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SPECIATION OF ARSENIC BY CHEMICAL SEPARATIONS AND NEUTRON ACTIVATION ANALYSIS

by

Youqing Shi

Submitted in partial fulfillment of the requirements
for the degree of Doctor of Philosophy
at
Dalhousie University
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DALHOUSIE UNIVERSITY

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The undersigned hereby certify that they have read and recommend to the Faculty of Graduate Studies for acceptance a thesis entitled "Speciation of Arsenic by Chemical Separations and Neutron Activation Analysis"

by Youqing Shi

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To

my wife

and

daughter

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ABSTRACT

The speciation of trace arsenic has received particular attention in recent years. A combination of several chemical separation methods and neutron activation analysis (NAA) has been developed here for the speciation of arsenic.

An open-column cation exchange chromatographic method has been developed to separate dimethylarsinic acid (DMA) and arsenobetaine (AsB) from the anionic arsenic species including arsenite (As(III)), arsenate (As(V)) and monomethylarsonic acid (MMA). The method has been used as a group separation step in the speciation analysis schemes of water and seafood samples.

A solvent extraction-NAA method using ammonium pyrrolinedithiocarbamate (APDC) and 4-methyl-2-pentanone (MIBK) has been developed for the separation of As(III), As(V), Sb(III), Sb(V) and Se(IV). The acidity, aqueous-to-organic phase volume ratio, stability of the complexes, and potential interferences have been investigated.

A solid-phase extraction (SPE)-NAA method using dibenzyldithiocarbamate (DBDC) impregnated Amberlite XAD-4 resin has been developed for the determination of As(III) and Sb(III). The adsorption rate, adsorption isotherms, and various factors affecting the coating of DBDC on XAD-4 have been investigated.

A HPLC-NAA method using an anion exchange column and phosphate buffer has been developed for the determination of As(III), As(V), MMA, DMA, and AsB. A 10-mM phosphate buffer at pH 6.5 has been selected for the separation of AsB and DMA, and the same buffer at 75 mM and pH 5.5 has been used to separate As(III), MMA, and As(V).

The above methods have been successfully applied for the determination of As(III), As(V) MMA, DMA, AsB, and organically bound arsenic (OBAs) in water samples, and of As(III), As(V) MMA, DMA, AsB, lipid-soluble arsenic, and total arsenic in several fish samples. AsB has been found to be the predominate arsenic species in fish and As(V) in water. The precision and accuracy of the speciation methods have been found to be good.

LIST OF ABBREVIATIONS AND SYMBOLS

σ	cross section
Φ	neutron flux
ε	detector efficiency
λ	decay constant
AAS	atomic absorption spectrometry
AES	atomic emission spectrometry
AFS	atomic fluorescence spectrometry
APDC	ammonium pyrrolidinedithiocarbamate
AsB	arsenobetaine
AsC	arsenocholine
ASV	anodic stripping voltammetry
b	barns
BDSA	benzene-1,2-disulfonic acid
C*	activated charcoal
CE	capillary electrophoresis
CE-UV	capillary electrophoresis-UV detection
CE-ICP-MS	capillary electrophoresis-inductively coupled plasma-mass spectrometry
c_L	limit of detection
CNAA	cyclic neutron activation analysis
CRM	certified reference materials
CSV	cathodic stripping voltammetry

CTA^+-$DBDC^-$/XAD-4	cetyltrimethylammonium-pyrrolidinedithiocarbamate ion pairs impregnated XAD-4 resin
CTA^+-PDC^-	cetyltrimethylammonium-pyrrolidinedithiocarbamate ion pairs
CTAB	cetyltrimethylammonium bromide
DBDC	dibenzyldithiocarbamate
$[DBDC]_{aq}$	DBDC concentration in aqueous phase
$[DBDC]_r$	DBDC concentration on resin phase
DCP	direct current plasma
DDAB	didodecyldimethylammonium bromide
DDDC	diethylammonium diethyldithiocarbamate
DDTC	diethyldithiocarbamate
DDTP	ammonium diethyldithiophosphate
DDW	distilled deionized water
DMA	dimethylarsinic acid
DMA(III)	dimethylarsonous acid
DMPS	2,3-dimercaptopropane-1-sulfonate
DPASV	differential pulse anodic stripping voltammetry
DPCSV	differential pulse cathodic stripping voltammetry
DPP	differential pulse polarography
EI	electrospray ionization
EINAA	epithermal instrumental neutron activation analysis
EOF	electrosmotic flow
ESI-MS	electronic spray ionization-mass spectrometry
ET	electrothermal

ET-AAS	electronic thermal-atomic absorption spectrometry
FID	flame ionization detector
GC	gas chromatography
GC-MS	gas chromatography-mass spectrometry
h	hour
HAOc	acetic acid
HDBDC	dibenzyldithiocarbamic acid
HDBDC/XAD-4	HDBDC impregnated XAD-4 resin
HG	hydride generation
HG-AAS	hydride generation-atomic absorption spectrometry
HG-CT-AAS	hydride generation-cryotrapping-atomic absorption spectrometry
HG-CT-GC-MS	hydride generation-cryotrapping-gas chromatography-mass spectrometry
HG-CT-ICP-MS	hydride generation-cryotrapping-inductively coupled plasma-mass spectrometry
HG-ICP-AES	hydride generation- inductively coupled plasma-atomic absorption spectrometry
HG-ICP-MS	inductively coupled plasma-mass spectrometry
HMDC	ammonium hexamethylenedithiocarbamate
HMDE	hanging mercury drop electrode
HP	high pressure
HPDC	pyrrolidinedithiocarbamic acid
HPDC/XAD-4	HPDC impregnated XAD-4 resin
HPLC	high-performance liquid chromatography
HPLC-AFS	high-performance liquid chromatograpy-atomic fluorescence

	spectrometry
HPLC-ET-AAS	high-performance liquid chromatography-electrothermal-atomic absorption spectrometry
HPLC-HG-AAS	high-performance liquid chromatography-hydride generation-atomic absorption spectrometry
HPLC-HG-ICP-MS	high-performance liquid chromatography-hydride generation-inductively coupled plasma-mass spectrometry
HPLC-ICP-MS	high-performance liquid chromatography-inductively coupled plasma-mass spectrometry
HPLC-MD-HG-AAS	high-performance liquid chromatography-microwave digestion-hydride generation-atomic absorption spectrometry
HPLC-NAA	high-performance liquid chromatography-neutron activation analysis
HPLC-UV-HG-AFS	high-performance liquid chromatography-UV decomposition - hydride generation-atomic fluorescence spectrometry
HPLC-UV-HG-ICP-AES	high-performance liquid chromatography-UV-hydride generation-inductively coupled plasma-atomic emission spectrometry
HPLC-UV-HG-ICP-MS	high-performance liquid chromatography-UV decomposition-hydride generation-inductively coupled plasma-mass spectrometry
I	resonance integral
ICP	inductively coupled plasma
ICP-AES	inductively coupled plasma-atomic emission spectrometry
ICP-MS	inductively coupled plasma-mass spectrometry
INAA	instrumental neutron activation analysis
IUPAC	international union of pure and applied chemistry
K_d	distribution ratio
keV	kilo electron volt

LaC*	La loaded activated charcoal
LC	liquid chromatography
L_D	Currie detection limit
LP	low pressure
MAA	molecular activation analysis
mb	millibarns
MeOH	methanol
MIBK	4-methyl-2-pentanone
min	minute
MIP	microwave induced plasma
MMA	monomethylarsonic acid, monomethylarsonate
MMA(III)	monomethylarsonous acid
MS	mass spectrometry
Na_2EDTA	disodium ethylenediaminetetraacetate
NAA	neutron activation analysis
NaBS	sodium 1- butanesulfonate
NaDS	sodium dodecylsulfonate
N_B	background counts
NMR	nuclear magnetic resonance
NRCC	National Research Council of Canada
OBAs	organically bound arsenic species
P_γ	branching ratio
p-APA	p-aminophenylarsenate
PDC	pyrroliidinedithiocarbamate

PU	polyurethane
RNAA	radiochemical neutron activation analysis
RSD	relative standard deviation
s	second
SOS	sodium octyl sulfate
SDS	sodium dodecyl sulfate
SEC	size-exclusion chromatography
SP	spectrophotometry
SPE	solid-phase extraction
SPE-ET-AAS	solid-phase extraction-electrothermal atomic absorption spectrometry
SPE-ICP-AES	solid-phase extraction-inductively coupled plasma-atomic emission spectrometry
SPE-NAA	solid-phase extraction-neutron activation analysis
$t_{1/2}$	half-life
TBA	tetrabutylammonium ion
TBAH	tetrabutylammoniom hydroxide
TBAP	tetrabutylammonium phosphate
t_c	counting time
t_d	decay time
TDT	toluene-3,4-dithiol
TEAH	tetraenthylammonium hydroxide
t_i	irradiation time
TMA	tetramethylarsonium ion
TMAm	tetramethylammonium ion

$TMAm^+$-$DBDC^-$/XAD-4	TMA^+-$DBDC^-$ impregnated XAD-4 resin
TMAH	tetramethylammonium hydroxide
TMAO	trimethylarsenic oxide
TINAA	thermal instrumental neutron activation analysis
VC*	V loaded activated charcoal
XPS	X-ray photoelectron spectroscopy
ZrC*	Zr loaded activated charcoal

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1. INTRODUCTION

1.1 Chemical Speciation

Trace elements seldom exert their biological activities and make environmental impacts as free atoms, but as ions, or as components of molecules and macromolecules. The detection, identification and quantification of elemental species have become more important than the total element in some cases [1-4]. This is evident from the rapidly increasing number of publications in recent years on speciation analysis. Caroli [5] compiled publications related to speciation analysis from 1983 to 1994, and found that 2060 publications could be identified as work mainly focused on elemental species. Since then a large number of papers have been published on this subject. The analytes cover most of the elements in the periodic table with emphasis on Groups 13, 14, 15, 16, 11, 12, 6 elements and on actinides. Among these elements, arsenic has been attracting much attention from both the environmental and human health fields. This is supported by the observation that about 10% of the papers presented at the First International Conference on Trace Element Speciation in Biological, Nutritional and Environmental Sciences held in 1998 related to the speciation of arsenic [6].

Recently, the International Union of Pure and Applied Chemistry (IUPAC) and the Standard Measurement and Testing Programme of the European Union (SM&T Programme of EU) have put forward certain definitions related to speciation [5-9]. According to these definitions, "a species is an element in a specific and unique molecular, electronic, or nuclear structure" [5]. Chemical speciation is "the clear identification and quantification of the element species in a real matrix or sample" [7]. Analytical activities for identifying and quantifying one or more chemical species of an element present in a sample may be called "speciation analysis", and "an element's occurrence in or distribution among different species makes the speciation of that individual element" [5]. Some researchers consider these definitions to be a little bit too strict to follow in all examples of speciation. In fact, one can always find some examples of speciation in the literature that have to be differentiated from "chemical speciation". Some of the examples can be classified as "operationally defined" species which are "a form of characterization of groups from different compounds, showing a similar behavior

towards a specific methodical operation" [7]. For example, "water-soluble", "lipid-soluble" or "EDTA-extractable" when the extraction procedures are necessary for obtaining the speciation information. Another group is called "functionally defined" species, such as "essential" or "toxic", indicating a form of characterization of groups from different compounds showing "a mutual biological impact or function within the organism" [7].

1.2 Speciation Techniques

Speciation analysis has become a novel branch in the field of analytical chemistry. The rapid development in this area is benefiting more and more from the development of innovative analytical techniques. Generally speaking, the potential success for the identification of a species of a given element depends on two factors. Firstly, the technique must be species selective. Second, the detection limit of the technique must be significantly lower than the analyte's level in the real samples. Intrinsically, mass or tandem mass spectrometry (MS), nuclear magnetic resonance spectrometry (NMR), and X-ray photoelectron spectroscopy (XPS) are not able to measure a species at trace levels in presence of a real sample matrix. The highly sensitive as well as widely used elemental determination techniques include hydride generation atomic absorption spectrometry (HG-AAS), electrothermal AAS (ET-AAS), inductively coupled plasma mass spectrometry (ICP-MS), ICP atomic emission spectrometry (ICP-AES), and neutron activation analysis (NAA). Since these techniques are generally element specific, chemical separation methods are required for speciation analysis. Coprecipitation, solvent and solid-phase extractions, cryotrapping and thermal desorption, and gas and liquid chromatography are useful separation methods. These methods can be coupled to the above detection techniques either on-line or off-line. Some of the commonly used separation and detection techniques are given in Table 1.1. Several authors [1, 4-5, 10-20] have comprehensively reviewed speciation analysis techniques. The techniques with emphasis on the speciation of arsenic are discussed in detail in Chapter 2 of this thesis.

Table 1.1. A Summary of Separation and Detection Techniques Used for Speciation Analysis

Separation Techniques	Detection Techniques
Solvent extraction	NMR
Solid-phase extraction	MS (ICP, EI)
GC	AAS (HG, ET, Flame)
Cryotrapping/thermal desorption	AES (ICP, DCP, MIP)
LC (LP, HP, CE, SEC)	Fluorescence
	NAA
	Electrochemical (CSV, ASC, DPP)

1.3 Neutron Activation Analysis

Neutron activation analysis (NAA), as an element determination technique, has been exclusively used in the present work. This technique has made a major contribution to the trace element analysis in life sciences and environmental protection primarily because of its unique specificity combined with superior sensitivity, high precision and accuracy, simultaneous multielement analysis capability, protection against contamination, and no interference from most of the biological and environmental matrices [21-24]. The NAA technique is based on the production of a radioactive nuclide through the absorption of neutrons by an isotope of an element in a sample, and the subsequent detection and measurement of the radiation emitted from the radioactive nuclide during its decay. Details of the theory of NAA have been described by De Soete, Gijbels and Hoste [24] and will not be described here.

Over the years, significant advances in the detection instrumentation and in the availability of low-power research reactors have made NAA more common. With the

development of high-resolution and high-efficiency detectors and computerized gamma-ray spectrometers, NAA has become one of the most powerful analytical techniques.

Like many analytical techniques, NAA is an element specific detection technique and not a species selective technique. However, by coupling with chemical separation techniques, NAA can become species specific. A technique called molecular activation analysis (MAA) has already been applied to speciation analysis of selenium, mercury, iodine, and rare earth elements [25-27]. Many of the elemental specific detection techniques (Table 1.1) can be coupled on-line or off-line to the separation techniques; however, the coupling of NAA with a separation technique can only be carried out off-line due to the special requirements of NAA.

1.4 Importance of Speciation of Arsenic

The toxic and medicinal properties of arsenic have been known for several centuries [28]. Research on the understanding of the processes that can generate various chemical forms of arsenic in biological organisms has intensified over the last twenty years or so, mainly due to the advent of improved analytical methods. Many arsenic species have been found to occur naturally in the environment, and they have different chemical and toxicological properties [29, 30]. Inorganic arsenic exhibits the highest toxicity; arsine is the most toxic species; arsenite is more toxic than arsenate. Simple methylated arsenic species are less toxic; arsenobetaine (AsB) and arsenocholine (AsC) are almost non-toxic. The names and chemical formulae of some of the commonly encountered as well as important arsenic species are shown in Fig. 1.1.

Arsenic is naturally present in igneous sedimentary rocks and ores mainly in the forms of sulfides, arsenides, and sulpharsenides [31]. Weathering, volcanic activities, dissolution in water, and biological activities can lead to the emission of arsenic into the atmosphere, biosphere and hydrosphere. Another important source of arsenic mobilization is human activities. It has long been recognized that the smelting of non-ferrous metals and the production of energy from fossil fuel combustion are the two leading industrial processes that introduce arsenic into the air and water at a significant rate [32].

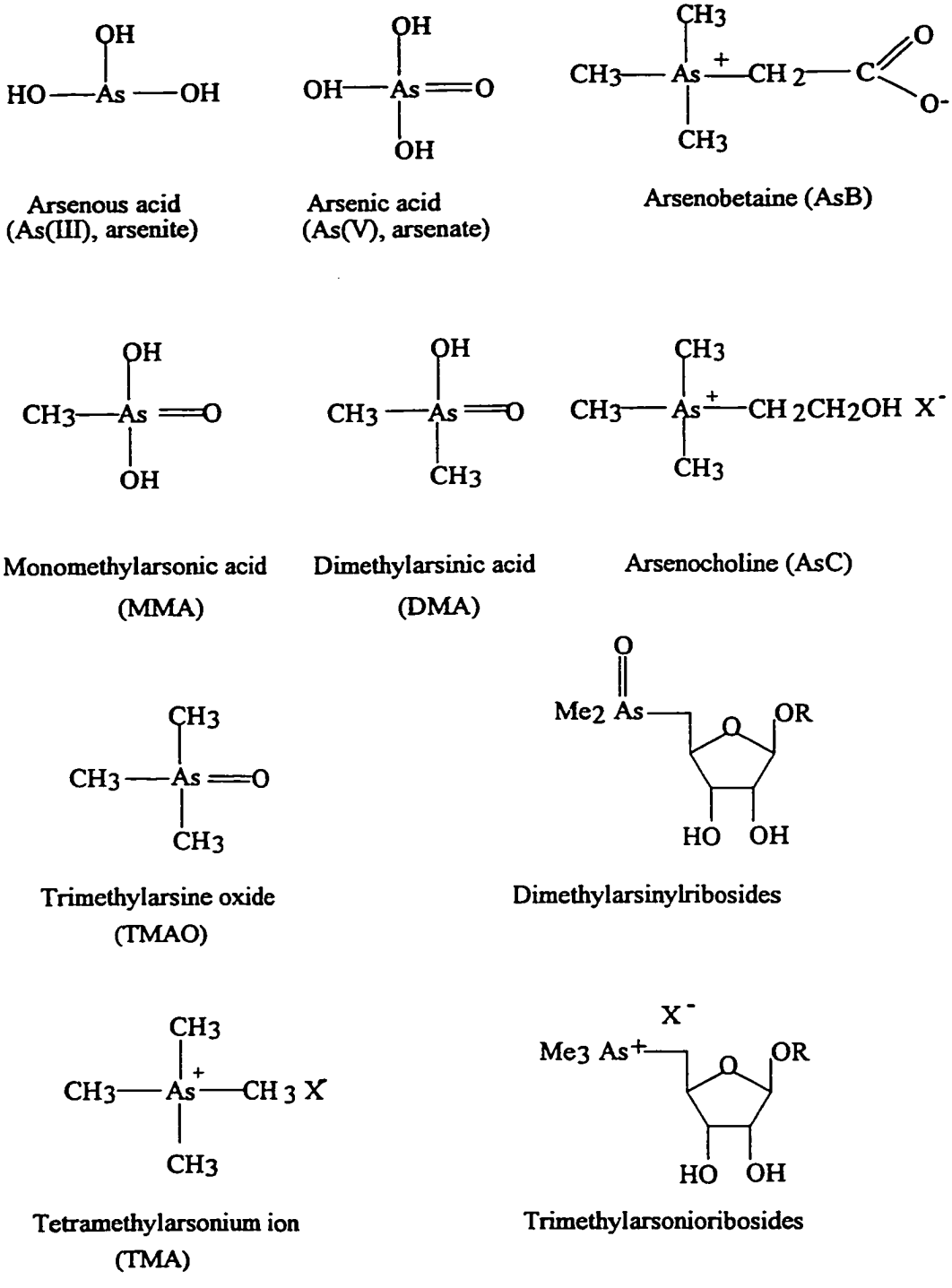

Fig. 1.1. Names and Structures of Selected Important Arsenic Compounds in the Environment [33, 49-52]

In unpolluted seawaters arsenic is present at a fairly uniform concentration of about 1 to 2 $\mu g\ L^{-1}$; however, the concentration of arsenic in fresh waters shows considerable variations with geological composition of the drainage area and the extent of anthropogenic input [34]. Compiled data for dissolved arsenic in some European and North as well as South American rivers were shown to vary between 0.1 and 75 $\mu g\ L^{-1}$ [35]. Arsenate, As(V), is the predominant form of arsenic in natural waters. Small quantities of monomethylarsonate (MMA) and dimethylarsinate (DMA) have also been found in natural waters [36-39]. Although in the literature, both MMA and MMAA have been used as the abbreviation of monomethylarsonic acid or monomethylarsonate, MMA is used exclusively in this thesis. Similarly, DMA is used as the abbreviation of dimethylarsinic acid or dimethylarsinate.

