPMS response between arsenic species seen in Table VIII may also be due to differences between the nebulisation efficiencies of these species. By using an internal standard, it might be possible to go some of the way towards correcting these variations along with variations in injected sample volume, rate of sample uptake into the ICPMS from the nebuliser and changing instrument response. Ideally, an internal standard comprised of a compound containing an element closely related in atomic mass to arsenic and not present in urine could be included in the terminal electrolyte.

Time of flight mass spectrometry - One disadvantage of including an internal standard would be a decrease in sensitivity once the internal standard element was monitored. When a quadrupole mass discriminator is used to monitor the counts generated at m/z 75 only, as was done in this project, the maximum possible signal for arsenic is obtained. With each additional element monitored, arsenic sensitivity would decrease as it takes a finite time to sweep across the mass range to detect transient peaks. If more than three elements were to be monitored, a 'time of flight' mass discriminator may give better sensitivity. These instruments are a relatively recent innovation in ICPMS detection and are theoretically well suited to measuring multiple elements in a transient signal. In time of flight ICPMS, elements are measured over fixed time intervals and are separated by the time they take to reach the detector. Sensitivity therefore remains the same regardless of the number of elements monitored.

Internal standard - Within the period of this project, spiking of samples with separate standard preparations of each arsenical was the only means used to confirm the identities of peaks. One or more internal standards could also be included in sample runs so that retention times could be used to assist in peak identification. To maintain optimum sensitivity an arsenic containing compound would be required. This arsenical would also need to be chemically synthesised and purified so as to be free from those found in nature. The choice should also be a compound unlikely to be excreted in urine. Phenylarsine oxide (PAO; C_6H_5AsO) is one such compound, available from Merck, that could be employed as an internal standard for CE / ICPMS arsenical estimations, both for identification of peaks by their retention times relative to that of PAO and also for quantitative calibration of the technique. The use of an internal standard in ICPMS is

also essential if results are to be accurately compared from between runs as the instrument sensitivity to an element will change over time. Numerous factors influence sensitivity such as altered tuning characteristics, ageing extraction lens, ageing detector, altered plasma parameters and worn sampler and skimmer cones.

To confirm that argon chloride formation (Section 4.2) had a negligible effect on the CE / ICPMS system, a 'Base Urine' (Section 2.2.1.1) with a total arsenic level < 0.1 µmol/L was processed. The results of the experiment are shown in Figure 12. From this graph, it can be seen that no interfering molecular species were detectable above baseline noise with chloride migrating before ICPMS measurements commence.
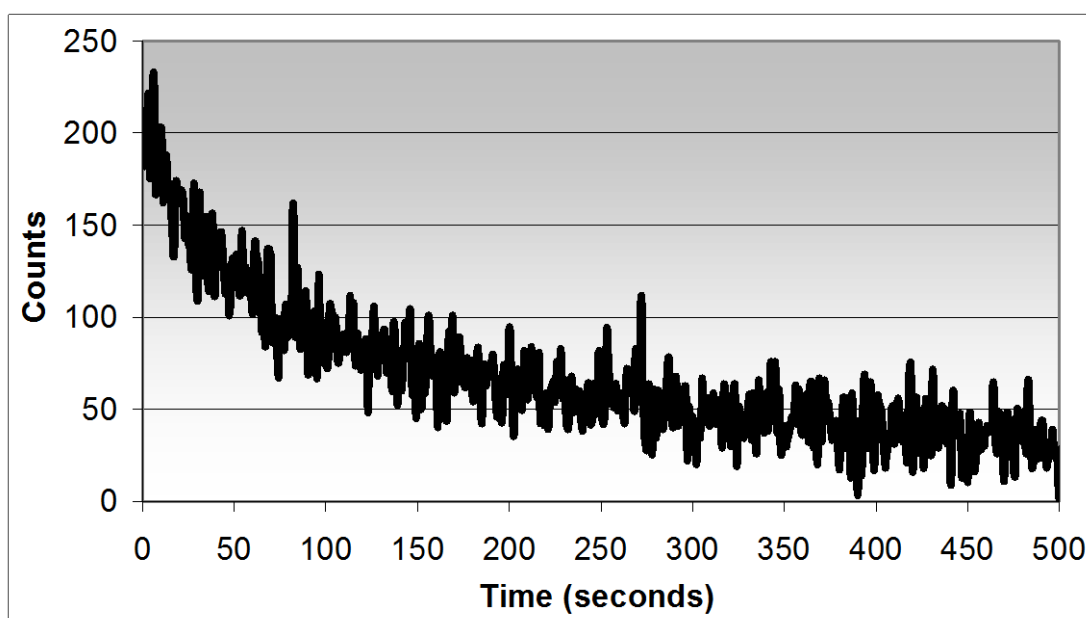
Figure 12 CE / ICPMS separation of a 'Base Urine' (Section 2.2.1.1) with a total arsenic level < 0.1 µmol/L. Analysis performed by CE using 5 mM HBA / 4 mM TTAOH electrolyte coupled to ICPMS monitoring at m/z 75, as described in Section 2.4.3.

The results from the analysis of a urine sample collected from a subject 12 hours after a seafood meal are shown in Figure 13a. An arsenobetaine peak of 17.1 µmol/L AsB is visible. The concentration of AsB in this sample was calculated from a peak area of 6745 counts using the counts obtained for the 7.7 µmol/L AsB standard (Table VII). Spiking of the sample collected following a seafood meal with 11.7 µmol/L AsB

(Figure 13b) was used to confirm the peak identity as AsB. The measured concentration of AsB in the spiked sample was 29.5 µmol/L indicating that analytical recovery of the standard addition was 106%.
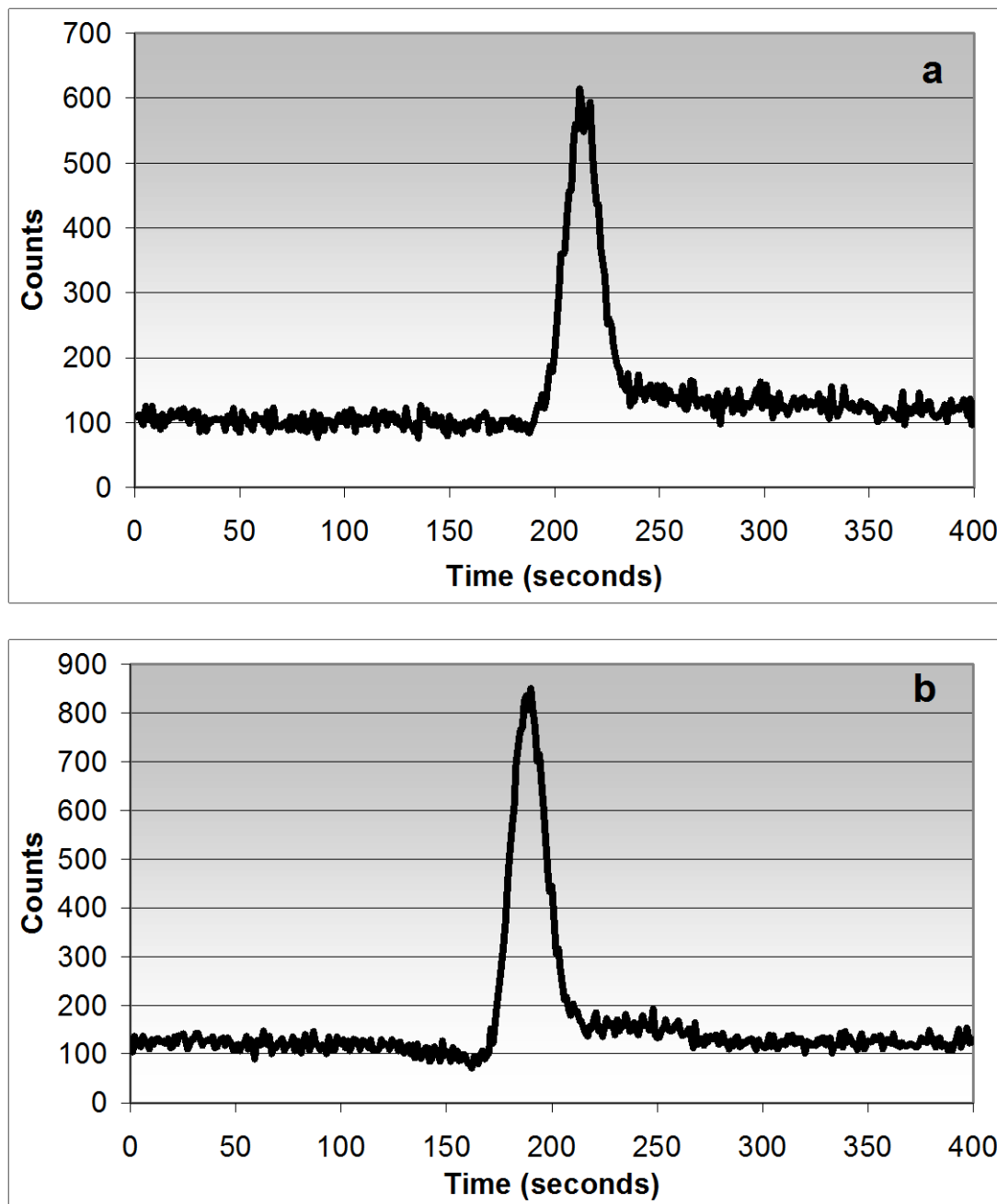
Figure 13 CE / ICPMS separation of urine. **(a)** Urine collected from subject 12 hours after a seafood meal showing peak of AsB. **(b)** Same sample spiked with 11.7 µmol/L AsB. Analysis performed by CE using 5 mM HBA / 4 mM TTAOH electrolyte coupled to ICPMS monitoring at m/z 75, as described in Section 2.4.3.