The concentrations of As(III), MMA, and DMA are higher in the photic zone of marine waters [40]. Andreae [41] reported a positive correlation between the minor arsenic species, such as As(III), MMA and DMA, and the indicators of the primary producers, such as chlorophyll concentration and ^{14}C uptake, and suggested that As(III) and methylated arsenic species might be produced in the photic zone by biological activities. The correlation between the biological activities and the concentrations of these minor arsenic species has also been reported by other researchers [42-44]. As(III), As(V), MMA and DMA are the only four arsenic species that have so far been identified in marine waters. However, this does not mean that additional arsenic species are not present in natural waters. The most commonly used analytical methods involve the generation of volatile arsine for determining the arsenic species in natural waters but it does not detect arsonium compounds ($R_4As^+X^-$) and most other organic arsenic compounds that occur in marine organisms, such as AsB, AsC and the arsenosugars. Using alternative analytical methods, so-called "hidden" arsenic species have been detected in natural waters [45-47]. Therefore, there is a need to develop and apply other analytical methods to natural water samples for the determination of additional arsenic species, and this problem has been regarded as one of the main research objectives of this thesis.

Since the first scientific paper published on arsenic in marine organisms [48], it has been known for many years that the arsenic concentrations in marine organisms are

considerably higher than that in the surrounding waters, but there have been no reports of arsenic poisoning from the consumption of seafood, even for the Japanese people who consume a substantial amount of marine organisms as food on a daily basis [49]. The relatively high arsenic levels in marine organisms facilitate the studies on the chemical forms of arsenic in marine environment. A large number of publications including several comprehensive review papers have been published in this area [49-52]. Characteristics and chemical forms of some arsenic in marine organisms are fairly well understood. They are mostly organic in nature and water-soluble [53, 54]. Since its discovery in the western rock lobster [55], arsenobetaine (AsB) has been found to be the main arsenic compound in a wide range of marine animals, including fish [56-77], crustaceans [78-82], mollusks [60, 83-87], and gastropods [88]. Tetramethylarsonium ion (TMA) has been found in the clam *Meretrix lusoria* [86], the gastropod *Tectus pyramis* [89], some species of bivalves [90], and possibly in the NRCC Dogfish Muscle reference material DORM-2 [91] at lower levels. Arsenocholine (AsC) has been found as a minor species in some species of shrimp [79, 80, 92], and even trimethylarsine oxide (TMAO) at low concentrations has been identified as a natural component in some types of marine animals [90, 93]. With the improvement of analytical techniques, there has been more and more evidence of the presence of AsC, TMA, TMAO, MMA, DMA and inorganic arsenic in marine animals [56, 57, 59, 65, 69, 88]. Besides the known arsenic species, unidentified arsenic chromatographic peaks are also observed in the analysis of some marine organisms [56, 57, 59, 60, 64, 67-69, 88, 94, 95]. Hanaoka and co-workers recently reported AsB as the major component together with AsC and TMA as minor components in two kinds of jellyfish [96]. The concentration of arsenic in marine algae is higher than that found in marine water and the main species are identified as a group of dimethylarsinoylribosides and trimethylarsonioribosides, known as arsenosugars [54, 64, 67, 88, 97-102]. It is also known that arsenosugars could be present in bivalves and crustaceans [97]. Recently, some dimethylarsinoylriboside derivatives have been found in the gonads of scallops [103].

Most of the arsenic compounds present in the environment undergo chemical transformation [104]. Many of the transformations are of a biochemical nature. Several biochemical pathways have been proposed to describe the transformation among arsenic

species in the marine environment [51, 52]. However, a complete understanding of the mechanisms of these transformations requires more evidence of the existence of the intermediates, which sometimes may be present at very low concentrations. Therefore, there is a need to develop more accurate and sensitive analytical methods capable of identifying and quantifying a wider range of arsenic species in environmental samples.

Although speciation information is needed to study the bioavailability of nutritionally important elements from dietary sources, the study of the contamination of foodstuff and the prospects for legislation on the basis of speciation for toxic elements are also becoming increasingly important. Until now most of the maximum permissible limits or guideline levels of trace elements in food are given by their total elemental contents. For example, the total daily intake of arsenic via food in Canada, Denmark, UK, and Japan has been estimated at 67, 118, 49, and 985 μg [105-108], respectively. Although these numbers look quite different, the levels of the toxic arsenic species, for example As(III), could be the same. As the toxicology of arsenic is well known with respect to the chemical forms, estimation done on the basis of species should be more practical. Most determinations of arsenic in foodstuffs in the past were for the total arsenic [109-112]; however, more and more attention on speciation of arsenic in foodstuffs, mostly seafood, is being paid in recent years [56, 113-116].

The assessment of exposure to inorganic arsenic, especially As(III), appears to be of much more concern since it is a well-established human carcinogen [117, 118]. Schoof *et al.* [119] recently reported a so-called "market basket survey" of inorganic arsenic in food. In the 40 commodities analyzed, the total arsenic concentration was found to be the highest in the seafood samples (ranging from 160 ng g^{-1} in fresh water fish to 2 360 ng g^{-1} in marine fish on wet basis). The concentration of inorganic arsenic in seafoods was reported as <1 ng g^{-1} to 2 ng g^{-1}. In contrast, the highest inorganic arsenic levels were found in grains and produce, such as, raw rice (74 ng g^{-1}), flour (11 ng g^{-1}), grape juice (9 ng g^{-1}) and cooked spinach (6 ng g^{-1}). Velez *et al.* [120] reported a lower percentage of AsB in canned samples than in fresh samples for several seafood products, namely sardines, cockles, squid and small squid. They also found a lower AsB percentage in frozen or preserved seafood products than in fresh seafood. Further research is then needed to confirm whether AsB is degraded or lost during the

manufacturing process. In order to observe this transformation, sensitive methods for the determination of many arsenic species are required.

1.5 Objectives

The overall objectives of this research project were: (i) to develop analytical methods for arsenic species at trace levels using NAA as a principal detection technique in combination with various separation methods, namely solvent extraction, solid-phase extraction, ion exchange chromatography and HPLC; (ii) to analyze natural water samples for arsenic species at very low levels using these methods; (iii) to analyze fish samples for as many arsenic species possible using the developed methods; and (iv) to develop methods as well as to simultaneously analyze other chemically similar elements, namely antimony and selenium, in natural waters.

A solvent extraction-NAA method and a solid-phase extraction-NAA method were developed mainly for the speciation of inorganic arsenic, antimony and selenium species at ultra low levels in natural waters. These separation techniques were chosen primarily because of their ability to yield higher enrichment factors of the analytes compared to other techniques.

A method using cation exchange chromatography was developed mainly for the group separation and preconcentration of cationic arsenic species from the neutral and anionic species. This method could serve as one of the important steps in the overall speciation scheme of natural waters and fish.

A HPLC method was used to separate As(III), As(V), MMA, DMA, and AsB in water as well as fish samples. Apparently, no one has yet explored the full potentials of the superior separation power of HPLC and the extremely high sensitivity of NAA for the speciation of arsenic. The coupling of HPLC-NAA was studied in depth.

A speciation scheme consisting of the above methods was designed for measuring arsenic species in natural waters. Speciation analyses of selected natural water samples as well as certified reference materials (CRMs) of water origin were performed. Efforts were also set to look for the existence of other (so called "hidden") arsenic species in addition to those already known to exist in natural waters. The expected results obtained

from the water samples were discussed and compared with that reported by other researchers.

Similarly, a speciation scheme for the determination of as many arsenic species as possible in fish samples was designed. Necessary sample pretreatment procedures and a method for the extraction of arsenic from fish using mixed solvents were explored. The determination of arsenic species as well as total arsenic in some Canadian fish samples was performed. Available CRMs of fish origin were used throughout the development of the methods as well as for the speciation analysis. The results obtained were discussed.

The current status of the analytical methods for speciation with emphasis on arsenic is reviewed in Chapter 2. The experimental details are described in Chapter 3. The development of methods for the speciation of arsenic as well as antimony and selenium are discussed in Chapter 4 in four sub-sections on cation exchange chromatography, solvent extraction, SPE, and HPLC. The results of the speciation of arsenic in natural waters and in seafoods are discussed in Chapter 5 and Chapter 6, respectively. The determinations of the inorganic antimony and selenium species in natural waters carried out together with the speciation of arsenic are discussed in Chapter 5 as well. Brief conclusions drawn from this research project and the recommendations for future work are given in Chapter 7.

2. LITERATURE SURVEY

A detailed survey of the literature published on the speciation analysis of arsenic is reviewed here. The separation techniques surveyed include solvent extraction, solid-phase extraction, coprecipitation, open-column ion-exchange chromatography, cryotrapping/thermal desorption, and HPLC. The determination techniques, namely AAS, AFS, ET-AAS, ICP-AES, ICP-MS, HG-AAS, HG-ICP-AES, HG-ICP-MS, electrochemical, and NAA, are also reviewed.

2.1 Solvent Extraction in Speciation Analysis of Arsenic

Solvent extraction, also known as liquid-liquid extraction, is a process of separating chemical compounds using two immiscible liquid phases. It is a simple and efficient separation method for handling large amounts of liquid samples. It can also be used to preconcentrate analytes at the same time. Although most of the solvent extraction methods for the speciation of arsenic are based on the formation of uncharged chemical species by chelation and/or ion association, there have been a few reports on methods based on the formation of relatively small covalent molecules such as As(III) halides. A summary of speciation method for arsenic based on solvent extraction is given in Table 2.1.

Yasui and coworkers [121] described a method in 1978 for the determination of As(III), As(V), and organic arsenic in water samples by solvent extraction. The As(III) species was first extracted into toluene from concentrated HCl solution in the form of $AsCl_3$; As(V) was also extracted by the same procedure after reduction with KI, while organic arsenic remained unaffected in the sample. After back-extraction into water, As(III) and As(V) were determined by HG-AAS. The total arsenic was also determined by HG-AAS after dissolution of the sample and oxidation to As(V); the organic arsenic content was obtained by subtraction of As(III)+As(V) from the total arsenic. This method was applied to algae, shark muscle, and orchard leaves. It was found that 90-100% of arsenic in algae and shark muscle was of organic nature, and all the arsenic in orchard leaves was in the inorganic form. Chappell *et al.* [122] used a similar extraction procedure for the speciation of arsenic in contaminated soil. The inorganic arsenic

Table 2.1. Speciation of Arsenic by Solvent Extraction

Species	Extraction procedures	Detection techniques	Applications	Ref.
As(III), As(V), organic arsenic	As(III): extracted with toluene in concentrated HCl solution and back extracted with H_2O; As(V): extracted by the same procedure after reduced to As(III) by KI.	HG-AAS	Algae, shark muscle, orchard leaves	121
As(III), As(V)	As(III): extracted with toluene in >9M HCl solution and back extracted with H_2O; As(V)+As(III): extracted after reduction of As(V) to As(III) by 50% KI at 60°C for 30 min.	HG-AAS	Soil extractant	122
As(III), As(V)	As(III): extracted with benzene in 10-12M HCl solution and back extracted with H_2O; As(V): extracted after reduction to As(III) by $TiCl_3$ at 60°C for 30 min.	AAS	Waste water	123
substoichiometric separation of As(III), As(V), MMA, DMA	As(III): extracted with benzene in 7M H_2SO_4/1 M NaCl; As(V): extracted with benzene in 7M H_2SO_4/1 M NaBr; MMA: extracted with benzene in 2M H_2SO_4/0.1MNaI/0.1M ascorbic acid; DMA: extracted with benzene in 2M H_2SO_4/0.1MNaI/0.1M $NaHSO_3$.	Isotope-dilution method	Marine organisms	125-127
As(III), Sb(III), As(V), Sb(V)	As(III), Sb(III): extracted with DDTC/$CHCl_3$ at pH 2.5 -4.0; As(V), Sb(V): collected by thionalide cocrystallization.	NAA	Seawater	131

Table 2.1. Speciation of Arsenic by Solvent Extraction (continued)

Species	Extraction procedures	Detection techniques	Applications	Ref.
As(III), As(V), Sb(III) and Sb(V)	As(III) and Sb(III): extracted with APDC/$CHCl_3$ at pH 3-6; As(V) and Sb(V): extracted after reduction to As(III) by $K_2S_2O_3$ and KI at pH 1.	NAA	Natural waters	132
As(III), As(V)	As(III): extracted by APDC/$CHCl_3$ at pH 1.5, As(V)+As(III): extracted after reduction of As(V) to As(III) by $Na_2S_2O_3$.	NAA	Seawater and marine organism	133
As(III), total arsenic	As(III): extracted with DDTC/MIBK in pH 4.6; Total arsenic: analyzed by ET-AAS.	ET-AAS	Marine sediment	135
As(III)	As(III): extracted by benzene after adding TDT solution, and then mixing, stirring, and warming at pH 2.	Isotope-dilution	Standard reference materials	139
As(III)+MMA(III)+ DMA(III), As(V) +MMA +DMA	As(III)+MMA(III)+DMA(III): extracted with DDDC/$CHCl_3$ at pH 5, then back-extracted with NaOH solution.	HG-AAS	Natural waters	150

DDTC: Sodium diethyldithiocarbamate; APDC: Ammonium pyrrolidinedithiocarbamate; TDT: Toluene-3,4-dithiol
DDDC: Diethylammonium diethyldithiocarbamate

species in these samples was leached first by HCl solution then separated using chloroform instead of toluene.

Izawa and coworkers [123] determined nanogram quantities of As(III) and As(V) in the oxidative degradation reaction products of wastewater containing DMA. The As(III) species was extracted first with benzene from highly concentrated HCl solution, and As(V) was extracted by the same procedure after reduction with $TiCl_3$. They concluded that 2 mL of 10% $TiCl_3$ at 60°C for 30 min could completely reduce 10 μg of As(V) to As(III).

Rohr and Michael [124] used HF-H_2SO_4 mixture for dissolution, $TiCl_3$ as the reducing agent, and toluene as the solvent to determine total arsenic in glass and raw materials.

The uses of As(III) bromide and iodide have also been reported. A series of solvent extractions have been applied [125-127] to the substoichiometric separation of As(III), As(V), MMA, and DMA. As(III) was extracted with benzene from a solution containing 7M H_2SO_4 and 1M NaCl, and As(V) with benzene after adjusting the aqueous phase to 7M H_2SO_4 and 1M NaBr. MMA was extracted into benzene after adjusting the solution to 2M H_2SO_4/0.1M NaI/0.1M ascorbic acid, and DMA from a 2M H_2SO_4/1M NaI/0.1M $NaHSO_3$ solution. These methods were applied to the analysis of a macro-algae (*Laminaria religiosa*) sample after acid digestion. However, the main arsenic species found here as DMA could actually be a digestion product of arsenosugars which have been reported to be the main form of arsenic in macro-algae.

Venkaji *et al.* [128] reported the extraction of arsenic as AsI_3 with MIBK for the determination of total arsenic in geological materials, steels and alloys after reduction of As(V) with ascorbic acid.

Tesfalidet and Irgum [129] used a cold trap to concentrate $AsCl_3$ and then re-evaporated it for determination by AAS. This method was applied for the interference-free determination of As(III) in steel alloys with a detection limit of 3.9 μg g^{-1}.

Li and coworkers [130] reported a technique called emulsion liquid membrane (ELM) for the separation of As(III) and As(V). The ELM consisted of surfactant L113A, liquid paraffin stabilizer and kerosene solvent, with HCl solution as the external phase

and KOH solution as the inner phase. As(III) present as $AsCl_3$ permeated through the ELM into the inner phase, whereas As(V) did not.

It has been mentioned before that most of the solvent extraction methods for arsenic speciation are based on the formation of uncharged chemical species by chelation and/or ion association. Dithiocarbamate has been widely employed as a chelating agent. Gohda [131] used diethyldithiocarbamate (DDTC) and NAA to study arsenic and antimony species in seawater. Mok and Wai [132] used ammonium pyrrolidine-dithiocarbamate (APDC) and NAA to determine arsenic and antimony species in sea and river water. Yusof *et al.* [133] also used APDC and NAA for the speciation analysis of inorganic arsenic in seawater and marine organisms.

Chung *et al.* [134] used similar solvent extraction methods for the speciation of Se (IV, VI) and Te (IV, VI) in conjunction with AAS. The AAS measurements were performed directly in the organic solvent. They successfully applied the method to seawater samples [134]. Bernejo-Barreara *et al.* [135] applied a similar method for sediment samples.

Other chelating agents, including ammonium N-nitrosophenyl hydroxylamine (cupferron) [136], dithiozone [137], lactic acid-malachite green [138], and toluene-3,4-dithiol [139], have been used for the solvent extraction separation of either Sb(III) from Sb(V) or As(III) from As(V). Some reductants, such as $K_2S_2O_3$ [133], mixture of $Na_2S_2O_3$ and KI [132, 140], $TiCl_3$ [134], KI in strongly acidic medium [141-143] or together with ascorbic acid [144], and L-cysteine [145-146] have been used to reduce the pentavalent species to trivalent. Similar reduction and extraction procedures have been used for the RNAA determination of total arsenic in some biological certified reference materials and in human autopsy samples of kidney, liver, heart, spleen and hair [141-143].

A speciation analytical method of on-line coupling of solvent extraction and ICP-AES detection has been reported by Garcia *et al.* [147] for the determination of Sb(III) and Sb(V) in spiked seawater samples. Again, APDC, MIBK and KI were chosen as the chelating agent, solvent, and reducing reagent, respectively.

Trivalent methylarsenicals, such as monomethylarsonous acid (MMA(III)) and dimethylarsinous acid (DMA(III)), are relatively unstable compared to their pentavalent

counterparts, namely MMA(V) and DMA(V). The first two compounds are thought to be possible intermediates in the biotransformation of arsenic among various species, where the methylation of inorganic arsenic proceeds through alternating reduction and oxidative methyl group transfer [148, 149]. The trivalent methylarsenicals can be prepared by reduction of their corresponding pentavalent species using H_2S, and they can exist for a considerable time under aerobic conditions. Hasegawa and co-workers [150] described a method to separate As(III), MMA(III), DMA(III) from As(V), MMA(V) and DMA(V) by solvent extraction using diethylammonium diethyldithiocarbamate (DDDC). The three trivalent species were determined by HG-AAS after cold trapping in a chromatographic column packed with Chromosorb. The detection limits of the trivalent species ranged between 0.013 and 0.017 nM. This is the only report claiming the measurement of trivalent methylarsenicals in natural water samples.

Of As(III) and As(V), the latter can form a heteropoly acid called as arsenomolybdic acid which can be quantitatively extracted into MIBK. In combination with an oxidation step of As(III) to As(V) by iodine-iodide solution, Kanke [151] developed a method for the determination of total arsenic by ET-AAS in steel, mineral water and river water samples. This is the only reported example of direct extraction of As(V) as a heteropoly acid.

In addition to the solvent extraction methods, which served to perform the separation of the different species as well as their preconcentration, sensitive detection methods are needed for the determination of very low levels of arsenic species. The NAA, HG-AAS, ET-AAS are the most widely used detection techniques for solvent extraction procedures. Although speciation methods based on solvent extraction suffer from certain limitations, such as the small number of species separated, the large volumes of solvents needed, usage of mixed organic-aqueous solvents, often cumbersome and time-consuming, and possible contamination, they have been extensively used for the separation of inorganic arsenic species in natural water samples because of their high preconcentration factors. In this thesis, methods using solvent extraction and NAA have been developed for the speciation of inorganic arsenic, antimony, and selenium in natural water samples.

2.2 Coprecipitation and Solid-Phase Extraction in Speciation Analysis of Arsenic

Separation as well as preconcentration methods based on coprecipitation and solid-phase extraction are relatively more suitable for NAA determinations than for other methods [152-155] because solid samples can be more conveniently irradiated in a reactor, although the HG-AAS and ET-AAS [156-159], ICP-AES [157, 160] can also be used after elution or dissolution of the species. Coprecipitation and solid-phase extraction methods published in the literature for studying the speciation of arsenic are listed at Table 2.2 and reviewed below.

Van Elteren and Das [161] used dibenzyldithiocarbamate (DBDC) and NAA to determine As(III) and As(V) by coprecipitation. Yusof *et al.* [162] also used DBDC and NAA to measure Se(IV) in marine sediments. Sun and Yang [163] reported a coprecipitation-NAA method for As(III), As(V), Sb(III), Sb(V), Se(IV), and Se(VI) by $Pb(PDC)_2$.

A combination of DBDC coprecipitation and cryotrapping thermal desorption was used by Ritsema [164] to separate As(III), As(V), MMA and DMA followed by their determinations by AAS.

A method of selective separation and preconcentration of As(III) from natural water by flotation technique was reported by Nakashima [156]. Thionalide and a mixture of sodium dodecylsulphate and sodium oleate surfactants were used to collect the precipitate. As(III) was determined by HG-AAS after the precipitate was dissolved in acetone and digested with HNO_3 and $HClO_4$ acids.

Solid-phase extraction (SPE) using modified inert support materials and ion-exchange resins with various chelating agents have been reported for arsenic speciation. Van Elteren and coworkers [157] reported a method of selective preconcentration of As(III). They loaded cetyltrimethyl ammonium-pyrrolidinedithiocarbamate (CTA^+-PDC^-) ion-pairs on a C_{18}-bound silica column. They also converted a strong anion exchange (SAX) resin column from the quaternary ammonium form into the pyrrolidine-dithiocarbamate (PDC^-) form. It was proposed that As(III) was retained in the form of As(III)-trispyrrolidinedithiocarbamate complex, $[As(PDC)_3]$, on the non-loaded part of the C_{18} cartridge or on an unprocessed C_{18} cartridge connected in series with the modified SAX cartridge. As(III) in the form $As(PDC)_3$ was eluted from the stationary phase with

Table 2.2. Speciation of Arsenic by Coprecipitation and Solid-phase Extraction

Species	Extraction procedures	Detection techniques	Applications	Ref.
As(III), As(V)	As(III): flotation of As(III)-thionalide precipitate at pH 6-7.7 with surfactants; As(V): flotation of As(III,V)-$Fe(OH)_3$ precipitate with surfactants.	AAS	Water, seawater	156
As(III)	As(III): extracted by CTA^+-PDC^- modified C_{18}-bound silica or SAX anion exchange columns at pH 3-6.	HG-AAS	Fresh water, sea water	157
As(III)	As(III): extracted by mercapto-modified silica gel at pH 1.5-8.5.	HG-AAS	Seawater	158
As(V)	As(V): extracted by $(C_8H_{17})_2SnCl_2$ modified Chromaton N-AW-HMDS at pH 2.5-3.5.	ET-AAS	Waste water, potable water	159
As(III), As(V)	As(III): precipitated by adding DBDC at pH 2; As(V): reduced by $Na_2S_2O_3$ to As(III) then precipitated by DBDC.	NAA	Reference materials, surface waters	161
As(III), As(V)	As(III): precipitated with DBDC at pH <1.	HG-AAS	Water	164

Table 2.2. Speciation of Arsenic by Coprecipitation and Solid-phase Extraction (continued)

Species	Extraction procedures	Detection techniques	Applications	Ref.
As(III)	As(III): SPE by PU immobilized HMDC at pH 1-5.	ICP-AES, ET-AAS	Spiked seawater	160
As(III), As(V), Sb(III), Sb(V), Se(IV), Se(VI)	As(III), Sb(III), Se(IV): co-precipitated with APDC and $Pb(PDC)_2$ at pH 2; As(V), Sb(V) and Se(VI): after reduction by $TiCl_3$ co-precipitated with APDC and $Pb(PDC)_2$.	NAA	Waters	163
As(III), As(V)	As(III): complexed with DDTP and adsorbed on C_{18}; As(V)+As(III): determined by ET-AAS after reduction by KI/ascorbic acid.	ET-AAS	Waters	165
As(III), As(V)	As(III): complexed with DMPS and adsorbed by C_{18}; As(V): same as As(III) after reduction by L-cysteine.	ET-AAS	Drinking water, air	166
As(III), As(V), DMA, PAS	PAS: SPE by VC* at pH6; As(V): SPE by LaC* at 7; As(III): coprecipitated with APDC and Fe^{3+} and SPE by C*; DMA: SPE by ZrC* at pH 7.	EDXRF	Synthetic water sample	167

DBDC: Dibenzyldithiocarbamate; CTA^+ -PDC^-: Cetyltrimethylammonium - Pyrrolidinedithiocarbamate ion-pairs; HMDC: Hexamethylenedithiocarbamate; PU: Polyurethane foam; DDTP: Diethyldithiophosphate; DMPS: 2,3-dimercaptopropane-1-sulfonate; C*: Activated charcoal; VC*, LaC*, ZrC*: Metal V, La, Zr loaded activated charcoal.

acetone, and subsequently determined by HG-AAS. Arpadjan *et al.* [160] used ammonium hexamethylenedithiocarbamate (HMDC) immobilized on polyurethane foam (PU) for the selective solid-phase extraction of As(III), Sb(III), and Se(IV). The column was then eluted with methanol and MIBK for the determination of the elements by ICP-AES and ET-AAS, respectively.

Howard *et al.* [158] used (3-mercaptopropyl)trimethoxysilane to modify inert silica gel. The reaction of silica gel with this reagent produced a surface mercapto-modified silica gel with a silver-capacity of about 0.9 mmol g^{-1}. This material could be used to selectively remove As(III) from samples that also contained As(V), MMA, and DMA.

Russeva *et al.* [159] employed $(C_8H_{17})_2SnCl_2$ to modify the inert support Chromaton N-AW-HMDS (silanized diatomite, 0.250-0.315 mm). This material selectively and quantitatively retained As(V) at pH 2.5-3.5, while As(III) passed through. The As(V) was then eluted from the column with 2M HCl. Both As(III) and As(V) were determined by ET-AAS.

Pozebon *et al.* [165] separated As(III) from As(V) by complexation with ammonium diethyldithiophosphate (DDTP) followed by sorption of As (III) on a C_{18}-bonded silica column and reported an ET-AAS detection limit of 0.15 μg L^{-1} for As(III) in water. Hsieh *et al.* [166] also used a C_{18}-bonded silica column but a different chelating agent, namely 2,3-dimercaptopropane-1-sulfonate (DMPS) to separate these two species. They reported the detection limits of 0.11 for As(III) and 0.15 μg L^{-1} for As(V) in drinking water.

Latva *et al.* [167] developed a 4-step separation method for As(III), As(V), DMA, and phenylarsonic acid (PAS) using metal-loaded activated charcoal as the solid support, APDC as the chelating agent, and EDXRF as the detection technique. They evaluated Al, Cr(III), Fe, Hf, Mn, W, Ce, La, Ti, V, In and Zr as matrix modifiers. The detection limits for all arsenic species were reported to be 0.02 mg L^{-1} which is not low enough for the analysis of natural water samples.

Yan *et al.* [168] used APDC on a knotted reactor made from a PTFE tube (1500 mm × 0.5 mm i.d.) and ICP-MS for the determination of As(III) and As(V) with detection limits of 0.021 and 0.029 μg L^{-1}, respectively.

Other SPE systems have also been reported in the literature. For example, Garbos *et al.* [169] selectively extracted Sb(III) from Sb(V) at pH 10 on a Polyorgs 31 sorbent containing mainly amidoxime and amine functional groups. They reported a preconcentration factor of about 50 (for a sample volume of 200 mL) and a detection limit range of 30-34 ng L^{-1} for tap water and snow samples. For the separation of Se(IV) and Se(VI), activated alumina [170], SAX anion exchanger [171], and Rhodamine B immobilized on a lipophilic Sephadex LH-20 gel [172]. Gomez-Ariza [171] also used a C_{18} column to separate dimethylselenide (DMSe), dimethyldiselenide (DMDSe), diethylselenide (DESe), and diethyldiselenide (DEDSe) using GC-MS.

In addition to the efficiency of SPE as a separation method for arsenic, antimony and selenium species, it is highly suitable for NAA because of the final solid form of the sample. Compared to solvent extraction, SPE requires less time for the separation and does not use organic solvents. In this thesis, a SPE method using DBDC as the chelating agent impregnated on XAD-4 has been developed for the separation of inorganic arsenic, antimony and selenium species.

2.3 Hydride Generation, Cryotrapping and Thermal Desorption in Speciation Analysis of Arsenic

One of the most widely used techniques for the separation of arsenic species is hydride generation (HG) followed by cryotrapping (CT) and thermal desorption (TD) of the hydrides. The most commonly used determination technique is AAS; other techniques such as AES, AFS, ICP-AES, ICP-MS, MS, GC-ECD, GC-FID, GC-MS, NAA, and spectrophotometry have also been applied. Since the first paper published by Braman and Foreback [173] on the separation of methylated arsenic species, namely MMA and DMA, in natural waters by HG-CT-AES, this method has been modified and used extensively in various types of samples. Howard [174] recently published a comprehensive review paper on (boro)hydride techniques in trace element speciation.

The HG-CT-AAS technique is generally used for the separation, preconcentration and on-line measurements of As(III), As(V), MMA, DMA, and TMAO. As(III) can be separated from the other arsenic species by adjusting the pH in the range of 4-6, adding $NaBH_4$, cryotrapping and re-evaporating the arsine produced. As(V), MMA, DMA, and

TMAO, on the other hand, can be reduced to arsine and corresponding methylated arsines at pH 1. The separation of the individual hydride species is carried out by passing them through a U-trap packed with a GC stationary phase and immersed in liquid nitrogen. When liquid nitrogen is removed, and the arsines pre-concentrated on the U-trap are re-evaporated by heating; the various species are separated according to their boiling points (AsH_3: -55°C; CH_3AsH_2: 2°C; $(CH_3)_2AsH$: 36°C; $(CH_3)_3As$: 55°C). A typical hydride generation system consists of a reduction vessel or a continuous flow injection unit for hydride formation, a trap to remove water, and a cryotrap fitted with a coil heater and containing a chromatographic stationary phase for the collection and separation of various arsines.

Andreae [39, 41] reported a method for the determination of As(III), As(V), MMA and DMA in natural waters using HG-CT-AAS and GC-FID as well as GC-ECD. The detection limits of this method were several ppb in natural waters. Shaikh and Tallman [175] used a bigger reaction vessel to obtain absolute detection limits of 1, 15, and 10 ng for inorganic arsenic, MMA and DMA, respectively. The respective RSD were 2%, 5%, and 4% at the 2 ppb level.

Van Cleuvenbergen and coworkers [176] evaluated many factors that could influence HG-CT-AAS measurements. These included pH of the reaction mixture, volume and concentration of $NaBH_4$ solution, carrier gas flow rate and collection time, various parameters for the collection trap, atomization support gases and cell parameters, and interferences form inorganic substances present in the sample. The transition metal ions at high oxidation states showed some serious interference which was masked by EDTA. The method was applied to natural waters with detection limits in the ppb range.

The $NaBH_4$ technique does not reduce compounds such as AsB, AsC, TMA, *etc.* Hence it can be effectively used for the measurement of other arsenic species such as As(III), As(V), MMA, DMA, and TMAO in presence of the above compounds in samples such as urine [177] and in marine organisms [178]. Recently, a HG-CT-AAS method was reported for the determination of methylated antimony species in some plant materials [179]. The HG-CT technique has also been in conjunction with gas-phase diode array molecular absorption spectrometry [180, 181] for the simultaneous determination of As(III), Sb(III) and Se(IV).

The hydride generation unit widely used nowadays is a continuous flow system in which a reaction cell is employed to mix the sample solution, a solution to adjust pH, and a $NaBH_4$ solution [178]. Reproducibility was reported to improve by this technique.

The reduction of certain arsenic species with L-cysteine was found to enhance analytical performance [182-184]. Le and coworkers [182] suggested that the use of L-cysteine could be helpful for the determination of total arsenic using one species as the standard. They also concluded [183] that the interference from other transition metals and hydride-forming elements could be substantially reduced by this procedure. Since a lower acidity is required for hydride generation in presence of L-cysteine, the acid blank can be lowered. Howard and Salou [184] found that thioglycollic acid could be as effective as L-cysteine for obtaining equal arsine yields from different arsenic species, and that the rate of reaction is faster than that of L-cysteine [184].

In HG-AAS, the commonly used reducing reagent tetrahydroborate, $NaBH_4$ or KBH_4, is not very stable. It is only stable for a few days even when prepared in alkaline solution and stored under refrigeration. The reagent itself is a source of contamination. Furthermore, the hydride generation using tetrahydroborates suffers from interference by transition metals. In order to overcome these problems, electrochemical reduction has been proposed [185, 186]. Pyell *et al.* [185] reported the selective determination of As(III) and Se(IV) using fibrous carbon as the cathodic material in an electrochemical hydride generation cell. Ding and Sturgeon [186] used lead as the cathode material in an electrochemical cell, and found that both Sb(III) and Sb(V) were equally converted into their hydrides with 92±4% efficiency.

Haywood and Riley [187] used $NaBH_4$ and then a trap containing I_2-KI solution to absorb arsine. They determined total arsenic and As(III) spectrophotometrically using the molybdenum blue method and reported a detection limit of 0.14 ppb. Crecelius [188] introduced three additional traps for absorbing H_2O, CO_2 and H_2S and used dc arc emission spectrometry for As(III), As(V), MMA and DMA in diverse environmental samples.

Van Elteren *et al.* [189] made a Teflon-lined stainless steel trap for rapid cooling and heating and it was packed with 10% OV-3 on Chromosorb W-AW DMCS (30-60 mesh). An on-line quartz tube atomizer was used for AAS detection. In this paper the

authors reconfirmed the formation and trapping of the MMA and DMA by using radioactive labeled compounds and collected them on active charcoal with a device connected to the hydride trap.