The retention time of AsB varied between samples with retention times of 170, 215 and 190 seconds for the results shown in Figures 11, 13a and 13b, respectively. Varying retention times are a characteristic of CE and can occur in response to changes in the concentration of dissolved solid in the sample. It highlights the need for the inclusion of a suitable internal standard in the run to aid in peak identification. The main cause for the differing retention times in the samples shown in Figure 13 was the failure of the argon plasma to ignite on the first attempt when analysing the unspiked urine. This resulted in a delay of around 30 seconds before the plasma could be successfully ignited and results collected.

The fact that AsB can be measured in a sample of undiluted urine highlights the advantages of element-specific detection over indirect UV detection. By monitoring only arsenic, the AsB peak was resolved from chloride and sulphate in the CE step. This permitted the arsenic to be monitored by ICPMS without interference from argon chloride. If the same measurement were attempted using indirect UV detection, AsB would be undetectable as the huge concentrations of chloride and sulphate relative to AsB would disrupt too much of the background electrolyte for AsB to be visible.

To achieve lower limits of detection, the use of a 100 µm ID capillary was investigated (Altria 1999). The set-up of the system was such that the injection of a 10 mg/L elemental arsenic solution (133 µmol/L) would deliver 0.3 ng of analyte into the capillary. Under the same run conditions using a 100 µm ID capillary, 0.9 ng of analyte would be delivered into the capillary giving a threefold increase in sensitivity (calculated using Beckman CE Expert software). This was confirmed experimentally (Figure 14) using Base Urine (Section 2.2.1.1) spiked to contain As^V, DMAA and As^{III}, each at approximately 27 µmol/L, plus 16 µmol/L AsB. Approximate concentrations of the arsenic species were calculated from peak areas using the 75 µm ID capillary (Figure 11a) and are presented in Table IX. In each case, improved sensitivity was observed. When the larger capillary was used, the low-pressure injection encountered less resistance and was able to force solution through the capillary faster. This meant that, when the CE parameters employed for the smaller ID capillary were used, the fastest moving peak (As^V) was expelled from the capillary before measurements were taken and was therefore not detected. This problem arose from lack of experience with

the 100 μm ID capillary. A shorter length of time spent performing the high-voltage separation is likely to permit detection of the As^V. Peak resolution would however, be expected to decrease as the high-voltage separation time decreased.
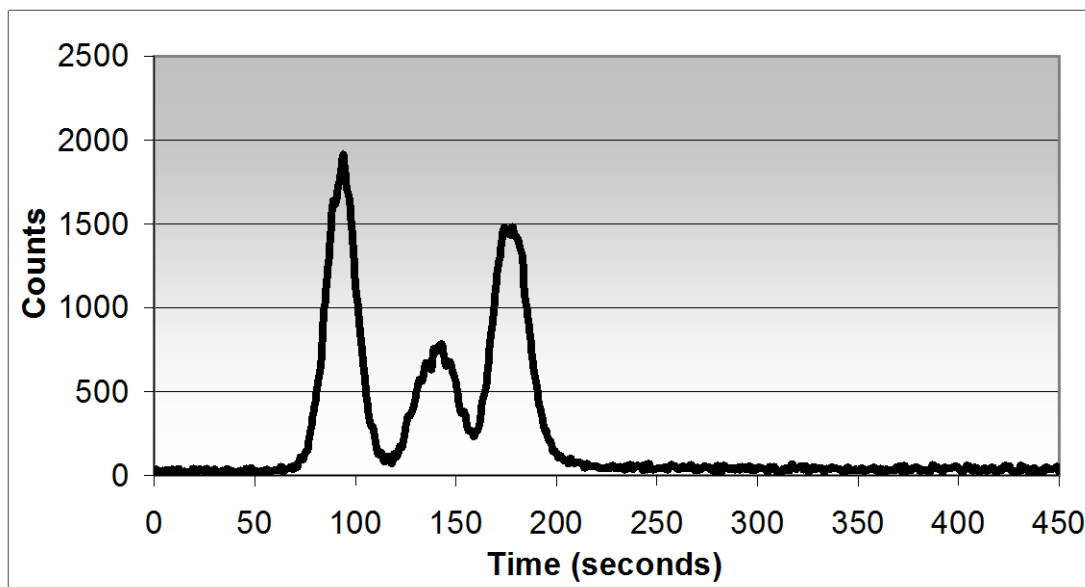
Figure 14 CE / ICPMS separation of Base Urine (2.2.1.1) spiked to contain 27 μmol/L arsenate (As^V), 27 μmol/L DMAA, 27 μmol/L arsenite (As^{III}) and 16 μmol/L AsB. Peaks shown (left to right) are DMAA, AsB and As^{III}. Analysis performed by CE in 100 μm internal diameter capillary using 5 mM HBA / 4 mM TTAOH electrolyte coupled to ICPMS monitoring at m/z 75, as described in Section 2.4.3. As^V peak emerged before counting commenced and was not detected.

Separation efficiencies decreased with increasing capillary diameter. Poorer peak resolution with broad, round peaks running close together can be seen in Figure 14, where the 100 μm ID capillary was used, in contrast to those in Figure 11a, where a 75 μm ID capillary was used. Compared to the 75 μm ID capillary results, the 100 μm ID capillary showed less variation in ICPMS response and gave an approximately threefold increase in sensitivity for DMAA and AsB, as predicted by the Beckman CE Expect software. However, for As^{III}, this ratio was only 2.288 (see ICPMS Response Ratios, Table IX). Some of the variation in response seen with As^{III} in the 100 μm ID capillary may have been the result of oxidation of As^{III} to As^V, since seven months

elapsed between the 75 µm ID and 100 µm ID capillary trials, during which the As^{III} standard was stored. The stability of the arsenic standards was not investigated but there is published evidence to suggest that they are not stable for more than 10 days unless steps are taken to optimise their storage conditions (Francesconi *et al*. 1994).

Nevertheless, this experiment demonstrated that up to threefold increases in sensitivity are achievable in the larger ID capillary, at some cost in resolution between different arsenic compounds. Using a longer 100 µm ID capillary could restore the resolution, but this would increase run times. Further improvements in sensitivity could also be achieved by injecting a larger sample volume. As sufficient sensitivity and separation were achieved using the run parameters described in Section 2.4.3 these other parameters were not investigated further.

Table IX Counts obtained from CE / ICPMS analyses of standard mixtures of As^V, DMAA, AsB and As^{III}, using 75 and 100 µm ID capillaries. The "ICPMS response" for each arsenical = (counts obtained) / (As concentration in sample, µmol/L). As^V peak emerged before counting commenced and was not detected. Data derived from Figures 11a and 14.

Analyte	75 µm ID capillary (Figure 11a)			100 µm ID capillary (Figure 14)			ICPMS response ratio 100/75
	As, µmol/L	Counts	ICPMS response	As, µmol/L	Counts	ICPMS response	
DMAA	133	49137	369.5	27	31266	1158.0	3.133
AsB	77	33116	430.1	16	19178	1198.6	2.787
As^{III}	133	71848	540.2	27	33372	1236.0	2.288

The main limitation of CE / ICPMS used in this project occurred because the CE power supply was earthed so that any voltage leakage from the outlet electrode resulted in the instrument shutting down to protect the operator. With the ICPMS running, electrolyte was transferred from the capillary outlet to the spray chamber, causing a decrease in electrolyte resistance. The CE instrument interpreted this as voltage leakage and shut down so that CE high-voltage separations and ICPMS nebuliser operation could not be

performed simultaneously. For this reason the CE / ICPMS had to be configured so that the CE high-voltage was stopped before the ICPMS nebuliser was started and the argon plasma ignited. This situation was less than ideal for ICPMS operation as the ICPMS argon plasma had to be extinguished and reignited for each successive sample.

Ignition of the argon plasma requires high radio frequency (RF) energy to be directed into a stream of argon inside a quartz torch. Once the plasma is ignited, the RF energy is decreased to a level necessary to maintain the argon plasma. Focusing of the RF energy into the argon to initially ignite the plasma is critical. In the ignition stage the RF energy is usually focused into the argon but can be attracted to other surfaces such as the quartz torch, sampler cones or induction coils. High RF energy arcing into any of these components will quickly melt them leading to expensive repairs and down time on the instrument. To minimise the risk of arcing, normal instrument operation is such that plasma ignition is into a stream of argon containing nebulised water.

For speciation by CE / ICPMS to be successful, plasma ignition had to be commenced as soon as the CE high-voltage was stopped. This meant that plasma ignition occurred into a stream of argon containing nebulised electrolyte rather than water. Under these conditions, the likelihood of RF arcing was increased. The likelihood of RF arcing was further increased by the need to reignite the plasma for each sample.

These run conditions also prevented optimum instrument stability being achieved before sample measurement, which had to be made as soon as possible after plasma ignition. Ideally, the argon plasma would be allowed to stabilise for at least 15 minutes before measurements were taken. The effect of not allowing sufficient plasma stabilisation is evident in Figure 12, where high initial background counts can be seen. The background counts decrease as the run progresses but a noisy baseline persists throughout.

A further disadvantage of stopping the high-voltage separation and relying on low-pressure to force separated arsenicals from the capillary is a decrease in resolution as peaks increase in width. Once the high-voltage is stopped, separated species gradually diffuse into the surrounding electrolyte. Sample analysis must therefore commence before the argon plasma has time to fully stabilise.