Other detection techniques such as AFS, ICP-AES [190], ICP-MS [191], GC-MS, and NAA have also been used in combination with hydride generation. Featherstone *et al.* [192] used AFS and reported detection limits for arsenic species comparable to that of AAS. Santosa and coworkers [40, 193] reported a HG-CT-ICP-MS method for the separation of inorganic arsenic, MMA and DMA in seawater with detection limits of 9.2, 0.2 and 0.7 ng L^{-1}, respectively.

Odanaka *et al.* [194] used an n-heptane trap in dry ice-acetone cold bath (-80°C) to collect the hydrides followed by GC-MIDMS (multiple ion detection mass spectrometry). They utilized both retention time and mass data to identify inorganic arsenic, MMA, DMA and TMA in soil and plant extracts, urine, and river water with detection limits of 0.2-0.4 ppb. Kaise and coworker [54] reported a method for the speciation of arsenic in marine organisms by on-line coupling of HG-CT-GC-MS. Their results showed that AsB was converted to TMAO and the ribosyl arsenic compounds to DMA by NaOH digestion. They measured the levels of inorganic arsenic, MMA, DMA, AsB and ribosyl arsenic compounds. Dodd *et al.* [195] also used both retention time and mass spectral data to identify hydride-forming antimony species in extracts of a freshwater plant.

Mester *et al.* [196] developed a GC-MS method and found it to be comparable to a HPLC-HG-AFS method for the determination of MMA and DMA in urine samples with detection limits of 0.8 and 0.95 μg L^{-1}, respectively. Ebdon *et al.* [197] also compared GC and HPLC methods with AAS, AFS, and ICP-AES detection systems. They concluded that the HG-CT-GC-AAS method had the lowest detection limits for arsenic species.

The trapping of arsine has been used in a radiochemical NAA (RNAA) method for the determination of low levels of arsenic in biological tissues by Orvini and Delfanti [198]. They digested the irradiated samples using concentrated HNO_3 and H_2SO_4 acids and then converted arsenic into arsine by adding KI and $SnCl_2$ solutions, and metallic

zinc. Finally, the arsine was trapped in $AgNO_3$ for counting. The method was applied to marine organism, in which arsenic was thought to be mainly in the AsB form.

The HG-CT separation technique is widely used for the speciation of arsenic. However, there are a few disadvantages associated with this technique. Firstly, it is obvious that HG-CT can be used to analyze only the hydride-forming species which include five species, namely As(III), As(V), MMA, DMA and TMAO. Several other important arsenic species, such as AsB, AsC, arsenosugars, and TMA do not form hydrides; hence, they cannot be directly analyzed by HG-CT. Secondly, the hydride generation reaction can suffer from the rearrangement of the methyl groups. For example, Talmi and Bostick [199] and Feldman [200] reported that at a higher acidity and in presence of dissolved oxygen, the reduction of MMA resulted in the partial production of AsH_3, $(CH_3)_2AsH$, and $(CH_3)_3As$; the reduction of DMA produced small amounts of $(CH_3)AsH_2$ [199, 200]. These side reactions can be minimized by controlling the experimental conditions. It might become serious as the matrix of the sample becomes more complicated. It has also been reported that the HG-CT technique can suffer interference from transition metals. One has to refer to the maximum tolerant concentrations of these interfering elements for reliable measurements. In addition to the above potential problems, the volatile arsenic hydrides must be absorbed on a solid non-interfering matrix such as activated charcoal before they can be irradiated in a reactor for NAA. For these reasons, the HG-CT technique was not considered as a preferred separation method in this thesis.

Some examples of the speciation of arsenic by hydride generation are listed in Table 2.3.

2.4 Ion-Exchange Chromatography in Speciation Analysis of Arsenic

Selected applications of open column ion-exchange chromatography for the speciation of arsenic are given in Table 2.4. Both cation and anion exchange chromatographic methods have been applied to the separation and preconcentration of arsenic species because the naturally occurring arsenic can exist as cationic, anionic or neutral species. The four arsenic species, namely arsenous acid, arsenic acid, MMA and DMA, found in natural waters are weak acids. The dissociation constants (pK_a) of these

Table 2.3. Speciation of Arsenic by Hydride Generation, Cryotrapping and Selective Volatilization

Species	Hydride generation method	Cryogenic trapping method	Detection techniques	Applications	Ref.
As(III), As(V), MMA, DMA	1.2 mL of 4% $NaBH_4$ at pH 6 for As(III); 2 mL of 4% $NaBH_4$ at pH 1 for As(V), MMA, DMA.	U-trap packed with glass wool, GC column packed with silicone oil DC-550 on Chromosorb WAW DMCS.	AAS, FID	Natural waters, marine algae	39, 41
As(III+V), MMA, DMA	4% $NaBH_4$, 1M HNO_3.	15% OV-3 on Chromosorb WAW DMCS (60-80 mesh).	ICP-MS	Seawater	40
As(III+V), MMA, DMA	2% $NaBH_4$, 10 % HCl.	HF etched glass beads.	AAS	Natural water	43
As(III+V), MMA, DMA	2% $NaBH_4$, 9M H_2SO_4.	3% OV-101 on Chromosorb WAM-DMCS	AAS	Natural waters	44
As(III+V), MMA, DMA	3% $NaBH_4$, 1.2 M HCl.	15%OV-3 on Chromosorb WAM-DMCS.	AAS	Natural waters	47
As(III), MMA, DMA, TMAO.	Continuous flow of 0.6M HCl and 2% of $NaBH_4$ at a speed of 6 mL min^{-1} to sample.	3% silicone OV-17 on CHRW-AW DMCS (80-100 mesh).	GC-MS	Marine organism	54

Table 2.3. Speciation of Arsenic by Hydride Generation, Cryotrapping and Selective Volatilization (continued)

Species	Hydride generation method	Cryogenic trapping method	Detection techniques	Applications	Ref.
As(III+V), MMA, DMA, TMAO	6 mL of 4M HCl, 2 mL methanol, and 3 mL of 10% $NaBH_4$ for 50 mL sample.	5% PEG-20M coated on Chromosorb 101 (80-100 mesh).	MS	River water, soil, plant extracts	125
As(III+V), MMA, DMA	6% $NaBH_4$, HCl (pH<1).	3% SP-2100 on Chromosorb G NAW.	AAS	Natural waters	164
As(III), As(V), MMA, DMA	pH 4-9 for As(III), pH 1 for As(V), MMA and DMA.	U- trap packed with glass beads.	AES	Waters, eggshells	173
As(III), As(V), MMA, DMA	pH 0 for As(III+V), MMA, and DMA; pH 5 for As(III).	Pyrex U-tube filled with glass beads (60-80 mesh).	AAS	Natural water	175
As(III), As(V), MMA, DMA	4% $NaBH_4$, pH 7.5 for As(III), 0.2M HCl for As(V), MMA and DMA	10% poly-m-phenylether on Chromosorb WAW DMCS (80-100 mesh).	AAS	Waters	176
As(III+V), MMA, DMA	0.06M HCl for As(III+V), MMA and DMA.	10% polyphenylether [5rings(PPE)] on Chromosorb DMCS (100-120 mesh).	AAS	Urine	177

Table 2.3. Speciation of Arsenic by Hydride Generation, Cryotrapping and Selective Volatilization (continued)

Species	Hydride generation method	Cryogenic trapping method	Detection techniques	Applications	Ref.
As(III+V), MMA, DMA	2% $NaBH_4$, 1M HCl and EDTA solution.	U-trap packed with glass beads.	AAS	Water, marine organism.	178
As(III), Sb(III), Se(IV)	Continuous flow of 4% $NaBH_4$ (4 mL min^{-1}) and analyte in 0.5 M HCl (35 mL min^{-1}).	Glass U-tube filled with glass beads.	SP	N/A	180
DMA	Continuous flow of 3% $NaBH_4$ (17 mL min^{-1}), 750 mL sample (0.25M HCl).	U-trap packed with glass beads.	SP	N/A	181
As(III), As(V)	4% $NaBH_4$, 0.08M H_2SO_4.	0.25% I_3^{-1} solution.	SP	Seawater, potable water	187
As(III), As(V), MMA, DMA	Continuous flow of sample (4.45 mL min^{-1}), 2.5% $NaBH_4$ (4.73 mL min^{-1}), and 2M HCl solution to a reaction coil.	Teflon-lined stainless tube filled with 50g 10% OV-3 on CHRW-AW DMCS.	AAS	Sediment interstitial water	189
As(III), As(V), MA, DMA	Deliver 2% $NaBH_4$ solution into 10 mL of sample acidified as 0.18M H_2SO_4.	U-trap with 3% OV-101 on Chromosorb WAW-DMCS.	AFS	Seawater	192

Table 2.3. Speciation of Arsenic by Hydride Generation, Cryotrapping and Selective Volatilization (continued)

Species	Hydride generation method	Cryogenic trapping method	Detection techniques	Applications	Ref.
As(III+V), MMA, DMA, TMAO	3 mL of 10% $NaBH_4$ reacted with 50 ml sample (acidified as 0.36M HCl)	3-5 mL n-heptane cooled at -80°C by a dry ice acetone bath.	GC-MS	Water, urine, soil and plant extract.	194
Total arsenic, As(III)	2% $NaBH_4$ and pH 5 for As(III); 2% $NaBH_4$ for As(III)+As(V) after reduction by 3 mL HCl and 1 mL of 5% KI.	No trap.	AAS	Soil and river sediments.	201
As(III), As(V), MMA, DMA	3% $NaBH_4$, pH 6 for As(III), 2M HCl for As(V), MMA and DMA.	40-cm U tube packed with 10% SE-30 on Supelcoport (80-100 mesh).	AAS	Natural waters	202
As(III), As(V), MMA, DMA and TMAO	4% KBH_4, pH6 for As(III), pH1 for As(V), MMA, DMA, TMAO.	U-tube partly filled with 15% OV-3 on Chromosorb WAW DMCS (60-80 mesh).	AAS	Marine interstitial water	203

Table 2.3. Speciation of Arsenic by Hydride Generation, Cryotrapping and Selective Volatilization (continued)

Species	Hydride generation method	Cryogenic trapping method	Detection techniques	Applications	Ref.
As(III), As(V), MMA, DMA	2% $NaBH_4$, 1% citric acid buffer for As(III), 2 M HNO_3 for As(V), MMA and DMA.	A PTFE coil knotted and sealed into a tube cooled by L-N_2.	AAS	Waters	204
As(III), As(V), MMA, DMA	3.7×10^{-2}M KBH_4; 4.0M HCl for As(III); 0.165M HCl with 1 mg L^{-1} $KMnO_4$ for MMA+DMA; 0.025M HCl with 1 mg L^{-1} $KMnO_4$ for MMA and DMA by different sensitivity; 0.85M tartaric acid with 1 mg L^{-1} $KMnO_4$ for all species.	No trap.	AAS	Standards	205
Total arsenic, toxic arsenic	HG with and without $K_2S_2O_8$ and microwave decomposition.	No trap.	AAS	Urine	206
As(III), As(V), MMA, DMA	2 % $NaBH_4$.	Glass beads.	AAS, and ICP-AES	Standard	207, 208

Table 2.4. Speciation of Arsenic by Open Column Ion Exchange Chromatography

Species	Columns	Mobile phases	Detection techniques	Applications	Ref.
As(III)+As(V)+ MMA, AsC+TMA, AsB+DMA,	Dowex 50W×8.	pH 1.6 for As(III)+As(V)+MMA; 4M ammonia for AsB+DMA; and 4M HCl for AsC+TMA.	ET-AAS, HG-AAS	Riverine water CRM	46
As(III), As(V), MMA, DMA	BDH SRA 70	pH 5.5 buffer for As(III) and DMA, pH 4.0-4.2 buffer for MMA and As(V).	HG-AAS	Sediment, interstitial water, and aquatic plants	209
As(III), As(V)	Dowex 1×8.	0.12M HCl.	ET-AAS	Estuarine sediments	210
As(III), As(V), MMA	AG50W×8 (100-200 mesh)	Water.	Colorimetry, AAS, and potentiometry	Purification of synthetic MMA	211
As(III), As(V), DMA	AG50W×8 (100-200 mesh)	Water and ammonia solution.	Colorimetry, AAS, and potentiometry	Purification of synthetic DMA	211

Table 2.4. Speciation of Arsenic by Open Column Ion exchange Chromatography (continued)

Species	Columns	Mobile phases	Detection techniques	Applications	Ref.
Inorganic arsenic, MMA, DMA	Dowex 50W×8.	0.5M HCl for inorganic arsenic, water for MMA, 1.5M ammonia for DMA.	HG-AAS	Marine algae, mollusk tissue, sediments	213
DMA	Dowex 50W×8.	0.1 M H_3PO_4 for inorganic As, 0.02M ammonia for MMA, 1M ammonia for DMA.	ET-AAS	Seawater	214
As(III)+As(V), MMA, DMA	AG50W×8.	0.5M HCl for As(III)+As(V); H_2O for MMA; 0.5% ammonia for other form; 2% ammonia for DMA.	HG-AAS	Urine	215
DMA, As(III), As(V), MMA	Dowex 50W×8 and AG1×8.	0.02M HOAc followed by 1.0M NaOH for DMA on cation exchange column, 0.01M and 1.0M HOAc/OAc^- buffer (pH 4.7) for MMA on anion exchange column.	Polarographic analysis	CRM, ash basin slurry samples	216

Table 2.4. Speciation of Arsenic by Open Column Ion exchange Chromatography (continued)

Species	Columns	Mobile phases	Detection techniques	Applications	Ref.
As(III), As(V), DMA, MMA	Dowex 50W×8 and AG1×8.	0.02M HOAc followed by 1.0M ammonia for DMA on cation exchange column, 0.01M and 0.5M HOAc/NH_4OAc buffer (pH 4.7) for MMA on anion exchange column.	ET-AAS	Standard	217
As(III), As(V), MMA, DMA	AG50W×8 and AG1×8 in series.	0.006M CCl_3COOH (pH 2.5) for As(III) and MMA, 0.2M CCl_3COOH for As(V), 1.5M ammonia followed by 0.2M CCl_3COOH for DMA.	ET-AAS	Sediment, interstitial water, and natural water	218
As(III+V), MMA, DMA, AsB	Dowex 50W×8 and Dowex 1×8.	0.5M HCl for inorganic arsenic, water for MMA, 0.75M ammonia followed by 3.0M ammonia for AsB and DMA mixture on cation exchange column; 12.5mM phosphate buffer (pH 8) for AsB and DMA on anion exchange column.	INAA	NRCC CRM (DORM-1)	219

Table 2.4. Speciation of Arsenic by Open Column Ion exchange Chromatography (continued)

Species	Columns	Mobile phases	Detection techniques	Applications	Ref.
As(III+V), MMA, DMA, AsB, TMA	Dowex 50W×8 and Dowex 1×8.	0.5M HCl for inorganic As; water for MMA; 0.75M and 3.0 M ammonia for AsB and DMA mixture; 0.5M and 6M HCl for TMA on cationic exchange column; 12.5 mM phosphate buffer (pH 8) for AsB and DMA on anion exchange column.	NAA and HG-AAS	Fresh and marine mussels, fish, sea-gull eggs, CRM	220
As(III), As(V), MMA, DMA	Dowex 1×8 and Dowex 50W×8.	1.5M ammonia followed by HCl solution for As(III), HCl solution followed by H_2O for As(V) and MMA.	HG-AAS	Marine sediment, tap water and seawater	221-223

Table 2.5. pK_a values of Some Weak Acids of Arsenic

Compounds	pK_a
Arsenous acid (As(III), H_3AsO_3)	pK_1=9.2
Arsenic Acid (As(V), H_3AsO_4)	pK_1=2.2, pK_2=6.9, pK_3=11.5
Monomethylarsonic acid [MMA, $(CH_3)AsO(OH)_2$]	pK_1=4.1, pK_2=9.1
Dimethylarsinic acid [DMA, $(CH_3)_2AsO(OH)$]	pK_1=6.3

acids vary from 2.2 to 11.5 as shown in Table 2.5, which indicate that they can possibly be separated by anion exchange chromatography.

Aggett and Kadwani [209] reported a relatively straightforward two-stage anion exchange method. In the first stage, As(III) and DMA were eluted in succession with a buffer at pH 5.5 from a 25cm column packed with Zerolit SRA 70 anion-exchange resin. The separation of MMA and As(V) was then carried out by elution with a buffer at pH 4.0-4.2. This method was applied to the analysis of interstitial waters and aquatic plants. Ficklin [210] used Dowex 1×8 (100-200 mesh) and 0.12M HCl to separate As(III) and As(V) in sediments. Both arsenic species were determined by ET-AAS.

Some arsenic species, such as TMA and AsC, are cationic and some species, such as AsB and DMA, act as cations in acidic and neutral solutions, although AsB has the zwitterionic nature and DMA is a weak acid. The reason for DMA to be cationic is thought to be the protonation of the compound under acidic conditions [211]. The behavior of As(III), As(V), MMA and DMA with a strongly acidic cation exchange resin was first investigated by Dietz and Perez [211]. They used Bio-Rad AG 50W-×8 resin and water as the eluting agent to separate inorganic arsenic in MMA samples, and MMA and inorganic arsenic in DMA samples. These methods were also used for the purification of MMA and DMA samples. Elton and Geiger [212] used cation exchange chromatography to overcome peak overlap in differential pulse polarograms of MMA and DMA.

In 1981, Maher [213] developed a method for inorganic arsenic, MMA and DMA using HG-AAS in combination with cation exchange column chromatography. These three species were separated by sequentially eluting with 0.5M HCl, water, and 2.5M NH_3 from a column packed with Dowex 50AG-x8 resin. They reported relatively high levels of DMA in marine algae (*laminaria, cystoseira tamariscifolia* and *halidrys siliquosa*) which might be due to the use of vigorous extractions by 0.1M NaOH solution. Kaise and coworkers [54] investigated the decomposition of ribosyl arsenic compounds (known as arsenosugars) and AsB into DMA and TMAO. They concluded that AsB was converted to TMAO, and dimethyl(ribosyl)arsine oxides decomposed to DMA under hot 2M NaOH.

Persson and Irgum [214] employed cation exchange chromatography to isolate DMA from seawater samples and ET-AAS for the determination. Blas *et al.* [215] used cation exchange and HG-AAS to separate inorganic arsenic, MMA and DMA in urine samples.

Sturgeon and coworkers [46] developed a method for the quantification of arsenic species in the NRCC SLRS-1 Riverine Water CRM. The procedure involved the following steps: (1) direct HG-AAS determination of As(III)+As(V)+MMA+DMA; (2) UV photolysis, oxidation, and HG-AAS determination of a total of seven species: As(III)+ As(V)+MMA+DMA+AsB+AsC+TMA; (3) adsorption of organically bound arsenic on a C_{18} column, followed by elution and ET-AAS determination; and (4) a cation exchange column separation involving several steps of HG-AAS and ET-AAS determinations of some arsenic species. It was concluded that As(III), As(V) and DMA accounted for 71% of the total arsenic. Negligible amounts (<5%) of AsC, TMA, MMA and organically bound arsenic were present. A significant fraction of arsenic (calculated 22%) existed as relatively inert unidentified arsenic species.

A combination of cation and anion exchange chromatography can provide useful information on both types of species. Henry and Thorpe [216] developed a method for separating DMA by cation exchange and MMA by anion exchange followed by their determinations by differential pulse polarography (DPP). They used DPP directly for measuring As(III), and As(V) after reduction. Pacey and Ford [217] employed a cation

exchange column for the separation of DMA and an anion exchange column for both MMA and As(V).

Grabinski [218] packed a 9-cm column with anion exchange resin (AG1-x8) and then placed a 26-cm of cation exchange resin (AG50W-x8) on top of it. He used an elution sequence of 0.006M CCl_3COOH to yield As(III) first and then MMA, 0.2M CCl_3COOH to give As(V), and finally 1.5M ammonia solution followed by 0.2M CCl_3COOH to yield DMA.

Slejkovec and coworkers [219, 220] reported the determination of several arsenic species by cation and anion exchange chromatography followed by NAA.

Soto and coworkers [221-223] used anion exchange to separate As(III) from As(V), MMA and DMA, and cation exchange for As(V) and MMA. They determined the levels of total arsenic, As(III), As(V), and MMA by HG-AAS, and then calculated the level of DMA by difference. They applied the method to tap water [221], seawater [222], and marine sediment [223].

It is convenient and inexpensive to use open column ion exchange chromatography for the separation of arsenic species. However, the low column efficiency due to large and less uniform resin particles leads to the use of large volumes of eluting agents. This could cause higher blanks and further restrict usage, in particular to ultra low-level analysis. An open column cation exchange chromatographic separation method for group separation of the cationic arsenic species from the neutral and anionic species has been developed in this thesis. This method has been found to be convenient and suitable as a pre-separation step for the HPLC-NAA method developed for the speciation of arsenic in natural water and fish samples.

2.5 Speciation of Arsenic by High-performance Liquid Chromatography

The HPLC technique, with its diversity of both stationary and mobile phases, can be used to separate arsenic species. As shown in Fig. 1.1, the arsenic species of interest are either anionic (*e.g.* As(III), As(V), MMA, and DMA), cationic (*e.g.* AsC, TMA, trimethylarsonioribosides), zwitterionic (*e.g.* AsB), or neutral (*e.g.* TMAO and dimethylarsinylribosides) in nature under normal conditions. Reversed-phase C_{18}, cation exchange and anion exchange columns have all been used to separate various arsenic

species by HPLC. In reversed-phase ion-pair chromatography, arsenic species can be combined with ion-pair reagents to form hydrophobic analyte-counter ion pairs which can then be separated using a non-polar stationary phase and a polar mobile phase. Selection of ion pair reagents, pH of the mobile phase and other experimental conditions depend on the arsenic species to be separated. Although theoretically ion-pair HPLC can be used to separate anionic, cationic, zwitterionic, and neutral species, in practice the separation by one column is not an easy task.

Le *et al.* [224] reported a separation of As(III), As(V), MMA, DMA and AsB on a C_{18} reversed-phase column using 10 mM sodium heptanesulfonate at pH 3.5. In another paper published by the same group [98], AsC and TMA in addition to the above five arsenic species were separated on the same C_{18} column using a mobile phase consisting of 10 mM hexanesulfonate, 1 mM tetraethylammonium hydroxide and 0.5% methanol at a column temperature of 70°C. Beauchemin *et al.* [62] reported that sodium dodecylsulphate was a suitable ion-pair reagent for the separation of AsB and DMA in biological samples containing high levels of salts. A reversed-phase polymeric resin (PRP-1) was reported to give good separation of arsenic species as well [68, 95, 225-228]. Six arsenic species were separated by 0.5 mM tetrabutylammonium phosphate at pH 9.0 [95, 228] and seven arsenic species were separated using 10 mM 2-hydroxy-5-sulphobenzoic acid at pH 3.5 [227]. Ding *et al.* [225] used a micellar mobile phase consisting of 50 mM cetyltrimethylammonium bromide, 10% propanol and 20 mM borate buffer at 40°C in a micellar liquid chromatography (MLC) method to separate As(III), As(V), MMA and DMA species in a urine sample.

The use of an anion exchange column in HPLC to separate some arsenic species is logical and practical. The four anionic arsenic species are weak acids and their pK_a values are shown in Table 2.3. They have different retention times on an anion exchange column, arsenite having the shortest time.

HPLC methods using only anion exchange columns cannot generally be employed to satisfactorily separate most cationic species, such as AsB, AsC and TMA [56, 66, 224, 229-231]. Similarly, cation exchange columns cannot be used to obtain a good separation of the anionic species, such as As(III), As(V) and MMA [56, 94, 229, 232]. Consequently, some researchers [56, 64, 88, 229, 233] used two columns, one

anion and one cation exchange, to separate most of the cationic and anionic arsenic species. Han and coworkers [234] alternatively employed a cation exchange and an anion exchange column in series. The separation of AsB from As(III) appears to be more difficult than other species by HPLC with an anion exchange column. This is particularly true for samples of marine organisms in which the concentration of AsB is usually much higher than that of As(III) [224, 230, 231]. Methods based on microwave digestion [224], thermal oxidation [230] and ultraviolet photooxidation [59, 60, 231, 235] have been reported to distinguish As(III) from AsB. Boucher *et al.* [236] used an amperometric detector for As(III) and an UV detector for both AsB and As(III) to distinguish AsB from As(III) on a C_{18} column.

Lopez *et al.* [237] separated AsB from As(III) by placing an additional anion cartridge before the analytical anion exchange column to pre-separate the cationic species from the anionic ones. Demesmay *et al.* [238] and Mckiernan *et al.* [67] improved the separation between AsB and As(III) using a gradient elution on an anion exchange column (PRP-X100). In addition, Mattusch and coworkers [65, 239] used the Ion Pac AS7 column (also an anion exchange column) and gradient elution at pH 1.3 and pH 3.3 to separate arsenic species.

Highly sensitive element-specific detectors, including ET-AAS, HG-AAS, ICP-AES, ICP-MS, AFS, and ESI-MS, have been used in HPLC. Brinckman and coworkers [240] utilized two interfaces to couple HPLC with ET-AAS. The first interface was a PTFE flow-through cell, the effluent from which was periodically sampled and injected into a graphite furnace in a pulsed-mode operation. In the second interface, termed survey-mode, the effluent was collected by an auto sampler and each fraction was analyzed by ET-AAS. Stockton and Irgolic [241] pointed out that this is not a truly "on-line" analysis system. However, its high sensitivity and the capability for determining several arsenic species has lead to its applications to natural waters by Han *et al.* [234] and to marine organism by Hanaoka *et al.* [96].

Hansen *et al.* [229] used a vented capillary tube for coupling HPLC with hydrogen-argon flame AAS. The venting balanced the difference between the HPLC flow rate (1 mL min^{-1}) and the AAS nebulizer uptake rate (5 mL min^{-1}). They evaluated five columns for their suitability. The four anionic species, namely As(III), As(V), MMA

and DMA, were separated well on the anion exchange column Merck Polysphere IC AN. Three cationic species, namely AsB, AsC and TMA, were resolved well by the cation exchange column Chrompack Ionospher-C. Han *et al.* [234] employed a combination of one Elite AS3 bifunctional ion-exchange column and one Dionex CAS 1 anion exchange column in series to separate seven cationic and anionic arsenic species.

Momplalsir *et al.* [57] described an improved interface for the on-line AAS detection of arsenic and selenium species in HPLC column eluate. This eluate was combusted in a hydrogen/oxygen atmosphere of a heated pyrolysis chamber in a fused silica T-tube. Finally, the pyrolytical products were carried into an optical tube of AAS. The detection limits were in the range of 0.6-1.0 ng for arsenic and 1.0-2.0 for selenium species.

The HPLC methods can be combined with other methods to enhance sensitivity. HPLC followed by hydride generation also allows measurements to be done on-line [97, 233, 242-246]. The main disadvantage of this combination is the inability to determine non-hydride-forming species, such as AsB, AsC and TMA. For these species, microwave assisted thermal digestion [58, 97, 224, 230, 237, 247, 248], UV photolysis digestion [249-251], and a combination of them [231, 232] have been employed.

Several researchers [244, 233, 252, 253] have evaluated HPLC-ICP-AES methods where the eluate from HPLC is directly injected to ICP-AES. Like AAS, the sensitivity can be improved by inserting hydride generation between HPLC and ICP-AES [254-256]. However, the non-hydride-forming species would not be detected. Attempts have been made to include an on-line UV photolysis step [59, 60, 235].

The ICP-MS is also a powerful technique for speciation analysis of trace elements preceded by HPLC separation. This is because ICP-MS has multielement capability, a wide linear dynamic range, relatively simple spectra, very low detection limits, and ability to obtain isotopic information of the analyte thus permitting isotope dilution analysis. As the effluent flow rate of an HPLC column matches the typical sample uptake flow rate of the commonly used nebulizers for ICP-MS, their coupling is quite straightforward. Even without the aid of hydride generation as the sample introduction technique, the detection limit of ICP-MS is comparatively low. The application of ICP-MS for speciation analysis has recently been reviewed [14, 257].

Generally speaking, the conventional pneumatic nebulizer employed as the sample introduction device in ICP-MS suffers from poor nebulization efficiency. The hydride-generation sample introduction technique possesses a number of benefits. It can considerably elevate the sample introduction efficiency through the formation of volatile arsenic hydrides. It allows a separation of the analyte from major components of the matrix. This, in turn, implies that there may be no limit to the salt content of the HPLC eluates. The main drawback of the coupling of HPLC with HG-ICP-MS for the speciation analysis of arsenic is perhaps the spectroscopic interference from $ArCl^+$ molecular ion (mass of 75 which is identical to that of ^{75}As) produced when HCl is used for the hydride generation and argon gas for the plasma. However, the use of HNO_3 could eliminate this problem [258, 259]. Some applications of the speciation of arsenic by HPLC-HG-ICP/MS are listed in Table 2.6.

The AFS detector is relatively simple and low-cost, and has also been used in conjunction with HPLC. The use of ultrasonic nebulization interface for the on-line detection of arsenic species, however, suffers from matrix interferences from the mobile phase [260-261]. Detection limits comparable to other detection techniques for the speciation of arsenic can be obtained by coupling HPLC with microwave or UV digestion hydride generation followed by AFS [69, 98, 116, 262, 263]. Other detectors such as UV [236, 264-265], amperometry [236], and microwave-induced plasma-AES (MIP-AES) [266] have also been reported in conjunction with HPLC separation of arsenic species.

There has been an increasing interest in the separation of ionic arsenic species using capillary electrophoresis (CE). The main advantages of CE include capability for high resolution, possibility of varying analytical conditions, simplicity, and analysis of complex samples. However, it should be noted that the detection limits of 10-50 ppb for arsenic species by CE-UV are not particularly good [267-270]. Attempts have been made to improve the sensitivity of CE by procedures such as on-column preconcentration with field amplified injection [269, 270], conductivity detection with a cationic surfactant as an electroosmotic flow (EOF) modifier [271], and pre-treatment of the capillary with an EOF modifier [267]. Perhaps the most effective procedure is to couple CE with ICP-MS [272-275] where the detection limits for arsenic species were reported to be as low as 1-2 ppb.

Table 2.6. Speciation of Arsenic by HPLC

Species	Column	Mobile phase	Detection technique	Applications	Ref.
AsB, TMAO, AsC, TMA, DMA, MMA, As(III), As(V)	Ionosphere-C (100×3 mm) and ION 120 (125×4.6 mm)	20 mM pyridinium ion (pH 2.65 with methanol) and 100 mM NH_4HCO_3/NH_4OH buffer (pH 10.3).	ICP-MS	Seafoods, CRM (DORM-1, DOLT-1)	56, 94
As(III), As(V), MMA, DMA, TMA, AsB, AsC	Supelcosil LC CN (5μm, 150×4.6 mm).	0.075% TMAH/0.01% HOAc and 0.1% HOAc.	AAS	NRCC CRM (DORM-1)	57
AsB, DMA, MMA, As(V)	Benson BAX-10	100 mM K_2SO_4 and 75 mM K_2SO_4 (pH 10.4)	TD-HG-AAS	Marine organism	58
As(III), MMA, AsB DMA, As(V), AsC	Hamilton PRP-X100 (10μm, 250×4.1 mm).	Gradient of 5 and 35 mM $NaH_2PO_4/$ Na_2HPO_4 buffer (pH 6.0).	UV-HG-ICP-AES	Marine organism	59, 60
As(III)+As(V)+ MMA, DMA, AsB, AsC	Pierce C_{18} (5μm, 30×4.6 mm)	10 mM NaDS /5% MeOH/ 2.5% HOAc.	ICP-MS	NRCC CRM (DORM-1)	61, 62
AsB, DMA, As(V), As(III), MMA	Benson SAX (7-10 μm, 125×4 mm).	1 to 50 mM K_2SO_4 (pH 10.5).	ICP-MS	Seafood, DORM-1	63

Table 2.6. Speciation of Arsenic by HPLC (continued)

Species	Column	Mobile phase	Detection technique	Applications	Ref.
As(III), As(V), MMA, DMA, AsB, AsC, TMA, TMAO	Supelcosil LC-SAX1 and LC-SAX (5 μm, 250×4.6 mm).	15 mM $NH_4H_2PO_4$/$(NH_4)_2HPO_4$ (pH 5.1) and 20 mM pyridine (pH 2.0, 2.5, 3.0).	ICP-MS	NRCC CRM (DORM-1, 2)	64
As(III), As(V), MMA, DMA, AsB, TMA, TMAO, AsC	Dionex Ion Pac AS 7 (10μm, 250×4 mm).	0.05 mM benzenedisulfonic acid gradient from pH 4 to pH 1.3.	ICP-MS	NRCC CRM (DORM-2), mushroom.	65
As(III), As(V), AsB, MMA, DMA, AsC.	Hamilton PRP X-100 (10μm, 250× 4.1 mm); Phenomenex C_{18} (50×2 mm)	30 mM $(NH_4)_2CO_3$/NH_4OH (pH 9); 25 mM citric acid/10 mM sodium pentanesulfonate.	ICP-MS	NRCC CRM (DROM-2), marine fish.	66
AsB, AsC, DMA, MMA, As(III), As(V)	Hamilton PRP-X100	Gradient elution with 5 mM NH_4NO_3/5 mM $(NH_4)_2CO_3$ (pH 9.0) and 50 mM NH_4NO_3/5 mM $(NH_4)_2CO_3$ (pH 9.0).	ICP-MS	DROM-2, marine fish.	67
As(V), MMA, DMA, AsB, AsC	Hamilton PRP 1 and PRP X-100 (10μm, 250×4.1 mm)	0.5 mM of TBAP (pH 9.5); 10 to 100 mM Na_2HPO_4/ NaH_2PO_4 (pH 6.5).	ICP-MS, ICP-AES	Marine organism	68

Table 2.6. Speciation of Arsenic by HPLC (continued)

Species	Column	Mobile phase	Detection technique	Applications	Ref.
As(III), As(V), DMA, MMA, AsB, AC, TMA, TMAO	PRP-X100 (250×4.1 mm); Adsorboaphere SCX 5U(250×4.6 mm).	15 mM HPO_4^{2-} / $H_2PO_4^-$ (pH 6.1); 2.5 mM pyridine/citric acid (pH 2.65).	UV-HG-AFS	CRM	69
AsB, MMA, DMA, As(V)	PRP -X100 and PRP-X200 (10 μm, 250× 4.1 mm).	Gradient elution with 5 and 100 mM $HPO_4^{2-}/H_2PO_4^-$ (pH 6); Gradient elution with 4 mM HNO_3 to 4 mM HNO_3/20 mM NH_4NO_3.	UV-HG-ICP-MS	Mussels, CRM	83, 84
As(III), As(V), AsC, MMA, DMA, AsB, TMAO, TMA.	Supelcosil LC-SAX1; Supelcosil LC-SAX; Hamilton PRP-X100.	15 mM $NH_4H_2PO_4/(NH_4)_2HPO_4$ (pH 5.1); 20 mM pyridine (pH 2.9); 30 mM $NH_4H_2PO_4/(NH_4)_2HPO_4$ (pH 6.0).	ICP-MS	Marine organism	88
AsB, TMA, AsC, DMA, TMAO	Inertsil ODS-1	TEAH or 1-butanesulfonate as pairing ion at pH 3.0-6.8.	ICP-MS	NRCC CRM	91
As(III), As(V), AsC, MMA, DMA, AsB	Hamilton PRP 1 (10μm, 250×4.6 mm)	0.5 mM TBAP/4 mM $H_2PO_4^-/HPO_4^{2-}$ (pH 9.0).	ICP-MS	Waters, fish and mussels.	95, 228

Table 2.6. Speciation of Arsenic by HPLC (continued)