Given the suboptimal measurement conditions and difficulties in igniting the argon plasma with potentially damaging effects to the instrument, only enough CE / ICPMS runs were performed to demonstrate the usefulness of this technique and validate solvent extraction / GFAAS as a suitable method for partial arsenic speciation. If CE / ICPMS were to be used more often than for doing the infrequent full speciation of arsenicals in patient urine, a different power supply would be required. By using a CE power supply that was not earthed on the outlet electrode, it would be possible to have the high-voltage running while the nebuliser was running. This would overcome the limitations of the existing CE / ICPMS method described above. The argon plasma could initially be ignited while nebulising water and then left continuously running while analysing samples so that good plasma stability would be achieved. By allowing the instrument to fully stabilise after plasma ignition, improved detection limits would follow from an improved signal to noise ratio. Peak broadening would be reduced as arsenic species could migrate all the way to the capillary outlet under the endosmotic and electromigrative forces created by the high-voltage. This would improve peak resolution and result in lower detection limits through a further improvement in the signal to noise ratio with taller, narrower peaks produced.

Even with the current set up, detection limits for the arsenic species investigated were estimated to be around 1.0 µmol/L. At these concentrations the system was sufficiently sensitive for the detection of arsenic species in urine at clinically significant levels. The detection limits found here approach those reported by other researchers. For example, Michalke and Schramel (1998) used CE / ICPMS for arsenic speciation with a laboratory designed interface connecting a 50 µm ID capillary. Detection limits for As^{III}, As^{V}, MAA and DMAA were reported as 0.2 µmol/L and detection limits for AsB and AsC were reported as 0.9 µmol/L.

4.4 Partial Speciation by Organic Solvent Extraction / GFAAS

This technique provides a rapid means of speciating clinically significant arsenicals in laboratories where expensive and technically demanding ICPMS and CE equipment are not available. The majority of high arsenic levels in the normal population are due to seafood consumption in the days preceding urine collection. Separation of these arsenicals from toxic arsenicals is usually all that is required to determine whether arsenic poisoning can be excluded in most subjects.

To verify that only arsenite (As^{III}), arsenate (As^V), MAA and DMAA were extracted into the organic (cyclohexane) phase during the partial speciation procedure (Section 2.4.4), urine samples from patients following a seafood meal (Seafood Urine; 2.2.1.3) and a patient treated with arsenic trioxide (Arsenic Trioxide Urine; 2.2.1.4) were analysed by solvent extraction / GFAAS. Total urine arsenic was also determined by ICPMS (Section 2.4.2) to ensure that the samples contained elevated levels of total arsenic and that both GFAAS and ICPMS instruments were calibrated to give the same total arsenic concentration. Control urine samples for analysis by both GFAAS and ICPMS were prepared using the 26.6 µmol/L sodium arsenite working standard (2.3.4.11). Four- and tenfold dilutions of this working standard in Base Urine (2.2.1.1) were performed to give urines with arsenic concentrations of 6.65 and 2.66 µmol/L, respectively. Additional control samples for solvent extraction / GFAAS analysis were prepared by spiking separate aliquots of Base Urine (2.2.1.1) with sodium arsenite (2.3.4.11), DMAA (2.3.4.15), AsB (2.3.4.17) and sodium arsenate (2.3.4.19) to give four urine samples with total arsenic concentrations of 1.80 µmol/L, 1.80 µmol/L, 4.40 µmol/L and 1.80 µmol/L, respectively. These four arsenic species were selected as controls so that the extraction system would be challenged with inorganic, methylated and organic arsenic species. Results for the analysis of patient and control samples are presented in Table X.

It can be seen that arsenic determinations on sodium arsenite standards by ICPMS and solvent extraction / GFAAS were reasonably well correlated, with arsenic recoveries agreeing to within 15% (Table X, Rows 1 and 2). Seafood Urines (2.2.1.3) yielded solvent extraction / GFAAS results which were less than 1% of the total arsenic content

of these samples by ICPMS (Table X, Rows 10-21). Solvent extraction / GFAAS was therefore able to efficiently separate marine arsenicals from toxic, solvent-extractable arsenic species. This finding was confirmed by a similar result for Base Urine spiked to contain AsB (Table X, Row 9). The initial analyses on urine from a leukaemic patient treated with arsenic trioxide (Arsenic Trioxide Urine, Run A) and Base Urine spiked with DMAA (Base Urine + 1.80 µmol/L DMAA, Run A) yielded lower than expected recoveries of 69% and 73%, respectively (Table X, Rows 3 and 5). Repeat analyses on the same samples (Run B for each) yielded recoveries of 71% and 70%, respectively, which confirmed this result (Table X, Rows 4 and 6). The results of all four estimations differed by less than 5% and suggest that the methylated arsenical / iodide complexes were not completely extracted into the organic phase, unlike the inorganic arsenicals As^{III} and As^{V} which showed recoveries of 100% and 99%, respectively (Table X, Rows 7 and 8).

An alternative explanation, as only around 70% of the total arsenic was recovered from the Arsenic Trioxide Urine by solvent extraction / GFAAS compared to ICPMS (Table X, Rows 3 and 4), was that 30% of the total arsenic in this urine may be from seafood consumption. Further characterisation of the Arsenic Trioxide Urine by CE / ICPMS (Figure 15) showed that only MAA and DMAA had been excreted. There were no other arsenic containing peaks evident to suggest recent seafood consumption as a cause of the lower than expected recovery. The fact that similar low arsenic recoveries were obtained by solvent extraction / GFAAS from the Arsenic Trioxide Urine and Base Urine spiked with DMAA is a strong indication that the mono- and dimethylated arsenical / iodide complexes partition during extraction between the organic and aqueous phases with a fairly constant efficiency of around 70%. Partitioning could also be occurring in the emulsion that formed when the extracted arsenicals were mixed with the nickel nitrate matrix modifier prior to analysis by GFAAS.

Table X Correlation between total arsenic by ICPMS and partial arsenic speciation by solvent extraction / GFAAS.

Row	Sample	Total arsenic by ICPMS		Solvent-extractable arsenic by GFAAS	
		µmol/L	% recovery	µmol/L	% recovery
1	NaAsO$_2$ standard (2.66 µmol/L)	2.66	100%	2.26	85%
2	NaAsO$_2$ standard (6.65 µmol/L)	6.58	99%	6.78	102%
3	Arsenic Trioxide Urine, Run A**	6.20	-	4.30	69%
4	Arsenic Trioxide Urine, Run B**	6.20	-	4.40	71%
5	Base Urine + 1.80 µmol/L DMAA, Run A	-	-	1.32	73%
6	Base Urine + 1.80 µmol/L DMAA, Run B	-	-	1.26	70%
7	Base Urine + 1.80 µmol/L NaAsO$_2$	-	-	1.80	100%
8	Base Urine + 1.80 µmol/L Na$_2$HAsO$_4$.7H$_2$O	-	-	1.79	99%
9	Base Urine + 4.40 µmol/L AsB	-	-	0.02	< 1%
10	Seafood Urine* 1	17.9	-	0.02	< 1%
11	Seafood Urine* 2	5.15	-	0.01	< 1%
12	Seafood Urine* 3	10.6	-	< 0.01	< 1%
13	Seafood Urine* 4	4.55	-	< 0.01	< 1%
14	Seafood Urine* 5	7.81	-	0.02	< 1%
15	Seafood Urine* 6	14.5	-	0.01	< 1%
16	Seafood Urine* 7	3.44	-	< 0.01	< 1%
17	Seafood Urine* 8	2.92	-	< 0.01	< 1%
18	Seafood Urine* 9	8.05	-	0.02	< 1%
19	Seafood Urine* 10	30.7	-	0.01	< 1%
20	Seafood Urine* 11	4.47	-	0.01	< 1%
21	Seafood Urine* 12	8.69	-	< 0.01	< 1%

* Urine samples passed by subjects after seafood ingestion (see 2.2.1.3).

** Both assays on the same urine from a leukaemic patient treated with arsenic trioxide (see 2.2.1.4).
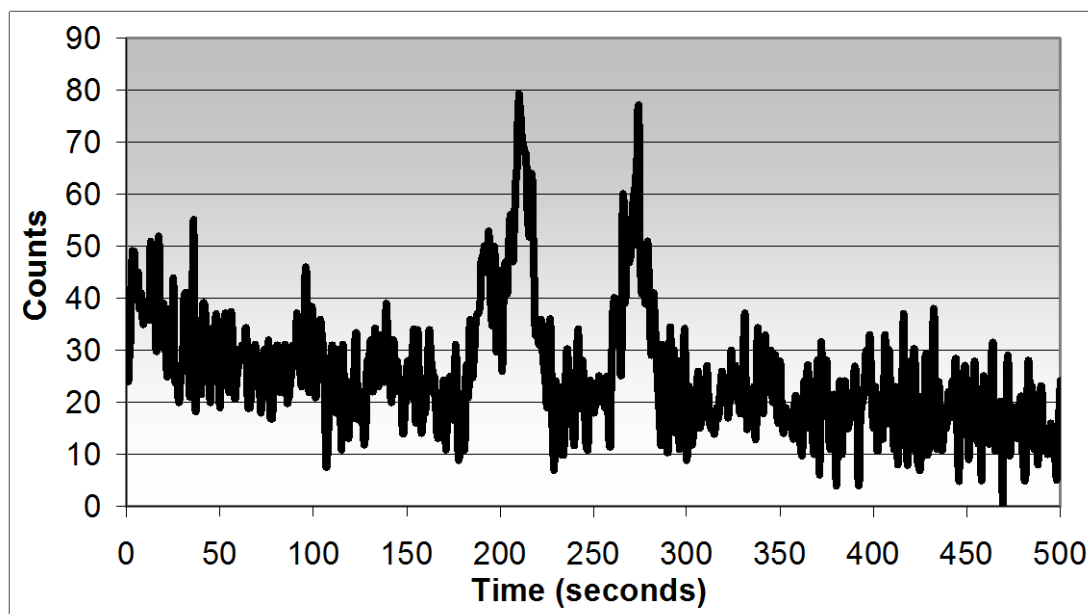
Figure 15 CE / ICPMS separation of Arsenic Trioxide Urine (2.2.1.4). Two arsenic peaks are present with MAA on the left and DMAA on the right. Analysis performed by CE using 5 mM HBA / 4 mM TTAOH electrolyte coupled to ICPMS monitoring at m/z 75, as described in Section 2.4.3.