Species	Column	Mobile phase	Detection technique	Applications	Ref.
As(V), DMA, arsenosugars, AsB, TMAO	10μ Bondclone C_{18} (300×3.9 mm); Inertsil ODS-2 (250×4.6 mm).	10 mM sodium heptanesulfonate/4.5 mM malanic acid (pH 6.8); 4 mM TMAH (pH 3.5).	ICP-MS, HG-AAS	Marine organisms	97
As(III), As(V), DMA, MMA, AsB, AC, TMA, arsenosugars	Phenomenex ODS (3) (5μm 250×4.6 mm) and C_{18} (10μm, 300×3.9 mm)	10 mM TEAH/4 mM Malonic acid/0.1% MeOH (pH 6.8) or 5 mM TBAH/1 mM hexanesulfonate /1 mM malonic acid/0.5% methanol (pH 5.5) and 10 mM hexanesulfonate /1 mM TEAH/0.5% MeOH (pH 4.0).	MW-HG-AFS	Urine	98
As(III), As(V), MMA, DMA, AsB, TMA, TMAO, AsC	Inertsil ODS (250× 4.6 mm); Supelcosil LC-SCX (250×4.6 mm).	10 mM TEAH/4.5 mM malonic acid/0.1% MeOH (pH 6.8 by HNO_3); 20 mM pyridine (pH 2.7 by formic acid).	ICP-MS	Scallops	103
As(III), As(V), DMA, MMA, AsB, AC, TMA, TMAO	PRP-X100 (250×4.1 mm); PRP-X200 (250×4.1 mm).	15 mM HPO_4^{2-} / $H_2PO_4^-$ (pH 6.1); 3 to 5 mM 3-Carboxy-4-hydroxybenzenesulphonic acid (pH 1.9) and 5 mM HCl (pH 1.9).	UV-HG-AFS	N/A	116

Table 2.6. Speciation of Arsenic by HPLC (continued)

Species	Column	Mobile phase	Detection technique	Applications	Ref.
As(III), MMA, DMA, As(V)	Benson SAX-10 (5μm, 250×5 mm).	100 mM $(NH_4)_2CO_3$ solution.	HG-AAS, ICP-AES, AFS	N/A	197
As(III), As(V), MMA, DMA, AsB	BDH PolySphere SAW (120×4.6 mm) and Phenomenex C18 (300×3.9 mm).	50 mM phosphate or carbonate buffer (pH 7.5, 9.0 or 10.3) and 10 mM heptanesulfonate/0.1% methanol (pH 3.5).	HG-AAS, TD-HG-AAS, ICP-MS	N/A	224
As(III), As(V), MMA, DMA	Hamilton PRP 1 (150×4.1 mm)	50 mM CTAB/20 mM borate buffer/10% propanol (pH 10.2).	ICP-MS	Urine, DORM-1	225, 226
As(III), As(V), MMA, DMA, AsB, AsC, TMAO, TMA	Hamilton PRP-1 (10μm, 250×4.1 mm)	10 mM 2-hydroxy-5-sulphobenzoic acid (pH 2.5-3.5).	ICP-MS	Blue mussels	227
As(III), As(V), MMA, DMA, AsB, AsC, TMA	Merck Polyspher IC AN 2 (10μm, 120× 4.6 mm); Ionospher-C (5μm, 100×3 mm).	100 mM $NaHCO_3/Na_2CO_3$ buffer (pH 10.25); 100 mM pyridine (pH 2.65 with formic acid).	AAS, ICP-MS	Soil extract	229

Table 2.6. Speciation of Arsenic by HPLC (continued)

Species	Column	Mobile phase	Detection technique	Applications	Ref.
As(III), MMA, DMA, As(V), AsB, AsC	Hamilton PRP-X100 (250×4.1 mm) and Waters IC-H.	17 mM and 5 mM $HPO_4^{2-}/H_2PO_4^-$ buffer (pH 6.0)	TD-HG-AAS	Natural and waste waters, sediment	230, 237
As(V), As(III), MMA, DMA, AsB	Spherisorb S SAX (120×4 mm); Supelcosil SAX 1 (250×4.6 mm); PRP-X100 (250×4 mm).	10 mM KH_2PO_4/K_2HPO_4 (pH 7.5-7.8); 20 mM K_2HPO_4 (pH 4.64); Gradient elution with KH_2PO_4/K_2HPO_4 (pH 6.22), 12 mM PO_4^{3-} and 24 mM PO_4^{3-}.	MW-UV-HG-AAS	Urine	231
As(V)+As(III)+MMA, DMA, AsB, AsC	Bio-Rad UNO S-1 (35×7 mm).	3-5 mM NaH_2PO_4 (pH 2.1-2.33 by adding H_3PO_4).	MW-UV-HG-AAS	Urine	232
As(III), MMA, DMA, As(V), AsB, AsC	Pharmacia Q Sepharose Fast Flow (300×8 mm) and SP-TSK 5PW (60×8 mm).	10 mM $HPO_4^{2-}/H_2PO_4^-$ (pH 5) and 100 mM sodium citrate (pH 5).	HG-AAS, ICP-AES	Urine	233
As(III), As(V), MMA, DMA, AsB, AsC, p-APA	Elite AS3 and Dionex CAS1 in series.	5 mM $NH_4H_2PO_4/(NH_4)_2HPO_4$ buffer (pH 5.8).	HG-AAS	Natural water	234

Table 2.6. Speciation of Arsenic by HPLC (continued)

Species	Column	Mobile phase	Detection technique	Applications	Ref.
As(III), MMA, AsB DMA, As(V), AsC	Hamilton PRP-X100 (10μm, 250×4.1 mm).	Gradient from 20 to 100 mM NaH_2PO_4/ Na_2HPO_4 buffer (pH 5.75).	UV-HG-ICP-AES	Spiked mineral water	235
As(III), As(V), MMA, DMA, AsB, AsC	C_{18} Lichrosphere (5μm, 250 mm).	5 mM TBAH (pH 7.3).	UV and Amperometry	N/A	236
As(III), MMA, DMA, As(V), AsB, AsC	Hamilton PRP- X100 (10μm, 250×4.1 mm).	Gradient elution with 10 mM $H_2PO_4^-$/ HPO_4^{2-}/2%CH_3CN (pH 6.5) to 100 mM $H_2PO_4^-$/HPO_4^{2-} (pH 7.95).	ICP-MS	Artificial fish extract	238
As(III), As(V), DMA, AsB, AsC	IonPac AS4A-SC; Dionex IonPac AS7.	5 mM Na_2CO_3/40 mM NaOH/4% MeOH; gradient elution with 0.5 mM HNO_3 (pH 3.3) to 50 mM HNO_3 (pH 1.3).	ICP-MS	NRCC CRM DORM-2, waters	239
As(III), MMA, As(V), DMA	BAX-10 (5μm, 250 ×5 mm).	10 mM $(NH_4)_2CO_3$.	HG-AAS	Soil pore water	242

Table 2.6. Speciation of Arsenic by HPLC (continued)

Species	Column	Mobile phase	Detection technique	Applications	Ref.
As(III), MMA, DMA, As(V)	Ionospher (10μm, 250×4.5 mm)	30 mM $H_2PO_4^-/HPO_4^{2-}$ buffer (pH 6.2-6.3).	HG-AAS	Urine	243
As(III), As(V), MMA, DMA	PRP-X100 (10μm, 250×4.1 mm)	Gradient elution with 20 and 100 mM $H_2PO_4^-/HPO_4^{2-}$ buffer (pH 6.0).	HG-AAS	N/A	244
As(III), As(V), MMA, DMA	PRP-X100 (10μm, 250×4.1 mm).	Isocratic 25 mM $HPO_4^{2-}/H_2PO_4^-$ (pH 6.0) or gradient elution with 25 and 100 mM $HPO_4^{2-}/H_2PO_4^-$ (pH 6.0-6.5).	HG-AAS	Natural waters	245
As(III), As(V), MMA, DMA	Two ChromSpher C_{18} (5μm, 100×3 mm).	20 mM phosphate/10 mM TBA.	HG-AAS	Urine	246
As(V), MMA, DMA, AsB, AsC	Hamilton PRP-X100 (250×4.1 mm).	17 mM NaH_2PO_4/Na_2HPO_4 (pH 6.0).	TD-HG-AAS	Water, urine	248
MMA, DMA, AsB, AsC	Dionex Ionpac CS 10 (250×4 mm).	100 mM HCl/50 mM NaH_2PO_4 (pH 1.3).	UV-HG-AAS	Human serum, urine	249, 250

Table 2.6. Speciation of Arsenic by HPLC (continued)

Species	Column	Mobile phase	Detection technique	Applications	Ref.
As(III), MMA, DMA, As(V)	Bio-Rad Aminex A-27 (15 μm, 100×8 mm).	Gradient elution from H_2O to 0.5M $(NH_4)_2CO_3$, and isocratic elution with 0.5M $(NH_4)_2CO_3$.	ICP-AES	Cultured cell suspension	252
As(III), MMA, DMA, As(V), Se(IV), Se(VI)	Partsil 5 ODS-3 (250×4.6 mm).	5 mM TBAP/5% MeOH.	ICP-AES	Shale oil	253
As(III), As(V), Se(IV), Se(VI)	Dionex AS4A (250×4 mm).	1.7 mM $NaHCO_3$/1.8 mM Na_2CO_3 (pH 8.98).	HG-ICP-AES	N/A	254
As(III), As(V), DMA, Se(VI)	Hamilton PRP-X100 (125×4 mm)	1 mM of *p*-hydoxybenzoate/0.4 mM benzoate/2.5% MeOH (pH 8.5).	HG-ICP-AES	N/A	255
As(III), MMA, DMA, As(V)	Nuclosil SB (5μm, 200 mm)	50 mM NaH_2PO_4/ Na_2HPO_4 buffer (pH 6.75).	ICP-AES	N/A	256
As(III), MMA, DMA, As(V)	Vydac 201TPC_{18} (5μm, 250×4.6 mm)	1 mM TBAP/2 mM NH_4OAc (pH 5.99).	HG-ICP-MS	Natural waters	258

Table 2.6. Speciation of Arsenic by HPLC (continued)

Species	Column	Mobile phase	Detection technique	Applications	Ref.
As(III), As(V), MMA, DMA	BST C_{18} Rutin (10μm, 250×4.6 mm)	20 mM Na_2HPO_4/NaH_2PO_4/0.1% of 10 mM DDAB /0.5% methanol (pH 6.0).	AFS	N/A	260
As(III), As(V), MMA, DMA	Phenomenex ODS (3) (30×4.6 mm)	5 mM TBAH/2 mM Malonic acid/5% MeOH (pH 6.0)	HG-AFS	Natural waters, urine	262
As(III), As(V), DMA, MMA, AsB, TMA	PRP-X100 (250×4.1 mm); PRP-X200 (250×4.1 mm).	15 mM HPO_4^{2-} / $H_2PO_4^-$ (pH 6.0); 10 mM HCl (pH 2.03).	UV-HG-AFS	Mushrooms	263
As(III), As(V), MMA, DMA, AsB, AsC	Vydac 302 IC and Partisil ODS3	1 mM HPO_4^{2-}/ $H_2PO_4^-$ (pH 5.3) and 5 mM TBAH (pH 7.3).	UV	N/A	264
As(III), As(V), MMA, DMA	DDAB modified Spherisorb ODS 2 (10μm, 250×4.6 mm).	5 mM of NaH_2PO_4/1% methanol/0.2 mM vesicles of DDAB (pH 5).	HG-MIP-AES	Natural waters, urine	266

Table 2.6. Speciation of Arsenic by HPLC (continued)

Species	Column	Mobile phase	Detection technique	Applications	Ref.
As(III), MMA, DMA, As(V), Se(IV), Se(VI)	Econospher C_{18} (5 μm, 250×4.6 mm)	5 mM TBAP/5% MeOH (pH 3.0).	ICP-MS	N/A	276
As(III), As(V), MMA, DMA	Adsorbosphere-NH_2 (5μm, 250×4.6 mm).	15 mM $NH_4H_2PO_4$/1.5 mM CH_3COONH_4/30% MeOH (pH 5.75).	ICP-MS	Urine	277
As(III), As(V), DMA, MMA	Wescan Anion/R-IC (250×4.1 mm).	5 mM phthalic acid (pH 2.55); Gradient elution with 2% propan-1-ol and 50 mM carbonate buffer (pH 7.5).	ICP-MS	Urine, club soda, wine	278, 279
As(III), MMA, DMA, As(V)	Inertsil ODS-2 (5μm, 100×1 mm)	5 mM heptyltriethyammonium phosphate /5-25% MeOH (pH 6.0).	ICP-MS	N/A	280
As(III), MMA, DMA, As(V)	Hamilton PRP-X100 (10μm, 150×4.1 mm)	5 mM $NH_4H_2PO_4$/$(NH_4)_2HPO_4$ (pH 6.0), and 10 mM citrate (pH 6.0) buffer.	ICP-MS	Sediment extractant	281

Table 2.6. Speciation of Arsenic by HPLC (continued)

Species	Column	Mobile phase	Detection technique	Applications	Ref.
As(III), As(V), Se(IV), Se(VI)	ANX1606As	5 mM Ammonium malonate (pH 8.5).	ICP-MS	Soil extract	282
As(III), As(V), MMA, DMA	ION 120 anion exchange column	45 mM $(NH_4)_2CO_3$/3%MeOH (pH 10.3)	ICP-MS	Soil and carrots,	283
As(III), As(V), MMA, DMA, AsB	Dionex IonPac AS14	Gradient elution with 2 mM TMAH and 10 mM $(NH_4)_2CO_3$ (pH 10.0).	ICP-MS	Urine	284
As(III), As(V), MMA, DMA	Supelcosil LC-SAX (5μm, 250×4.6 mm).	30 mM $NH_4H_2PO_4/(NH_4)_2HPO_4$ (pH 5.1).	ICP-MS	Urine	285
As(III), As(V), MMA, DMA, Se(IV), Se(VI), SeMet, Sb(III), Sb(V)	Dionex IonPac AS 14 (250× 4 mm).	Gradient elution with 2 mM NH_4HCO_3/2.2 mM tartaric acid (pH 8.2) and 2 mM NH_4HCO_3/45 mM tartaric acid (pH 8.2).	ICP-MS	N/A	286
As(III), As(V)	ANX-1606-AS	5 mM ammonium malonate.	ICP-MS	Water	287

Table 2.6. Speciation of Arsenic by HPLC (continued)

Species	Column	Mobile phase	Detection technique	Applications	Ref.
As(III), As(V), MMA, DMA	Waters μ-Bondapak C_{18}	5 mM TBA /5% MeOH.	ET-AAS	Clams	288
As(III), MMA, DMA, As(V)	Nucleosil 5SB or Hamilton PRP-X100	20 mM $HPO_4^{2-}/H_2PO_4^-$ (pH 6) buffer	ICP-AES	Artificial fish extract	289
As(III), As(V), MMA	Shim-pack SCR-102H (300 mm).	Diluted trifluoroacetic acid solution (pH 2.1).	HG-ICP-MS	Natural waters	290
As(III), As(V), MMA, DMA	Dionex AG7 and Dionex AS7.	Not mentioned.	HG-ICP/MS, GC-MS	Soil extract	291

BDSA: benzene-1,2-disulfonic acid ; DDAB: Didodecyldimethylammonium bromide; NaBS: Sodium 1- butanesulfonate; TBAP: Tetrabutylammonium phosphate; SDS; sodium dodecyl sulfate; SOS: sodium octyl sulfate; NaDS: Sodium dodecylsulfonate; TBA: Tetrabutylammonium ion; TBAH: Tetrabutylammonium hydroxide; TMAH: Tetramethylammonium hydroxide; TEAH: Tetraethylammonium hydroxide; CTAB: Cetyltrimethylammonium bromide.

It is evident from the above discussions that HPLC can be used to separate many species rapidly and efficiently in one run compared to open column chromatography. It also does not require complicated steps to synthesize volatile derivatives of the analytes like GC [196, 197]. Retention times from the HPLC chromatogram can be used to identify the species of interest. The detection limits of the species after HPLC separation can be significantly lowered by using element-specific detectors. In this thesis, we have used HPLC for the separation of various arsenic species followed by NAA in order to further improve the detection limits.

Some of the HPLC methods in conjunction with a variety of detectors reported in the literature are listed in Table 2.6.

2.6 Speciation of Arsenic by Electrochemical Techniques

Electrochemical techniques provide a sensitive method for the determination of As(III). The instrumentation required is relatively simple and generally costs far less than that required for the methods described in the previous sections. Zima and van den Berg [292] reported a method for the determination of As(III) and As(III+V) in seawater using differential pulse cathodic stripping voltammetry (DPCSV) at a hanging mercury drop electrode (HMDE) in the presence of pyrroliidinedithiocarbamate (PDC). The detection limit of the method was reported as 3 nM arsenic (equivalent to 0.225 ng mL^{-1}) and the method was applied to NRCC CASS-2 CRM and Atlantic Ocean water. Li and Smart [293] used a square wave cathodic stripping voltammetric method also at a HMDE for the determination of As(III) in natural waters. Lower detection limits of 0.2 and 0.06 nM arsenic were obtained when deposition times of 1 and 10 min were used, respectively. Using a combination of acid digestion and potassium iodide reduction, Eguiarte *et al.* [294] determined the total arsenic in soil by DPCSV at a HMDE in presence of copper. Henze *et al.* [295] found that As(V) becomes electroactive when mannitol is used as an activator for the electro-reduction of As(V) to As(III). They reported an analytical procedure which permits the discrimination between As(III) and As(V) in fresh water samples.

Anodic stripping voltammetry (ASV) can be applied for the determination of As(III) when gold or gold plated carbon electrodes are used as the working electrodes.

Sun *et al.* [296] prepared a rotating gold-film glassy-carbon electrode and applied it to the determination of As(III) and As(V) in seawater by ASV. As(III) was directly measured and As(V) was reduced to As(III) by gaseous SO_2 prior to its ASV determination. The detection limit of the method was 0.19 ng mL^{-1} for a deposition time of 4 min. Kopanica and Novotny [297] obtained a similar detection limit of 0.15 ng mL^{-1} using a gold disc electrode for the determination of As(III) by differential pulse anodic stripping voltammetry (DPASV). Jurica *et al.* [298] reported a calibrationless flow-through anodic stripping coulometric determination of As(III) as well as total arsenic in contaminated water samples with a detection limit of 0.15 ng mL^{-1}. The reduction of As(V) to As(III) was evaluated using three reagents, namely L-cysteine, hydroxylamine hydrochloride and hydrazine, in a microwave oven. Although electrochemical techniques are sensitive, the direct determination of only As(III) and As(V) has been reported so far.