Further work is required to establish where this partitioning is occurring and to develop methodology to reduce its effect. In the interim, a better calibration protocol for the solvent extraction / GFAAS method would be to calibrate against DMAA and not sodium arsenite, as is presently done. This point is made more salient with the knowledge that very little As^{III} is likely to be excreted in the urine of a person exposed to inorganic arsenic except in cases of severe poisoning (Benramdane *et al.* 1999). DMAA is likely to be the arsenic species in the highest concentration in a urine following exposure to inorganic arsenic, with MAA the next most abundant arsenic species (Section 1.4). Though MAA can be purchased from commercial suppliers, none was available when the experiments were performed in this project. Commercially prepared MAA will need to be purchased (Sigma-Aldrich catalogue) to demonstrate satisfactory recovery of MAA spiked into urine samples.

4.5 Extraction of Arsenicals from Marine Biota

To demonstrate that the method of partial speciation isolated only the arsenic species arsenite (As^{III}), arsenate (As^{V}), MAA and DMAA, an attempt was made to challenge the system with arsenicals from a wide range of marine sources. Seaweeds contain a mixture of arsenosugars and arsenolipids (Shiomi 1994). To extract these arsenicals two different digestion procedures were performed on three different types of edible seaweed (Section 2.2.2). One digestion procedure involved the heating of samples in concentrated nitric acid, a procedure routinely used by this laboratory to digest samples to a point where total arsenic can be determined by ICPMS (Section 2.4.5). A weaker digestion procedure using 2 M KOH in methanol was also used to extract arsenicals while still maintaining their chemical structure (Section 2.4.5). The total arsenic concentrations following nitric acid digestion were compared to the arsenic concentrations following extraction with 2 M KOH in methanol to evaluate the digestive efficiency of this method (Table XI). An ideal digestion system would completely extract arsenic species while maintaining their chemical structure. The weaker digestion procedure led to lower extraction of arsenic containing compounds in the moist green seaweed and kelp with extraction efficiencies of 93% and 33%, respectively.

Table XI Arsenic concentrations, determined by ICPMS, in extracts of three different types of edible seaweed. Based on HNO_3 and KOH extraction procedures (Section 2.4.5).

Seaweed Sample	HNO$_3$ digestion		KOH extraction		% Recovery in KOH procedure
	Sample mass (g)	Arsenic (µg/g)	Sample mass (g)	Arsenic (µg/g)	
1. Dry green	0.75	2.7	1.03	2.8	102%
2. Moist green	0.11	14.0	0.19	13.0	93%
3. Kelp	0.19	39.9	0.36	25.8	33%

A selection of nitric acid and KOH / methanol digests was then analysed by solvent extraction / GFAAS to verify that, like AsB, arsenosugars are not measured by this technique. Variable results were however obtained. Poor sample replicates (Table XII) and unpredictable recoveries of AsB and DMAA (Table XIII) were noted. To investigate these anomalous results further, the KOH / methanol digest obtained from the moist green seaweed (Sample 2) was analysed by CE / ICPMS (Section 2.4.3). Only one peak (Figure 16a) with a retention time similar to that of AsB was detected, even though Francesconi and Edmonds (1994) have reported that AsB has not been detected in marine algae. To confirm that the peak obtained in Figure 16a was indeed AsB, Sample 2 was spiked with an equal volume of 100 µmol/L synthetically prepared AsB (2.3.4.16). Sample 2 was previously found to contain 8.3 µmol/L total arsenic by ICPMS. As only one arsenic containing peak after spiking with AsB was visible (Figure 16b), Sample 2 is assumed to contain 8.3 µmol/L AsB. Spike recovery is estimated from peak heights of Sample 2 and the spiked sample (Figure 16) given that after spiking Sample 2 will contain 4.15 µmol/L arsenic plus a 50 µmol/L arsenic spike as equal volumes of Sample 2 and the AsB spiking solution are mixed together. The spiked sample therefore contains 54.15 µmol/L AsB and produces a peak with a height of 28000 counts (Figure 16b). The unspiked sample (Figure 16a) with a peak height of 3700 counts is therefore estimated to contain 7.2 µmol/L AsB.

Table XII Replicate determinations of arsenic concentrations by solvent extraction / GFAAS in HNO_3 and KOH extracts of edible moist green seaweed (Sample 2).

Seaweed Sample	HNO_3 digestion		KOH extraction	
	Sample mass (g)	Arsenic (µg/g)	Sample mass (g)	Arsenic (µg/g)
Weed 2. (Moist green seaweed) - Replicate* 1	0.11	11.6	0.19	7.7
Weed 2. (Moist green seaweed) - Replicate* 2	0.11	10.2	0.19	9.4

* Repeat analysis of sample within same analytical run.
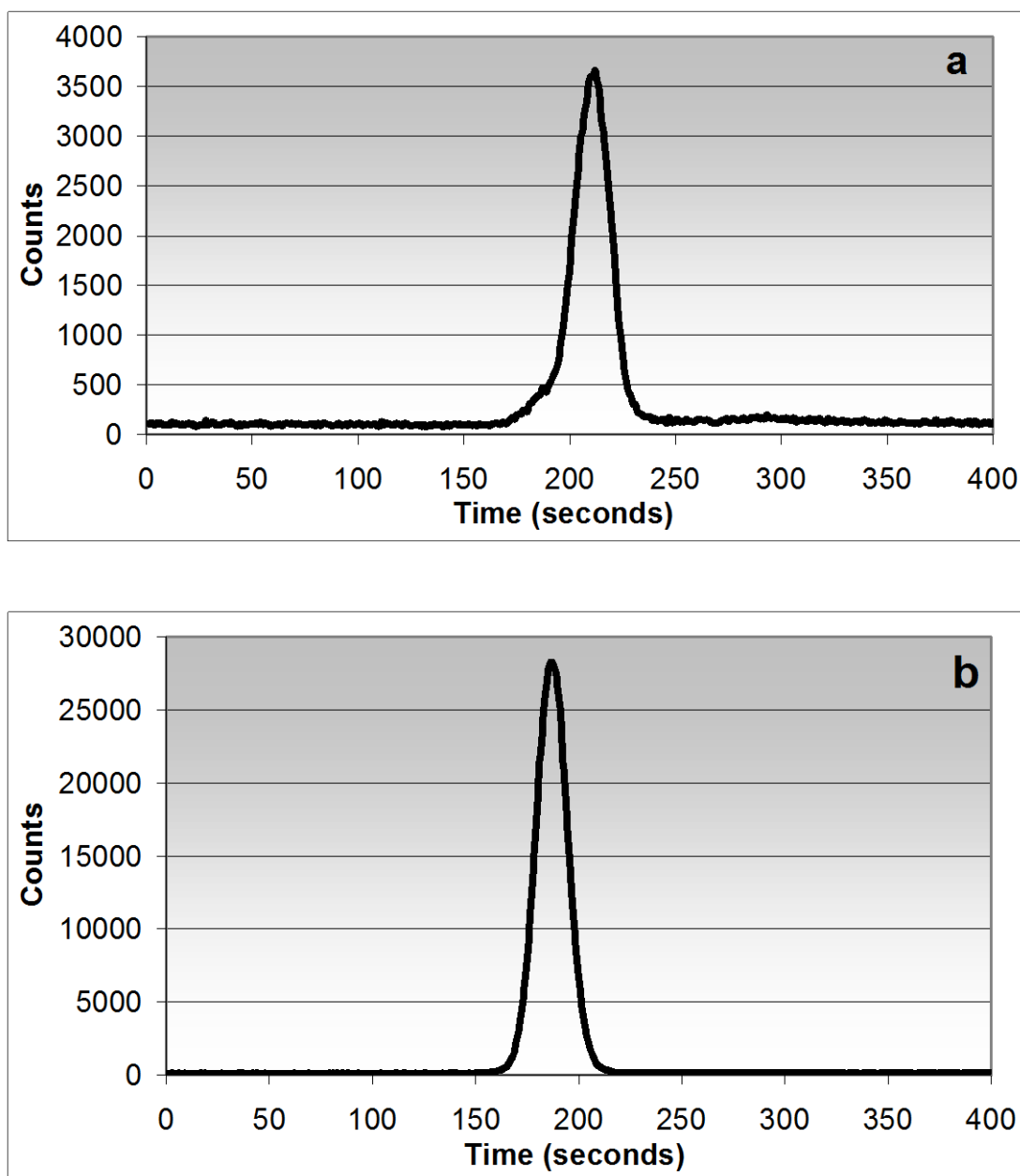
Figure 16 CE / ICPMS electrophoretograms of KOH / methanol digest of edible seaweed Sample 2 (Moist green seaweed). **(a)** Unmodified digested containing 8.3 µmol/L total arsenic; **(b)** Digest after spiking with a standard addition of 50 µmol/L AsB. Analysis performed by CE using 5 mM HBA / 4 mM TTAOH electrolyte coupled to ICPMS monitoring at m/z 75, as described in Section 2.4.3.

Table XIII Spike recoveries of arsenic by solvent extraction / GFAAS in KOH extracts of edible seaweed (Samples 1 and 2).