The use of electrochemical techniques as a detection device preceded by the separation of arsenic species has also been reported. For example, differential pulse polarography (DPP) was used as a detection method in combination with ion exchange chromatography for the determination of As(III), As(V), MMA, and DMA [216]. An amperometric detector in combination with a UV detector was used in HPLC for the determination of six arsenic species, namely As(III), As(V), MMA, DMA, AsB, and AsC [236].

2.7 Methods for Speciation of Arsenic Based on NAA

Neutron activation analysis (NAA) is one of the most sensitive techniques for the determination of arsenic. Since NAA has been used in this thesis for the measurement of total as well as species-specific concentrations, the published literature on NAA is reviewed in this section and summarized in Table 2.7. Arsenic has only one naturally occurring stable isotope, ^{75}As. It has a thermal neutron capture cross section (σ) of 4.5 b which is fairly high compared to the cross sections of the isotopes of most other elements which are generally of the order of mb. The product radioactive nuclide ^{76}As has a half-

Table 2.7. Speciation of Arsenic by NAA

Species	Method	Fluence (n cm^{-2}); t_d-t_c	Applications	Ref.
As(III), Sb(III), As(V), Sb(V)	As(III), Sb(III): extracted with DDTC/$CHCl_3$ at pH 2.5-4.0; As(V), Sb(V): collected by thionalide cocrystallization.	3.6×10^{16}; decay curve analysis.	Seawater	131
As(III), As(V), Sb(III), Sb(V)	As(III), Sb(III): extracted with APDC/$CHCl_3$ at pH 3-6; As(V), Sb(V): extracted after reduction to As(III) by $K_2S_2O_3$ and KI at pH 1.	6.48×10^{16}; 24 h-8000 s.	Natural waters	132
As(III), As(V)	As(III): extracted by APDC/$CHCl_3$ at pH 1.5, As(V)+As(III): extracted after reduction of As(V) to As(III) by $Na_2S_2O_3$.	8.64×10^{16}; 3 d - 3600 s.	Seawater, marine organism	133
As(III), As(V)	As(III): precipitated by adding DBDC at pH 2; As(V): reduced by $Na_2S_2O_3$ to As(III) then precipitated by DBDC.	2.16×10^{15}-4.5×10^{16}; 24 h-2000 s.	CRM, surface waters	161
As(III), As(V), Sb(III), Sb(V), Se(IV), Se(VI)	As(III), Sb(III), Se(IV): coprecipitated with APDC and $Pb(PDC)_2$ at pH 2; As(V), Sb(V) and Se(VI): after reduction by $TiCl_3$ coprecipitated with APDC and $Pb(PDC)_2$.	2.16×10^{20}; 5 d-1800 s.	Waters	163

Table 2.7. Speciation of Arsenic by NAA (continued)

Species	Method	Fluence (n cm^{-2}); t_d-t_c	Applications	Ref.
Inorganic As, MMA, DMA, AsB	0.5M HCl for inorganic arsenic, water for MMA, 0.75M ammonia followed by 3M ammonia for AsB and DMA mixture on Dowex 50W×8 cation exchange column; 12.5mM phosphate buffer (pH 8) for AsB and DMA on Dowex 1×8 anion exchange column.	3.6×10^{15}; not mentioned.	NRCC CRM (DORM-1)	219
Inorganic As, MMA, DMA, AsB, TMA	0.5M HCl for inorganic As; water for MMA; 0.75M and 3M ammonia for AsB and DMA mixture; 0.5M and 6M HCl for TMA on a Dowex 50W×8 cationic exchange column; 12.5 mM phosphate buffer (pH 8) for AsB and DMA on a Dowex 1×8 anion exchange column.	Neutron flux: $1.8\text{-}4\times10^{16}$ (cm^{-2} s^{-1}).	Marine mussels, CRM	220

life of 25.9 h and it decays to ^{76}Se which is stable. The most intense gamma-ray emission at 559.1 keV has been employed in this work for assaying arsenic levels [299].

Solvent extraction, coprecipitation, and ion exchange methods have been used in conjunction with NAA. One of the first papers using solvent extraction and NAA to study arsenic and antimony species in seawater was published in 1975 by Gohda [131]. In this procedure, As(III) and Sb(III) were extracted with diethyldithiocarbamate (DDTC) in chloroform from freshly collected seawater samples while As(V) and Sb(V) were not. As(III) and Sb(III) were then back-extracted into dilute nitric acid, cocrystallized with thionalide, and determined by NAA. The As(V) and Sb(V) were also determined by NAA after thionalide cocrystallization from the original seawater sample. The samples were irradiated for 30 min at a flux of 2×10^{13} cm^{-2} s^{-1} and counted using a well-type NaI(Tl) detector. Seawater samples were found to contain 1.56 to 2.45 μg L^{-1} of total arsenic and 0.37 to 0.60 μg L^{-1} of total antimony. About 80-97% of arsenic and 70-94% of antimony were found to exist as the pentavalent species. The solvent extraction method also effectively removed the major interfering elements, such as bromine, chlorine and sodium, and thereby dramatically improved the detection limits of NAA.

Mok and Wai [132] used APDC in chloroform at pH 3-6 to simultaneously extract As(III) and Sb(III). As(V) and Sb(V) were first reduced with $K_2S_2O_3$ and KI at pH 1 then extracted. The arsenic- and antimony-APDC complexes in the organic phase were then back-extracted into nitric acid solution and analyzed by NAA. The detection limits for both arsenic and antimony in water were 0.001 μg L^{-1}. The same method [140] gave detection limits of 0.005 μg g^{-1} for arsenic and 0.006 μg g^{-1} for antimony in biological samples using a Ge(Li) detector.

Another solvent extraction NAA method involving APDC in chloroform was reported by Yusof *et al.* [133] for As(III) and As(V) in seawater and marine organisms. They reported concentrations of As(III) and As(V) in seawater samples which agreed with the published values, and in marine organisms, namely swimming crab (*Portunus pelagicus*), blood clam (*Anadara granosa*), which were caught in the same locations as the water samples. They digested NRCC Lobster Hepatopancreas CRM (TORT-1) in 70% nitric acid, used the solvent extraction NAA method, and reported total arsenic content. It appears that they summed up As(III) and As(V) to obtain the total arsenic

content. However, in marine organisms arsenic has been reported to exist mainly as AsB. It is then possible that AsB was converted to inorganic arsenic during the digestion.

Compared to solvent extraction, coprecipitation yields a solid sample which can be easily irradiated and handled in NAA [152, 161, 163]. The chelating agents used in solvent extraction can also be used to separate total arsenic, As(III) and As(V) by coprecipitation. Van Elteren and Das [161] coprecipitated As(III) with dibenzyl-dithiocarbamate (DBDC) and As(V) with the same chelating reagent after reduction with $K_2S_2O_3$ and KI, collected the precipitates on 0.45 μm membrane filters, and determined the arsenic content by NAA. Further studies indicated that neither MMA nor DMA were coprecipitated with As(III), but significant amounts of MMA were coprecipitated with As(V) after its reduction to As(III). Therefore, at least in principle, MMA could interfere with the determination of As(V). However, it should be noted that the levels of MMA are generally lower than that of As(V) by at least two orders of magnitude in natural waters. The authors did not give a reason for the coprecipitation of MMA after the reduction. Yusof *et al.* [162] used DBDC and phenolphthalein to coprecipitate Se(IV) from marine sediment and then determined it by NAA. Sun and Yang [163] reported a method for the speciation of As(III), As(V), Sb(III), Sb(V), Se(IV), and Se(VI) by coprecipitation with $Pb(PDC)_2$ followed by NAA.

Slejkovec and coworkers [219] determined several arsenic species by ion exchange chromatography and NAA. They used a cation exchange column to separate inorganic arsenic (As(III) and As(V) together), MMA, AsC or TMA-ion, and a fraction containing both AsB and DMA. Then they used an anion exchange column to further separate AsB from DMA. In a separate paper [220], they reported the separation of a fraction which could be TMAO. They used these methods to determine various arsenic species in marine mussels, freshwater mussels, fish, shrimp, sea-gull eggs, and in reference materials, such as NRCC Dogfish Muscle (DORM-1) and Dogfish Liver (DOLT-1), and NIST Oyster Tissue (SRM 1566).

Van Elteren *et al.* [189] employed radiolabelled MMA and DMA and carried out a HG-CT procedure. Then they trapped AsH_3, CH_3AsH_2 and $(CH_3)_2AsH$ separately on activated charcoals and counted [189]. Although NAA was not used here, their work suggests that it may be possible to couple the NAA with HG-CT.

Slejkovec *et al.* [300] recently reported the separation of eight radiolabelled arsenic compounds by HPLC. Since the neutron irradiation of organoarsenic compounds can lead to their decomposition and since the degree of decomposition depends on the irradiation time and other conditions, it is impossible to perform speciation analysis after irradiation. However, it may be possible to perform the speciation analysis by HPLC separation followed by NAA. This approach was chosen in the work done in this thesis for the speciation of arsenic.

3. EXPERIMENTAL

3.1 Neutron Activation Analysis

3.1.1 Neutron Irradiation Facility and Counting Systems

All samples and standards were irradiated in the pneumatic transfer irradiation sites of the Dalhousie University SLOWPOKE-2 Reactor (DUSR). The composition, stability, homogeneity, and reproducibility of the DUSR neutron flux in all the irradiation sites have previously been described [301-303]. Briefly, the five inner sites, where most irradiations were performed, can hold medium-size polyethylene irradiation vials of 7 mL capacity. Samples irradiated in any of these five sites are subjected to the highest available neutron flux. Under normal operating power level of 8 kW, which is one-half of the maximum attainable power level, the thermal neutron flux in the inner sites is 5×10^{11} cm^{-2} s^{-1}. There are two outer sites which are designed to hold polyethylene irradiation vials of 27 mL capacity; the flux in these sites is 2.5×10^{11} cm^{-2} s^{-1} at 8 kW. There is an additional outer site which is shielded with 1-mm-thick cadmium. Samples irradiated in this site are essentially exposed to an *epi*-cadmium neutron flux of 5×10^{10} cm^{-2} s^{-1}.

The samples and comparator standards were counted under identical geometry conditions using three different gamma-ray spectrometry systems depending on factors such as the trace element concentration of interest, the matrix and interfering elements, and the irradiation conditions. One of these systems consisted of a 60 cm^3 APTEC Ge(Li) semiconductor detector, with a resolution of 1.9 keV and a peak-to-Compton ratio of 35:1 both measured at the 1332-keV photopeak of ^{60}Co, and an efficiency of 9.5%, and an APTEC personal computer based multichannel analyzer (PCMCA); it is designated as "System#1" in our laboratory. This system was mainly used for the development of the speciation methods. The second unit ("System#2") contained an EG&G ORTEC 92X Spectrum Master spectroscopy system with GammaVision-32 gamma-ray analysis and MCA emulator software. It had a HPGe coaxial detector with a resolution of 1.89 keV, a peak-to-Compton ratio of 56:1, and an efficiency of 32.3%. It was mainly used for assaying samples with low arsenic and antimony levels because of the higher efficiency and peak-to-Compton ratio than the System#1. The "System#3" was a Compton

suppression spectrometer consisting of an ORTEC HPGe detector surrounded by a 10"×10" NaI(Tl) guard detector with 3"×3" NaI(Tl) plug; The HPGe coaxial detector had a resolution of 1.79 keV, a peak-to-Compton ratio of 58:1, and an efficiency of 25%. The entire system had a peak-to-Compton ratio of 590:1; it has been described elsewhere in detail [304]. This special system was used for the determination of very low levels of arsenic and antimony in presence of high activities from ^{24}Na and ^{82}Br.

The nuclear data of the elements of interest as well as of the possible interfering elements are given in Table 3.1 [305]. The 559.16 keV, 564.3 keV and 162 keV gamma-rays of ^{76}As, ^{122}Sb and ^{77m}Se, respectively, were used for assaying arsenic, antimony and selenium levels in water and fish samples.

Table 3.1. Nuclear Data for Some Elements of Interest

Element	Na	As	Sb	Se	Br
Target isotope	^{23}Na	^{75}As	^{121}Sb	^{76}Se	^{81}Br
Abundance (%)	100	100	57.25	9.02	49.31
Thermal neutron cross-section, σ (b)	0.530	4.3	6.25	21	2.69
Resonance integral, I	0.34	41 b	127 b	16 b	48 b
Nuclide produced	^{24}Na	^{76}As	^{122}Sb	^{77}Se	^{82}Br
Half-life	14.96 h	26.3 h	64.8 h	17.5 s	35.4 h
Gamma-ray used (keV)	1 368.6	559.1	564.1	161.9	554.3

3.1.2 Elemental Comparator Standards for NAA

Ultrapure elemental comparator standards were used for the development of NAA methods as well as for quality assurance. Elemental comparator standards of different geometry and different physical states were prepared in the following manner. Stock solutions of 1 to 10 ppm were prepared by diluting 1000-ppm plasma emission grade

elemental standards (Spex Inc.) with distilled de-ionized water (DDW). The liquid comparator standards were prepared by pipeting a measured amount of the stock solution into precleaned polyethylene irradiation vials, then adding DDW to it for achieving the desired geometry. These standards were mainly used for the development of the separation methods, namely solvent extraction, solid-phase extraction, ion exchange chromatography, and HPLC where they were irradiated for a short time. The same arsenic elemental comparator standards were used for the determination of total arsenic, lipid soluble arsenic, and arsenic in the residue after extraction from fish samples. The solid comparator standards were prepared by pipeting a measured amount of the stock solution onto a measured amount of solid XAD-4 resin in the precleaned vials. If necessary, a little amount of water was added to homogenize the standard solution and the solid resin. All standard solutions were dried under an IR lamp and then the vials were heat-sealed.

3.1.3 Comparator Standards for Speciation Analysis

Comparator standards with specific species of an element are required when the speciation analysis is to be done by NAA in combination with chemical separations. The stock solutions of all the trace element species of interest were prepared or purchased as described in Section 3.2.1.

In the solvent extraction NAA method for speciation of arsenic in natural waters, the comparator standards were prepared in the following manner. A single layer of Whatman ashless filter paper was first placed on the bottom of a vial (medium size), then a known amount of the stock solutions was pipetted onto it. The standards were then allowed to dry under an IR lamp. Once dried, a trimmed cap was placed inside the vial to keep the piece of filter paper in position, and the vials were heat-sealed.

For the comparator standards to be used for the solid-phase extraction NAA, a certain amount of the stock solution was pipetted onto a known amount of precleaned XAD-4 resin or Dowex 50W cation exchange resin in precleaned vials (medium size). The drying, capping, and heat-sealing were the same as described above.

The preparation of the standards for speciation of arsenic by HPLC-NAA methods was a little bit more complicated because the sample injection loop in the HPLC

system needed to be calibrated. Stock solutions were injected into HPLC and the separation was carried out in the same manner as for real samples. The effluent was fraction collected in precleaned vials and dried under an IR lamp. Once dried, a trimmed cap was placed inside the vial to keep the small solid in position for keeping the geometry constant; the vials were then heat-sealed.

3.2 Materials and Apparatus for Separation Methods

3.2.1 Speciation Standards

Five different arsenic compounds were used in various experiments. They were sodium arsenite (NaH_2AsO_3, ACS certified grade, Fisher), potassium arsenate (KH_2AsO_4, Sigma), cacodylic acid ($C_2H_6AsO_2H$, >99%, Fluka), sodium monomethylarsonate ($CH_3AsO_3Na_2$, synthesized by the author, see Section 3.4), and arsenobetaine (1031±6 mg kg^{-1} in solution, CRM 626 prepared by the SM&T progamme of EU) [77]. Potassium antimonyl tartrate ($KSbC_4H_4O_4$, 99.95%, Aldrich), potassium hexahydroxyantimonate ($KSb(OH)_6$ 99.99%, Aldrich), sodium selenite (Na_2SeO_3 99.999%, Aldrich) and sodium selenate (Na_2SeO_4 99.999%, Aldrich) were used as the speciation standards for antimony and selenium, respectively.

Stock solutions of 1 000 μg mL^{-1} arsenic in forms of As(III), As(V), MMA and DMA were prepared by dissolving the corresponding compounds in DDW. Stock solutions of 1 000 μg mL^{-1} antimony and selenium in forms of Sb(III), Sb(V), Se(IV) and Se(VI) were also prepared by dissolving the corresponding compounds in DDW. The arsenobetaine standard solution was used as the stock solution without further treatment.

3.2.2 Reagents

Two chelating reagents, namely ammonium pyrrolidinedithiocarbamate (APDC) and sodium dibenzyldithiocarbamate (DBDC), were purchased from Sigma and Aldrich, respectively. A 5%(w/v) of APDC aqueous solution was prepared and it was purified by extraction with 5 mL of 4-methyl-2-pentanone (MIBK). This purification step also served as a step to co-equilibrate the aqueous APDC solution with MIBK. This solution was found to be stable for more than one month when it stored in a dark and cool place.

A 2% DBDC solution in 75% methyl alcohol was prepared, and this solution was also purified and co-equilibrated with MIBK.

Solvents, MIBK (99.5+%, HPLC grade), methyl alcohol (99.9+%, PRA grade) and acetonitrile (99.93%, HPLC grade) were purchased from Aldrich. Chloroform, *o*-xylene and 2,6-dimethyl-4-heptanone (DIBK) were purchased from Caledon. Cyclohexane (certified A.C.S.) and toluene (AR) were purchased from Fisher and BDH, respectively. These solvents were used without any treatment.

L-cysteine (>99.5%) and potassium thiosulphate (hydrate) were purchased from Fluka and Sigma, respectively. A 5%(w/v) solutions of these two chemicals were freshly prepared before use and purified by extraction with 1 mL of 5% APDC and 5 mL of MIBK. Disodium ethylenediaminetetraacetate (Na_2EDTA, AR) was purchased from Fisher. A 6%(w/v) solution was prepared and purified by APDC/MIBK extraction method as described above.