Seaweed Sample	Arsenic concentration (µmol/L)	Arsenic recovered
1. Dry green seaweed	1.9	-
1. Dry green seaweed + 4.4 µmol/L AsB spike	2.5	13.6%
2. Moist green seaweed	9.8	-
2. Moist green seaweed + 1.9 µmol/L DMAA spike	11.0	92.9%
2. Moist green seaweed + 4.4 µmol/L AsB spike	7.4	- 54.5%

From the absence of an additional peak (Figure 16b) and an 86% spike recovery (estimated AsB 7.2 µmol/L compared to measured AsB of 8.3 µmol/L), it can be inferred (see following paragraph) that sample digestion in concentrated nitric acid or 2 M KOH / methanol had cleaved the sugar and lipid components from the arsenosugar and arsenolipid compounds in the marine algae to form AsB. This finding was consistent with that of Francesconi *et al*. (1994) where very mild extraction procedures are recommended to maintain arsenic species chemical structure. These results also indicate that the solvent extraction / GFAAS method is not suitable for partially speciating arsenicals in samples where the matrix is vastly different to that found in urine samples. The digestion of seaweed in concentrated nitric acid and 2 M KOH / methanol had created conditions where the chemistry of the solvent extraction method was altered so that variable amounts of AsB could be extracted into the organic phase.

One assumption made when using sample spiking as a form of peak identification was that all organoarsenic compounds were fully resolved by CE. This may not be the case if two or more organoarsenic compounds have the same or very similar retention times. It has been reported that TMAO is formed in frozen fish from the oxidation of AsB, resulting in as much as 40% of the total arsenic in this food being TMAO (Shiomi

1994). As the AsB used in these experiments was at least 9 years old and derived from a frozen stock solution it would seem probable that a significant portion, if not all of the AsB, had been oxidised to TMAO. In the limited number of CE / ICPMS experiments performed where AsB had been spiked into a fresh urine sample from a patient following a seafood meal, only one peak of AsB of increased size was observed. This would indicate that TMAO and AsB are so similar in their chemical structures and properties that they are not resolved by the CE conditions used here. The solvent extraction / GFAAS method could therefore be said to exclude both AsB and TMAO, as demonstrated in Section 3.4, though this observation would need to be proved through further experimentation. Since both AsB and TMAO have no clinical significance with respect to their toxicity (Table II) and they have been observed to behave identically throughout these experiments, further characterisation of these two compounds is not considered relevant.

It was thought that seaweed digestion using 2M KOH in methanol would provide a sufficiently gentle digestion system to extract arsenic species while maintaining their chemical structure. From the results obtained it is apparent that this was not the case. If arsenosugars were to be extracted from seaweed to demonstrate the effectiveness of the solvent extraction / GFAAS method an extraction procedure such as that described by Christakopoulos *et al*. (1988) would be required. Incomplete recovery of organoarsenic compounds can be expected (Cullen and Reimer 1989). Unlike the arsenic species isolated from marine animals, recently reported work has shown that arsenosugars from seaweeds can be metabolised before excretion (Ma and Le 1998). Consequently, spiking urine samples with arsenosugars extracted from seaweed would not necessarily produce a urine containing organoarsenic compounds the same as those excreted in urine following the consumption of seaweed. Sample spiking with seaweed extracts can therefore not be used to demonstrate that solvent extraction / GFAAS is suitable for partially speciating arsenicals in patients following seaweed consumption. An extensive investigation is required which determines the arsenicals present in each seaweed and those excreted by human subjects after consumption of that seaweed. This was considered to be beyond the scope of the present project and was not pursued further.

4.6 Recommended Protocols for Arsenic Investigation

As discussed in the previous Section, KOH / methanol extraction of seaweeds and analysis of the resulting extracts by CE / ICPMS indicated breakdown of organoarsenic species during extraction to form AsB. Recent published work has demonstrated that arsenosugars consumed by humans from edible seaweeds can be metabolised to form a variety of arsenic species. These can include DMAA and the metabolic products excreted in urine can vary between individuals (Ma and Le 1998). Given that DMAA is measured by solvent extraction / GFAAS as a marker for exposure to inorganic arsenic, seaweed consumption should be considered a potential cause of an elevated level of arsenic in the solvent extractable fraction obtained after partial speciation. Full speciation by CE / ICPMS of urine samples shown to be positive by partial speciation should clarify the source of arsenic. It is likely that in urine samples containing DMAA and one or more additional organoarsenic species, the DMAA is derived from seaweed. In contrast, when an elevated total arsenic level and solvent-extractable organoarsenic species are absent, it can be concluded that the DMAA is derived from exposure to inorganic arsenic. In subjects exposed to inorganic arsenic, the urine will contain both DMAA and MAA (Section 1.4). Urine samples containing only DMAA suggest a subject who has been exposed to DMAA only.

It is proposed that the investigation of arsenic in urine specimens should be taken through the following protocol: -

Step 1. Total arsenic is determined by ICPMS or GFAAS. When the arsenic result is < 2.0 µmol/L, the total arsenic is reported and no further action is taken. When the arsenic level is ≥ 2.0 µmol/L, the result is reported and the sample is further investigated in the next step.

Step 2. The sample undergoes partial arsenic speciation by solvent extraction / GFAAS. Samples yielding an organic phase containing < 1.0 µmol arsenic after cyclohexane extraction (equivalent to 1.33 µmol/L solvent-extractable arsenic in the original urine) are classed as "containing predominantly non-toxic arsenicals of marine origin". This is the finding for almost all of the urine samples received

in the PaLMS Trace Elements Laboratory that contain elevated total arsenic levels (see Step 1 above). When the organic phase contains $\geq 1.0\,\mu\text{mol/L}$ arsenic, the sample is treated as described in Step 3 below.

The organic phase derived from each sample after solvent extraction is not assayed for arsenic content by ICPMS. The high chloride levels could result in argon chloride interference in the ICPMS instrument and the high iodine concentrations would compromise the instrument for subsequent iodine estimations.

Step 3. Full arsenic speciation of an aliquot of the original urine sample by CE / ICPMS. The proportions of the various arsenic species are reported.

4.7 Clinical Cases Requiring Arsenic Speciation

Two clinical cases demonstrating the usefulness of arsenic speciation are discussed below.

Case 1.

A urine specimen from a 52 year old male was referred to PaLMS for speciation after analysis by an outside laboratory revealed a high total arsenic content. The patient suspected poisoning and denied consuming seafood in the days prior to the urine sample collection. The total arsenic determined by ICPMS was 18.0 µmol/L. Partial speciation indicated that the combined arsenic concentration present as As^{III}, As^{V}, MAA and DMAA was < 0.2 µmol/L. This suggested that the total arsenic measured by ICPMS was of marine origin. CE / ICPMS confirmed the predominant arsenic species as arsenobetaine. The recommended abstinence from seafood consumption prior to urine collection is 2–3 days (Vahter 1994). Compliance in abstaining from seafood consumption can be difficult to achieve. Some foods may contain seafood additives such as fish stocks or oyster sauce to enhance flavour and can lead to the inadvertent consumption of organoarsenicals. Patients may also consume marine organisms such as crayfish or oysters that they might not think of as being seafood. This case highlights the difficulty of obtaining organoarsenical-free samples and, therefore, the usefulness of the solvent extraction / GFAAS partial speciation method evaluated in this report.

Case 2.

A urine sample from a leukaemic patient who had received therapeutic doses of arsenic trioxide (Arsenic Trioxide Urine; 2.2.1.4) was referred for total arsenic determination. As samples from subjects with acute exposure to inorganic arsenic are rare, this sample provided a valuable opportunity to study human arsenic metabolism. Arsenic trioxide has only recently been rediscovered as a treatment of acute promyelocytic leukaemia (Soignet *et al.* 1998). Analysis of this sample by CE / ICPMS showed that the administered arsenite (As^{III}) was rapidly metabolised to form the methylated arsenicals MAA and DMAA so that 24 hours after treatment only these arsenicals were excreted in the urine (Figure 15). The arsenic containing species were identified as DMAA by

sample spiking and MAA on the basis of retention time from work done on arsenic speciation under similar electrophoretic conditions (Lin *et al.* 1995).

Using the counts for DMAA in a 13.3 µmol/L solution (Table VII), the concentrations of MAA and DMAA in this urine were calculated. These results are presented in Table XIV. By adding the calculated arsenic concentration for the two arsenic peaks, the total arsenic in this urine was 6.2 µmol/L. This compared favourably with the total arsenic of 6.4 µmol/L, determined by ICPMS (Section 2.4.2). These values indicate that, after exposure to arsenic trioxide, this patient excreted 66% of the injected arsenic as MAA and 34% as DMAA, giving a urinary MAA:DMAA ratio of 2:1. Although the patient was seriously ill, this ratio was consistent with other studies in healthy humans that have shown that MAA was the major metabolite after acute As^{III} exposure, whereas DMAA was the major metabolite in chronic As^{III} exposure (Benramdane *et al.* 1999). Partial speciation of this sample by solvent extraction / GFAAS confirmed that the predominant arsenicals were methylated metabolites with a solvent-extractable arsenic value of 4.3 µmol/L (Table X).

Table XIV Calculation of arsenic in MAA and DMAA in urine from patient treated with arsenic trioxide (2.2.1.4). Arsenic concentrations calculated using data from 10x diluted DMAA standard (Figure 11b; Table VII).

Analyte	Patient urine sample (Figure 15)	
	Counts	**As, µmol/L**
MAA	1616	4.1
DMAA	830	2.1

CHAPTER 5. FUTURE RESEARCH AND CONCLUSIONS

5.1 Future Research

5.1.1 Arsenic Compounds

This study has shown that the partial speciation of arsenicals in biological samples by solvent extraction / GFAAS may result in different extraction efficiencies for different types of arsenicals. To eliminate the possibility of false negatives when screening human subjects for inorganic arsenic toxicity, samples should demonstrate recovery of both MAA and DMAA. It has been shown that recoveries of only 72% were obtained for DMAA. It is therefore proposed that solvent extraction / GFAAS assays include additional calibration tubes incorporating 'standard additions' of DMAA. MAA will also be purchased and its recovery in this partial speciation method investigated.

To fully realise the analytical potential, for arsenicals, of the CE / ICPMS system described in this Report, the problems encountered with earthing of the high-voltage CE power supply (Section 4.3) must be overcome and this requires a new power supply. Only two suitable units are currently in Australia. Both were at Melbourne University and unavailable for this project. The purchase price of a new power supply from Beckman Coulter exceeded the budget of this project. As the need to provide full speciation of urine arsenicals is rare it was decided that perseverance with the current set up is sufficient. With a new power supply, experiments could continue to find CE conditions capable of resolving AsB and TMAO. It would then be possible to see whether TMAO was indeed present in the synthetically prepared AsB standard used in this present work.

An upgraded power supply would also enable deficiencies in the data presented in this project to be addressed. To properly assess reliability and accurately define detection limits for CE / ICPMS would require within and between batch precision estimates. This would require collation of results from several analytical runs. As current system power supply operation is detrimental to analyser function, replicate runs were not performed. Only sufficient data to demonstrate a working method have been accumulated.

5.1.2 Other Elements

By coupling CE with ICPMS detection, an element-specific and highly sensitive technique was created which may have biomedical applications beyond arsenic speciation (Hughes *et al.* 1995; Sutton *et al.* 1997; Tomlinson *et al.* 1995). To investigate metalloproteins and other anionic species by CE / ICPMS, an upgraded CE power supply is needed. Alterations to the power supply are not required when separating cationic species. Using a different electrolyte to that prepared for arsenic speciation (Shi and Fritz 1994) and reversing the polarity of the CE electrodes, will enable the separation of cationic molecules by CE. By changing the polarity of the CE electrodes the CE unit is earthed at the outlet so that voltage leakage is no longer detected. This technique may prove useful in the separation of vanadium (V) from the less toxic vanadium (IV).

CE may also be able to separate chromium from carbon containing molecules so that chromium can be successfully measured in plasma and urine by ICPMS without the results being compromised by the molecular interferant, argon carbide. An added bonus would be the separation of chromium (III) from chromium (VI). Chromium (III) is an essential nutrient because an organochromium complex synthesised from dietary chromium helps to activate insulin (Tomlinson *et al.* 1995). Deficiency of this complex results in glucose intolerance as well as reduced efficiency of insulin, thereby affecting the carbohydrate and lipid biochemistry in the body. Exposure to chromium (VI) can result in lesions, lung disease and various forms of cancer (Hughes *et al.* 1995).

CE / ICPMS would also seem an ideal way to measure organotin compounds. These compounds have widespread industrial uses resulting in their release into the environment. Organotin toxicity is dependant upon the type of substituent linked to the metal atom. Tetra- and triorganotin compounds are the most toxic forms. Ethyl-, methyl-, propyl, and butyltin compounds are more toxic than the compounds with phenyl- and octyl- substituents. CE could be used to separate organotin compounds by substituent type with ICPMS used to selectively monitor the emergence of these tin containing compounds from the CE capillary.

5.2 Conclusions

By determining total urine arsenic levels by ICPMS, partially speciating arsenicals by organic solvent extraction followed by GFAAS and fully speciating samples by CE / ICPMS it has been shown that it is possible to create a protocol for the complete investigation of patients suspected of arsenic poisoning. Organic solvent extraction followed by GFAAS provides a relatively inexpensive and rapid means of isolating those arsenicals associated with toxicity. As the majority of elevated urine arsenic levels are due to the presence of non-toxic arsenicals following seafood consumption, solvent extraction / GFAAS can exclude most cases of suspected arsenic poisoning. Urines which yield, after the partial speciation procedure, an elevated level of arsenic in the organic phase can be referred to a laboratory with CE / ICPMS capabilities for result confirmation and full arsenic speciation.

When running arsenicals through the ICPMS as they emerge from the CE capillary under a low-pressure injection, it is possible to detect arsenic species spiked into undiluted urine to an arsenic concentration of 0.1 ppm (1.3 µmol/L). This level of detection is adequate for patients suspected of arsenic poisoning where arsenic concentrations greater than 2 µmol/L are considered significant. To achieve lower limits of detection and make the most use of CE / ICPMS as an analytical technique would require upgrading the CE power supply. It is hoped that with further research this versatile technique will prove clinically useful in the speciation of other elements such as chromium, vanadium and tin.

CHAPTER 6. REFERENCES

Altria, K.D. (1999). Optimization of sensitivity in capillary electrophoresis. *LC·GC Asia Pacific*, **2**, (4), 28–32.

Beckman (1987). P/ACE 2000 Series instrument manual.

Benramdane, L., Accominotti, M., Fanton, L., Malicier, D., Vallon, J-J. (1999). Arsenic speciation in human organs following fatal arsenic trioxide poisoning - a case report. *Clinical Chemistry*, **45**, (2), 301–306.

Buchet, J.P., Lauwerys, R., Roels, H. (1980). Comparsion of several methods for the determination of arsenic compounds in water and in urine. *International Archives of Occupational and Environmental Health*, **46**, 11–29.

Cannon, J.R. Edmonds, J.S., Francesconi, K.A., Raston, C.L., Saunders, J.B., Skelton, B.W., White, A.H. (1981). Isolation, crystal structure and synthesis of arsenobetaine, a constituent of the western rock lobster, the dusky shark, and some samples of human urine. *Australian Journal of Chemistry*, **34**, 787–798.

Christakopoulos, A., Hamasur, B., Norin, H., Nordgren, I. (1988). Quantitative determination of arsenocholine and acetylarsenocholine in aquatic organisms using pyrolysis and gas chromatography / mass spectrometry. *Biomedical and Environmental Mass Spectrometry*, **15**, 67–74.

Clarkson, T.W. (1991). Arsenic. In: Hayes, W.J. Jr, Laws, E.R. Jr. eds. *HANDBOOK OF PESTICIDE TOXICOLOGY. Volume 2. Classes of pesticides*. San Diego, California: Academic Press, Inc. (Ch.12.12, pp 545–552).

Cullen, W.R., Reimer, K.J. (1989). Arsenic speciation in the environment. *Chemical Reviews*, **89**, (4), 713–764.

Date, A.R. (1983). An introduction to inductively coupled plasma source mass spectrometry. *Trends in Analytical Chemistry*, **2**, (10), 225–230.

Delves, H.T. (1988). Biomedical applications of ICP-MS. *Chemistry in Britain*, 1009–1012.

Ding, M., Wang, J., Dorsey, J.G., Caruso, J.A. (1995). Arsenic speciation by micellar liquid chromatography with inductively coupled plasma mass spectrometric detection. *Journal of Chromatography A*, **694**, (2), 425–431.

Edmonds, J.S., Francesconi, K.A. (1981). Arseno-sugars from brown kelp (*Ecklonia radiata*) as intermediates in cycling of arsenic in a marine ecosystem. *Nature*, **289**, 602–604.

Farmer, J.G., Johnson, L.R. (1990). Assessment of occupational exposure to inorganic arsenic based on urinary concentrations and speciation of arsenic. *British Journal of Industrial Medicine*, **47**, 342–348.

Francesconi, K.A., Edmonds, J.S. (1994). Biotransformation of arsenic in the marine environment. In: Nriagu, J.O. ed. *Arsenic in the environment. Part I: cycling and characterisation*. New York: John Wiley & Sons, Inc. (Ch.10, pp 221–261).

Francesconi, K.A., Edmonds, J.S., Morita, M. (1994). Determination of arsenic and arsenic species in marine environmental samples. In: Nriagu, J.O. ed. *Arsenic in the environment. Part I: cycling and characterisation*. New York: John Wiley & Sons, Inc. (Ch.9, pp 189–219).

Goessler, W. (1997). Arsenic compounds in a marine food chain. *Fresenius Journal of Analytical Chemistry*, **359**, 434–437.

Hanna, C.P., Tyson, J.F., McIntosh, S. (1993). Determination of inorganic arsenic and its metabolites in urine by flow-injection hydride generation atomic absorption spectrometry. *Clinical Chemistry*, **39**, (8), 1662–1667.

He, X., Guoquan, G., Hui, Z., Hu-Lin, L. (1997). Fluorometric determination of arsenic(III) with fluorescein. *Microchemical Journal*, **56**, 327–331.

Hindmarsh, J.T., McCurdy, R.F. (1986). Clinical and environmental aspects of arsenic toxicity. *Critical Reviews in Clinical Laboratory Sciences*, **23**, (4), 315–347.

Holmes, R.P. (1995). Measurement of urinary oxalate and citrate by capillary electrophoresis and indirect ultraviolet absorbance. *Clinical Chemistry*, **41**, (9), 1297–1301.

Houk, R.S. (1986). Mass spectrometry of inductively coupled plasmas. *Analytical Chemistry*, **58**, (1), 97A–105A.

Hughes, K., Meek, M.E., Newhook, R., Chan, P.K.L. (1995). Speciation in health risk assessments of metals: evaluation of effects associated with forms present in the environment. *Regulatory Toxicology and Pharmacology*, **22,** 213–220.

Jackson, P.E., Haddad, P.R. (1993). Capillary electrophoresis of inorganic ions and low-molecular-mass ionic solutes. *Trends in Analytical Chemistry*, **12**, (6), 231–238.

Jandik, P., Bonn, G. (1993a). Application examples of coelectroosmotic capillary electrophoresis. In: *Capillary electrophoresis of small molecules and ions*. New York: VCH Publishers, Inc. (Ch.5, pp 257–290).

Jandik, P., Bonn, G. (1993b). Fundamentals of capillary electrophoresis. In: *Capillary electrophoresis of small molecules and ions*. USA: VCH Publishers, Inc. (Ch.2, pp 5–78).

Jandik, P., Jones, W.R. (1991). Optimization of detection sensitivity in the capillary electrophoresis of inorganic anions. *Journal of Chromatography*, **546**, 431–443.

Jandik, P., Jones, W.R., Weston, A., Brown, P.R. (1991). Electrophoretic capillary ion analysis: origins, principles, and applications. *LC·GC Asia Pacific*, **9**, (9), 634–645.

Kuban, P., Karlberg, B. (1998). Simultaneous determination of small cations and anions by capillary electrophoresis. *Analytical Chemistry*, **70**, (2), 360–365.

Larsen, E.H., Pritzl, G., Hansen, S.H. (1993). Arsenic speciation in seafood samples with emphasis on minor constituents: an investigation using high-performance liquid chromatography with detection by inductively coupled plasma mass spectrometry. *Journal of Analytical Atomic Spectrometry*, **8**, 1075–1084.

Le, X-C., Cullen, W.R., Reimer, K.J. (1994). Human urinary arsenic excretion after one-time ingestion of seaweed, crab, and shrimp. *Clinical Chemistry*, **40**, (4), 617–624.

Lin, L., Wang, J., Caruso, J. (1995). Arsenic speciation using capillary zone electrophoresis with indirect ultraviolet detection. *Journal of Chromatographic Science*, **33**, (4), 177–180.

Liu, Y., Lopez-Avila, V., Zhu, J.J., Wiederin, D.R., Beckert, W.F. (1995). Capillary electrophoresis coupled on-line with inductively coupled plasma mass spectrometry for elemental speciation. *Analytical Chemistry*, **67**, (13), 2020–2025.

Lu, Q., Barnes, R.M. (1996). Evaluation of an ultrasonic nebuliser interface for capillary electrophoresis and inductively coupled plasma mass spectrometry. *Microchemical Journal*, **54**, 129–143.

Lu, Q., Bird, S.M., Barnes, R.M. (1995). Interface for capillary electrophoresis and inductively coupled plasma mass spectrometry. *Analytical Chemistry*, **67**, (17), 2949–2956.

Ma, M., Le, X.C. (1998). Effect of arsenosugar ingestion on urinary arsenic speciation. *Clinical Chemistry*, **44**, (3), 539–550.

Michalke, B., Schramel, P. (1998). Capillary electrophoresis interfaced to inductively coupled plasma mass spectrometry for element selective detection in arsenic speciation. *Electrophoresis*, **19,** 2220–2225.

Nixon, D.E., Moyer, T.P. (1992). Arsenic analysis II: rapid separation and quantification of inorganic arsenic plus metabolites and arsenobetaine from urine. *Clinical Chemistry*, **38**, (12), 2479–2483.

Oehrle, S. A. (1996). Analysis of ionic impurities in circuit board extracts using capillary electrophoresis. *LC·GC Asia Pacific*, **14**, (3), 226–229.

Peters, G.R., McCurdy, R.F., Hindmarsh, J.T. (1996). Environmental aspects of arsenic toxicity. *Critical Reviews in Clinical Laboratory Sciences*, **33**, (6), 457–493.

Phillips, D.J.H. (1994). The chemical forms of arsenic in aquatic organisms and their interralationships. In: Nriagu, J.O. ed. *Arsenic in the environment. Part I: cycling and characterisation*. New York: John Wiley & Sons, Inc. (Ch.11, pp 263–288).

Potter, J.M. ed. (1995). Clinical Pharmacology and Toxicology: Laboratory aspects of therapeutic and toxicological drug investigations. AACB/RCPA, Brisbane. ISBN No. 0 9593355 1X. (pp 223–227).

Raie, R.M. (1996). Regional variation in As, Cu, Hg, and Se and interaction between them. *Ecotoxicity and Environmental Safety*, **35,** 248–252.

Shi, Y., Fritz, J.S. (1994). New electrolyte systems for the determination of metal cations by CZE. *Journal of Chromatography A*, **671**, 429–435.

Shiomi, K. (1994). Arsenic in marine organisms: chemical forms and toxicological aspects. In: Nriagu, J.O. ed. *Arsenic in the environment. Part II: human health and ecosystem effects*. New York: John Wiley & Sons, Inc. (Ch.12, pp 261–282).

Soignet, S.L., Maslak, P., Wang, Z-G., Jhanwar. S., Calleja, E., Dardashti, L.J., Corso, D., DeBlasio, A., Gabrilove, J., Scheinberg, D.A., Pandolfi, P.P., Warrell, R.P. Jr. (1998). Complete remission after treatment of acute promyelocytic leukemia with arsenic trioxide. *The New England Journal of Medicine*, **339**, (19), 1341–1348.

Spall, W.D., Lynn, J.G., Andersen, J.L., Valdez, J.G., Gurley, L.R. (1986). High-performance liquid chromatographic separation of biologically important arsenic species utilising on-line inductively coupled plasma atomic emission spectrometric detection. *Analytical Chemistry*, **58**, 1340–1344.

Subramanian, K.S. (1988). Determination of arsenic in urine by graphite platform-in-furnace atomic absorption spectrometry. *Canadian Journal of Spectroscopy*, **33**, (6), 173–181.

Subramanian, K.S. (1989). Determination of arsenic in urine by graphite platform furnace atomization atomic absorption spectrometry. *The Science of the Total Environment*, **89**, 311–315.

Sutton, K., Sutton, R.M.C., Caruso, J.A. (1997). Inductively coupled plasma mass spectrometric detection for chromatography and capillary electrophoresis. *Journal of Chromatography A*, **789**, 85–216.

Tan, S.H., Horlick, G. (1986). Background spectral features in inductively coupled plasma / mass spectrometry. *Applied Spectroscopy*, **40**, (4), 445–460.

Tangen, A., Trones, R., Greibrokk, T., Lund, W. (1997). Micro concentric nebuliser for the coupling of micro liquid chromatography and capillary zone electrophoresis with inductively coupled plasma spectrometry. *Journal of Analytical Atomic Spectrometry*, **12**, 667–670.

Tomlinson, M.J., Lin, L., Caruso, J.A. (1995). Plasma mass spectrometry as a detector for chemical speciation studies. *Analyst*, **120**, 583–589.

Vahter, M.E. (1988). Arsenic. In: Clarkson, T.W., Friberg, L., Nordberg, G.F., Sager, P.R. Eds. *Biological Monitoring of Toxic Metals*. New York: Plenum Press. (pp 303–321).

Vahter, M. (1994). What are the chemical forms of arsenic in urine, and what can they tell us about exposure? *Clinical Chemistry*, **40**, (5), 679–680.

Vanhaecke, F., Vanhoe, H., Dams, R., Vandecasteele, C. (1992). The use of internal standards in ICP-MS. *Talanta*, **39**, (7), 737–742.

Varian (1988). Analytical methods for graphite tube atomizers. Varian publication No. 85-100848-00.

Varian (1993). SpectrAA-800 operation manual. Varian publication No. 85-101043-00.

Varian (1997). UltraMass spectrometer system operation manual. Varian publication No. 85-101204-00.

Vaughan, M.A., Horlick, G. (1986). Oxide, hydroxide, and doubly charged analyte species in inductively coupled plasma / mass spectrometry. *Applied Spectroscopy*, **40**, (4), 434–445.

CHAPTER 7. APPENDICES

Appendix A - CE Instrument Parameters

Solution placement:

Carousel position	1	Outlet electrolyte solution
	2	Waste vial
	11–30	Samples
	31	Inlet electrolyte solution
	32	100 mM NaOH (2.3.1.5)
	33	Water

Capillary conditioning: Select high-pressure rinse to purge the capillary (2.3.1.6) with electrolyte for 2 minutes. At the completion of analysis purge the capillary with 100 mM NaOH (2.3.1.5) for 2 minutes followed by water for 5 minutes.

Detection: Indirect

Wavelength: 254 nm

Response factor: 0.1

Analysis time: 8.0 minutes

Stop data: 8.0 minutes

Re-zero: 2.5 minutes

Pre-rinse: Vial 31 to vial 2 for 1 minute at high-pressure.

Separation voltage: 15 kV

Sample injection: Pressure injection (0.5 psi for 7 seconds). Outlet vial 1.

Peak identification parameters:

Minimum peak height 0.00049

2.96 Peak integration

3.12 Negative Peak logic ON

7.90 Negative Peak logic OFF

Appendix B - ICPMS Instrument Parameters

Measurement Parameters

Analysis Modes	Analysis type:	Quantitative
	Acquisition mode:	Steady State
	Scan mode:	Peak Hopping
	Spacing:	0.100 AMU
	Points/Peak:	1
	Scans/Replicate:	20
	Replicates/Sample:	5
	Scan time:	70 milliseconds
	Replicate time:	1.40 seconds
	MaxiRange:	Off
Plasma	Plasma flow:	15.5 L/min
	Auxiliary flow:	1.75 L/min
	Nebuliser flow:	0.84 L/min
	Sampling depth:	5.0 mm
	Horizontal alignment:	-0.8 mm
	Vertical alignment:	-1.2 mm
	RF power:	1.20 kW
Ion Optics	Extraction lens:	-600 volts
	First lens:	-300 volts
	Second lens:	-12.6 volts
	Third lens:	0.0 volts
	Fourth lens:	-80 volts
	Photon stop:	-13.8 volts
	Entrance plate:	0.0 volts
	Exit plate:	0 volts
Quadrupole	Detector:	1700 volts
	Delta resolution high:	-1.955 volts
	Delta resolution low:	-2.5 volts
	High resolution:	0.76 AMU
	Low resolution:	0.79 AMU
	Pole bias:	0.000 volts

	Sampling	Aerosol generation:	Nebuliser
		Source:	Autosampler
		Pump rate:	10 rpm
		Fast pump during sample delay/rinse:	On
		Enable device control:	Off
		Sample uptake delay:	60 seconds
		Stabilisation time:	10 seconds
		Probe height:	0 mm
		Agitation time:	0 seconds
		Rinse time:	0 seconds

Analytes (1)

^{75}As

Semi-quantitative Analytes (0)

Internal Standards (1)

^{45}Sc

Default Exclusions (7)

^{40}Ar, ^{40}Ar$_2$, ^{14}N, ^{14}N^{1}H, ^{16}O, ^{16}O^{1}H, ^{40}Ar^{1}H

User-specified exclusions (0)

Scan Segments (2)

Start (m/z)	Stop (m/z)	Dwell (μsec)	Start (m/z)	Stop (m/z)	Dwell (μsec)
45	45	20000	75	75	20000

Standard Concentrations

Element	Units	C1	C2	C3	C4
As	ppb	0.1700	1.1700	5.1700	10.1700

Calibration Parameters

Analyte	Curve type	Thru blank	Weighted fit	Max % error
Arsenic	Linear	No	No	25.0

Appendix C - CE / ICPMS Instrument Parameters

<u>**CE Instrument Parameters**</u>

Solution placement:

Carousel position	11–30	Samples
	31	Inlet electrolyte solution (2.3.3.13)
	32	100 mM NaOH (2.3.3.9)
	33	Water
	1	Empty vial

Capillary conditioning: Select high-pressure rinse to purge the capillary (2.3.3.10) with electrolyte (2.3.3.13) for 2 minutes. At the completion of analysis purge the capillary with 100 mM NaOH (2.3.3.9) for 2 minutes followed by water for 5 minutes.

Detection: Indirect

Wavelength: 254 nm

Response factor: 0.1

Analysis time: 18.0 minutes

Stop data: 7.0 minutes

Re-zero: 2.5 minutes

Pre-rinse: Vial 31 to vial 1 for 1 minute at high.

Separation voltage: 10 kV from time 0–7 minutes.

Low-pressure separation: From time 7–18 minutes.

Sample injection: Pressure injection (0.5 psi for 10 seconds). Outlet vial 1.

Peak identification parameters:

Minimum peak height 0.00049

2.96 Peak integration

3.12 Negative Peak logic ON

6.90 Negative Peak logic OFF

ICPMS Instrument Parameters

Measurement Parameters

Analysis Modes	Analysis type:	Quantitative
	Acquisition mode:	Time resolved
	Scan mode:	Peak Hopping
	Spacing:	0.025 AMU
	Points/Peak:	1
	Scan time:	1020 milliseconds
	Sampling time:	600.00 seconds
	MaxiRange:	Off
Plasma	Plasma flow:	15.0 L/min
	Auxiliary flow:	1.65 L/min
	Nebuliser flow:	0.80 L/min
	Sampling depth:	5.0 mm
	Horizontal alignment:	-0.8 mm
	Vertical alignment:	-1.2 mm
	RF power:	1.20 kW
Ion Optics	Extraction lens:	-600 volts
	First lens:	-260 volts
	Second lens:	-11.8 volts
	Third lens:	0.0 volts
	Fourth lens:	-60 volts
	Photon stop:	-11.2 volts
	Entrance plate:	0.0 volts
	Exit plate:	0 volts
Quadrupole	Detector:	1700 volts
	Delta resolution high:	-1.955 volts
	Delta resolution low:	-2.5 volts
	High resolution:	0.76 AMU
	Low resolution:	0.79 AMU
	Pole bias:	0.000 volts

Sampling   Aerosol generation: Laser

                  Source: Manual

                  Pump rate: 10 rpm

                  Enable device control:Off

                  Sample uptake delay: 0 seconds

                  Stabilisation time: 0 seconds

Analytes (1)

^{75}As

Semi-quantitative Analytes (0)

Internal Standards (0)

Default Exclusions (7)

^{40}Ar, ^{40}Ar$_2$, ^{14}N, ^{14}N^{1}H, ^{16}O, ^{16}O^{1}H, ^{40}Ar^{1}H

User-specified exclusions (0)

Scan Segments (1)

Start (m/z)	Stop (m/z)	Dwell (μsec)
75	75	1000000

Appendix D - GFAAS Operating Parameters

Element – Matrix:	As – Urine
Instrument Type:	Zeeman
Conc. Units:	µmol/L
Instrument Mode:	Absorbance
Sampling Mode:	Autonormal
Calibration Mode:	Concentration
Measurement Mode:	Peak Area
Replicates Standard:	2
Replicates Sample:	2
Expansion Factor:	1.0
Minimum Reading:	Disabled
Smoothing:	5 point
Conc. Dec. Places:	2
Wavelength:	193.7 nm
Slit Width:	1.0 nm
EHT:	373 volts
Lamp Current:	8.0 mA
Lamp Position:	1
Background Correction:	BC On
STANDARD 1:	0.90 µmol/L
STANDARD 2:	1.80 µmol/L
STANDARD 3:	2.70 µmol/L
Reslope Rate:	100
Reslope Standard No.:	2
Reslope Lower Limit:	75.0%
Reslope Upper Limit:	125.0%
Recalibration Rate:	1000
Calibration Algorithm:	New Rational
Cal. Lower Limit:	20.0%
Cal. Upper Limit:	150.0%
Workhead Height:	0.0 mm
Total Volume:	20 µL

Sample Volume: 20 µL

Vol. Reduction Factor: 2

Makeup Vial No.:   52

Hot Inject: On

Hot Inject Temperature: 80°C

Hot Inject Rate: 5

CAL ZERO Position: 52

STANDARD 1 Position: 41

STANDARD 2 Position: 42

STANDARD 3 Position: 43

Correlation Coefficient Limit:0.9950

Step	Temp (^{o}C)	Time (s)	Flow (L/min)	Gas Type	Read	Signal Storage
1	85	5.0	3.0	Normal	No	No
2	100	10.0	3.0	Normal	No	No
3	800	5.0	3.0	Normal	No	No
4	800	5.0	3.0	Normal	No	No
5	800	2.0	0.0	Normal	No	Yes
6	2600	0.9	0.0	Normal	Yes	Yes
7	2600	2.0	0.0	Normal	Yes	Yes
8	2600	10.0	3.0	Normal	No	Yes

Appendix E - Calculation of Peak Area from CE / ICPMS Data

Time resolved CE / ICPMS data are exported to an Excel spreadsheet so that electrophoretograms can be drawn and peak areas calculated. Figure 11* shows an example of a CE / ICPMS electrophoretogram while an example of calculated peak areas are shown in Table VII[*]. Data from the ICPMS collected at m/z 75 to span the period during which the arsenate (As[V]) peak appears (Figure 11a*) are shown in Table XV* to demonstrate how peak areas were calculated. The peak area was estimated from counts where they exceeded the background counts. Counts from 18.54 seconds to 57.68 seconds were considered to exceed the background level. The total peak area was found by adding the counts over this time period to give a value of 65478 counts. Background corrections were performed by selecting counts at time intervals before and after the peak so that before the peak, at 17.51 seconds, a value of 200 counts was obtained and after the peak, at 58.71 seconds, a value of 287 counts was obtained. As the peak extended over 34 readings, background counts over this period were taken to be the average of the counts before and after the peak at 17.51 seconds and 58.71 seconds i.e. 243.5 counts. The peak background was calculated by multiplying 243.5 by 34 to give a value of 8279 counts. Peak area, corrected for background, was 57199 counts after subtracting total peak area (65478 counts) from background (8279 counts).

---

* See body of the Report. Page numbers 60 and 100.

Table XV Section of data for the arsenate (As^V) peak from the CE / ICPMS separation of an aqueous standard mixture containing arsenate (As$^\mathrm{V}$), DMAA and arsenite (As$^\mathrm{III}$) at 133 µmol/L and AsB at 77 µmol/L, as shown in Figure 11a[*]. Analysis performed by CE using 5 mM HBA / 4 mM TTAOH electrolyte coupled to ICPMS monitoring at m/z 75, as described in Section 2.4.3*.

Time (Seconds)	Counts at m/z 75	Time (Seconds)	Counts at m/z 75
1.03	223	38.11	3370
2.06	191	39.14	3059
3.09	186	40.17	2534
4.12	204	41.20	2123
5.15	246	42.23	1832
6.18	185	43.26	1554
7.21	242	44.29	1184
8.24	223	45.32	988
9.27	225	46.35	790
10.30	241	47.38	706
11.33	168	48.41	611
12.36	241	49.44	562
13.39	207	50.47	454
14.42	250	51.50	363
15.45	204	52.53	391
16.48	290	53.56	400
17.51	200	54.59	287
18.54	333	55.62	308
19.57	290	56.65	295
20.60	384	57.68	327
21.63	457	58.71	287
22.66	709	59.74	294
23.69	891	60.77	279
24.72	1222	61.80	261
25.75	1434	62.83	232
26.78	1997	63.86	256
27.81	2386	64.89	271
28.84	3006	65.92	279
29.87	3372	66.95	230
30.90	3639	67.98	239
31.93	3919	69.01	331
32.96	4364	70.04	295
33.99	4204	71.07	257
35.02	4152	72.10	243
36.05	4029	73.13	224
37.08	3769	74.16	203

* See body of the Report. Page numbers 37-39 and 60.