Reagent grade nitric acid was purchased from Fisher. Quartz sub-boiling distilled nitric acid (67-70%) and acetic acid (glacial, >99%) were purchased from Seastar. Ammonia solution (20-22%) was also purchased from Seastar. The DDW was prepared by passing quartz-distilled water through a mixed-bed deionizing water purification column supplied by Fisher. A pH 5.5 acetic acid-acetate buffer solution was prepared from 0.05M acetic acid and 1M ammonia solution and further purified by APDC/MIBK extraction.

Ion pairing reagents, namely 99% pure cetyltrimethylammonium hydrogen sulfate (CTA) and tetramethylammonium hydrogen sulphate, were purchased from Aldrich and Fluka, respectively. Ammonium dihydrogen phosphate (99.999%) and ammonium hydrogen phosphate (99.99+%) were purchased from Aldrich. These chemicals were used for making HPLC buffers.

Chemicals used for synthesis of MMA, including iodomethane (99%), arsenic (III) oxide (99%) and sodium hydroxide (97+%), were all purchased from Aldrich as well.

Amberlite XAD-4 nonionic polymeric adsorbent (surface area 725 $m^2\ g^{-1}$, pore size 40 Å, particle size 60-80 mesh) and Dowex 50W×8-200 strongly acidic cation exchange resin (cross-linkage 8%, particle size 100-200 mesh) were purchased from

Sigma. Extract-Clean C_{18} cartridges (500 mg per 2.8mL) were purchased from Alltech, and were used to clean up the aqueous extract of fish samples.

3.2.3 HPLC System

The HPLC system used in this work consisted of a pump (Beckman, model 110A), a Rheodyne type injection valve with either a 100-μL or 200-μL loop, a Hamilton PRP-X100 anion exchange column (250 mm×4.1 mm i.d.), and a Hamilton PRP-X100 guard column. A fraction collector (Eldex, universal type) was connected to the output end of the column. The $NH_4H_2PO_4/(NH_4)_2HPO_4$ buffer solutions were prepared by mixing certain amounts of $(NH_4)_2HPO_4$ solution of the required concentration with $NH_4H_2PO_4$ solution of the same concentration to obtain the desired pH value. Buffers were filtered through a Nuclepore membrane filter of 0.2 μm pore size and were degassed using a sonic bath before use. The HPLC column was regenerated by pumping 50-mL of methanol solution containing 0.5 mL 6M HNO_3. The column generally needed regeneration after either several separations of fish extracts or a long waiting period between separations.

3.2.4 Other Apparatus and Equipment

The columns used for solid-phase extractions were Bio-Rad Econo-Columns of 10-cm length and a 0.7-cm i.d. The flow rate was controlled using a removable Teflon stopcock (Bio-Rad).

The polyethylene irradiation vials (Olympic Plastic Company, Inc.) used for irradiations were of two sizes with approximate volumes of 1.1 mL (small) and 7 mL (medium). The vials were precleaned by soaking them in ultrapure 4M HNO_3 for 1-2 days, rinsing thoroughly with DDW, and drying at room temperature.

3.3 Certified Reference Materials

Two certified reference materials (CRM) of natural origin for trace metals prepared by the National Research Council of Canada (NRCC), namely Riverine water SLRS-4 and Seawater NASS-1, were used for evaluating the accuracy of the methods developed in this work for arsenic and antimony. It should be noted here that only the

total elemental concentrations of these materials are certified; no information, certified or not, is available for the individual species of any of the two elements.

Two certified reference materials of marine organism origin for trace metals also prepared by the NRCC, namely Dogfish Muscle DORM-2 and Dogfish Liver DOLT-2, were used to evaluate the total arsenic determination method by INAA.

A CRM of total arsenic, AsB and DMA in tuna fish tissue (CRM 627) prepared by the SM&T Programme of the European Union was used to evaluate the speciation analysis of fish samples [77, 306, 307]. This newly prepared CRM is the only CRM which gives information on arsenic speciation.

3.4 Preparation of Disodium Monomethylarsonate

Monomethylarsonic acid (disodium salt, MMA), which was used in the experiment as an arsenic speciation standard, was synthesized by the author because it was not commercially available. The synthesis was based on the Meyer reaction as shown below and the actual procedure was modified from those published in the literature [308, 309].

$$Na_3AsO_3 + CH_3I \longrightarrow CH_3AsO_3Na_2 + NaI$$

3.4.1 Synthesis and Purification of MMA

About 19.8 g of arsenic (III) oxide (As_2O_3, Aldrich, 99%) and 24.5 g of sodium hydroxide pellets (NaOH, 97+%, Aldrich) were dissolved in 200 mL distilled water. After about 30 to 60 min, when the temperature of the mixture was down to 30°C, the mixture was transferred into a three-neck flask, connected with a condenser, a thermometer, and an opening for adding iodomethane and taking samples for analysis. The flask was placed on a hot plate and magnetic stirring device. About 28.9 g of iodomethane was added to the mixture in the flask, the stirring was turned on, and the mixture was warmed to about 30-40°C. The reaction lasted for 7 h, with occasional yield test by iodometry to see if the reaction had stopped. During the first 5 h of the reaction a few grams of additional iodomethane were added in order to compensate for its loss by

evaporation. It was found that the reaction stopped after 5.5 h., and the yield was as high as 97 %.

After the reaction stopped, the unreacted iodomethane was removed by heating the solution. Also, through this step of heating, the volume of the solution was reduced to about 100 mL. About 200 mL of ethyl alcohol was added into the solution after the solution was cooled down to room temperature to precipitate the disodium salt of MMA. Sodium iodide remaining in the solution was removed by suction filtration.

The product was purified four more times by using the following re-crystallization procedure. The MMA disodium salt was first dissolved in a small amount of DDW, and then about 150 mL of ethyl alcohol were added to precipitate the MMA disodium salt. The precipitate was filtered by suction 20 min after the addition of ethyl alcohol. Small amount of ethyl alcohol was used to wash the crystals. Finally, the product was dried in air and kept in a desiccator. The overall yield was >75%.

3.4.2 Identification of MMA

The white crystals of MMA disodium salt were analyzed for water of hydration by a gravimetric method. It was found that the compound contained 5 to 6 moles water, which is in a very good agreement with reported value in the literature [310].

The relative contents of sodium and arsenic in the properly dried MMA disodium salt were determined by INAA. The product was dried at 110°C for 12 h to remove all the hydrated water and possible moisture before the analysis. It was found that the compound contained 40.75% of arsenic and 25.15% of sodium, which agrees well with the calculated values (40.75% of arsenic and 25.00% of sodium).

The dried MMA disodium salt was also analyzed using NMR for the presence of the methyl group using a Bruker AC-250 NMR spectrometer operating at 250.13 and 62.90 MHz for ^{1}H and ^{13}C NMR spectra, respectively. Absorption at about 4.82 ppm corresponding to three H signal in ^{1}H NMR spectrum and an absorption at 19.70 ppm in ^{13}C NMR spectrum were found, as expected.

3.5 Sample Collection and Preparation

3.5.1 Natural Water Sample Collection

All containers and apparatus used for the collection and storage of the water samples were carefully precleaned. The cleaning procedure comprised washing the container with laboratory grade detergent, soaking in 4M reagent grade HNO_3 for 24 h, washing with tap water, rinsing with DDW, and drying. The natural water samples for speciation analysis were collected from different sources. A commercially available natural spring water sample (Evian) was purchased from the local grocery store. A seawater sample was collected from one of the seawater taps located in the Department of Oceanography of Dalhousie University. A lake surface water sample was collected from the Morris Lake located in Dartmouth, Nova Scotia in 1999 September. A rain water sample was collected in the Studley campus of Dalhousie University in July1999.

3.5.2 Filtration and Acidification of Water Samples

The natural water samples were suction filtered soon after collection using a Nuclepore membrane of 0.2 μm pore size to remove the particulate matter. The speciation analysis on the soluble fraction of the water was performed soon after the filtration. In some cases, if the analysis was delayed, the water samples were stored in a refrigerator before the analysis. If the sample needed to be stored for a longer period and for the determination of the total elemental content, then it was acidified to pH 3 with 20% HNO_3 solution.

3.5.3 Lyophilization of Fish Samples

The fish samples were collected by Health Canada and were categorized under four items as marine fish, fresh water fish, canned fish, and shellfish. The samples were received in crushed pieces, stored in polyethylene bottles, and kept frozen. The freeze-drying was done in the following manner. The samples were thawed at room temperature for about 24 h before weighing; then they were weighed into 125 mL precleaned Teflon jars for freeze-drying. The Nalgene jars and the Teflon coated spatulas used to transfer the samples were carefully acid-washed before using. A piece of ultraclean Teflon sheet (Clean Room Products Inc.,10 cm×10 cm) with two small holes was used to cover each

jar, and a Teflon sheet was used to line the freeze-drier trays to prevent contamination during the freeze drying. The freeze-drying was performed in a tray-type freeze-dryer (Virtis model 50SRC6) at the Institute of Marine Biosciences, NRCC, Halifax. The samples were freeze dried for about one week. Afterwards, the samples were re-weighed and the moisture content of the samples was calculated.

3.5.4 Homogenization of Fish Samples

Homogenization was carried out using a precleaned porcelain mortar and a pestle. The homogenized samples were stored in the Nalgene jars and capped tightly to avoid to absorption of water.

3.6 General Procedures for Solvent Extraction

The sample or standard solution, containing the species of interest at a selected volume and pH, was added into either an extraction tube of 25-mL size or a separatory funnel of up to 250 mL. A chelating agent solution and an organic solvent were added to it and the mixture was then vigorously shaken for 10 min on a wrist action shaker. After the phase separation, about 1 mL of the organic solvent was transferred into a small-size irradiation vial, and heat-sealed for NAA. If a back-extraction was involved, a certain amount of the organic solvent after the phase separation was transferred into a new extraction tube or a new separatory funnel and back-extracted with suitable aqueous solution by vigorously shaking for 10 min on a wrist action shaker. After the phase separation, about 1 mL of the aqueous solution was pipetted into a small-size irradiation vial for NAA. If a solid form is required for longer irradiation, this aqueous solution was transferred to a medium-size irradiation vial, dried under an IR lamp, and sealed for NAA.

A few important factors affecting the solvent extraction were optimized. These factors included types of chelating agent and solvent, pH of the aqueous phase, extraction volume ratio, back extraction conditions and the volume ratio, stability of the metal complex, and reduction method for the species in the higher oxidation state.

For assaying arsenic and antimony, samples in liquid forms (MIBK or HNO_3 solution) were irradiated for 10 to 30 min and counted for 10 to 30 min after a minimum

decay time of 5 h. For solid samples, the irradiation time was 2 to 5 h, and the counting time varied from 2 to 10 h depending on the levels of the species after a decay time of about 40 h. The determination of selenium was carried out by irradiation of the liquid MIBK following a time scheme of t_i-t_d-t_c=30-10-30 s. If a CINAA is desired, several irradiation-decay-counting cycles were performed on the same sample, and the spectra were summed [311].

3.7 General Procedure for Solid-phase Extraction

3.7.1 Adsorption of HDBDC on XAD-4 Resin

The XAD-4 resin was carefully cleaned using 4M HNO_3 solution, 2M NH_3 solution and DDW in sequence, and then dried at 80°C. About 1 g of the cleaned XAD-4 resin was equilibrated with 100 mL of the chelating reagent solutions at concentrations ranging from 10 to 20 mM by vigorous shaking on a mechanical shaker for 3 h. Then the resin was suction filtered and washed with DDW several times to remove the unadsorbed chelating reagent solution. This ligand-impregnated resin was kept in DDW for the solid-phase extraction.

3.7.2 Solid-phase Extraction Experiment

The solid-phase extraction experiment on column was carried out by slurry packing 1.2 g of the prepared resin into a column and passing the sample or standard species solutions at a selected pH through the column. After flushing the column with an additional 5 to 10 mL of DDW, the resin inside the column was air dried and transferred to an irradiation vial for NAA.

3.8 General Procedure for Ion-exchange Chromatography

3.8.1 Static Distribution Experiments

The Dowex 50W×8-200 resin was first slurry packed into a column and cleaned by washing it with 8 column volumes of 6M HNO_3 solution followed by 8 column volumes of DDW. The resin was then transferred to a beaker and dried at 80°C. About 0.4 g of the dried resin was equilibrated with 5 mL solutions containing the species of interest in different concentrations of HNO_3, NH_3 and DDW. After shaking for 1 h, the

resin and the solution were separated by centrifugation, and a certain amount of the solution was pipetted to the irradiation vial for NAA. The static distribution coefficient of the species was calculated as the ratio of the concentration of the species in the resin phase (mmol g^{-1}) to that in the solution phase (mmol mL^{-1}).

3.8.2 Column Separation

About 2 g of the Dowex 50W×8-200 resin was slurry packed in the Bio-Rad Econo column as described above. Up to 1 mL of the arsenic species standard solution to be tested was loaded on the column first, then the column was eluted by an appropriate eluting reagent and the eluate was fraction collected directly in the small-size irradiation vials, the volume of each fraction varied depending on the total volume collected. For example, for the weakly retained species, such as As(III), As(V) and MMA, the volume of each fraction was set at 0.5 mL or less. The vials were then heat-sealed and analyzed by NAA. The elution curve was constructed by plotting the counts at the 559.1-keV photopeak of ^{76}As (or the arsenic concentration) against the elution volume. For the strongly retained species, such as AsB and DMA, the collection volume was set at 1 mL every 10 to 40 mL. These 1-mL portions were heat-sealed in small-size vials and analyzed by NAA for arsenic. The elution curve was again plotted.

3.9 General Procedure for HPLC Separation

The HPLC column was conditioned with a suitable buffer solution first, then the sample solution containing the species of interest was loaded into the sample loop of the injector and it was injected onto the column. The fraction collector was activated immediately after the injection and 0.25-mL fractions were collected in precleaned small-size irradiation vials which were then heat-sealed for NAA.

The following factors were optimized in HPLC separation: the concentration of the $NH_4H_2PO_4/(NH_4)_2HPO_4$ buffer, the pH of the buffer, and the volume of each fraction. They were optimized by injecting 200 μL of 50 μg mL^{-1} standard solutions of all five arsenic species. The collected fractions were heat-sealed in small-size irradiation vials and analyzed by NAA. Short irradiation and counting times were used for standard

because of the relatively high concentrations of arsenic. Long irradiation and counting times of 2 to 5 h were used in the analyses of natural water or fish samples.

4. DEVELOPMENT OF METHODS FOR THE SPECIATION OF ARSENIC

This chapter describes the development of a number of analytical methods for the determination of inorganic and organic arsenic species, and of inorganic antimony and selenium species. These methods include open-column cation exchange chromatography for the separation of DMA and AsB from other arsenic species, solvent extraction and solid-phase extraction for inorganic arsenic, antimony and selenium species, and HPLC using an anion exchange column for As(III), As(V), MMA, DMA and AsB species. The evaluation of various parameters related to the separation and the determination by NAA are discussed below. The advantages and disadvantages of the methods developed are also described.

4.1 Separation of Arsenic Species by Cation Exchange Chromatography

It is evident from the literature survey (Section 2.4) that ion exchange chromatography is useful for the separation of cationic, anionic and neutral arsenic species. We have developed an open-column cation exchange chromatographic method for the separation of cationic arsenic species from the other species. The details of this method development are given below.

4.1.1 Distribution Coefficients of Arsenic, Antimony and Selenium Species

In order to determine the distribution coefficients (K_d) of various arsenic species, and inorganic antimony as well as selenium species, static experiments were carried out using a cation exchange resin and aqueous phases of different acidity. Since sodium ion is one of the interfering species in NAA and since it is present at high concentrations in both seawater and seafoods, it was also included in these experiments. Dilute solutions (10 $\mu g\ mL^{-1}$) of these species were prepared from stock solutions, and the acid or base solutions were added. Then 10 mL of these solutions were equilibrated with 0.4 g of Dowex 50W×8 resin for 10 h. The mixture was centrifuged and the supernatant solution was decanted. About 0.75 mL of the decanted solution was directly pipetted into a small-size irradiation vial, heat-sealed, and analyzed for the elements by NAA. The

concentrations of each species in both phases were calculated. The K_d of a species can be defined as:

$$K_d = [S]_r / [S]_a \quad (4.1)$$

where $[S]_r$ refers to the concentration of the species in the resin phase while $[S]_a$ is that in the aqueous phase. The K_d values of As(III), As(V), DMA, MMA, AsB, Sb(III), Sb(V), Se(IV), Se(VI), and Na(I) were calculated and are shown in Table 4.1.

In general, high K_d values were observed under slightly basic to acidic conditions for almost all cationic species tested. Among all the cationic species, AsB had the highest K_d of 2 540 in water (DDW). The amphiprotic nature of MMA and DMA provides an explanation for their higher K_d on cation exchange resin compared to the inorganic arsenic species as pointed out by Diets and Perez [211]. A K_d of 2.5 was obtained for MMA in water in this thesis, which agrees well with the reported value of 3; the corresponding value for DMA was not measured and reported simply as very large [211]. It was also found that Sb(III) is basic enough to exist as a cation in the slightly acidic to slightly basic solutions. The K_d values shown in Table 4.1 suggest that cation exchange chromatography could be conveniently used for the group separation of AsB and DMA from As(III), As(V) and MMA. Although Diets and Perez [211] were able to separate inorganic arsenic species from MMA by using a very long column containing 200 g of AG 50W×8 [211] for checking the purity of synthesized MMA, this method is not well suited for the separation of MMA from As(III) and As(V) at low levels in real samples because of the large amount of the resin as well as the large volume of eluent needed.

4.1.2 Elution of Arsenic Species from the Cation Exchange Column

The elution curves of four selected arsenic species, namely As(III), As(V), MMA and DMA, on the cation exchange column by water and 0.025M acetic acid solution (pH 3.2) are presented in Figs. 4.1 through 4.4. Both As(III) and As(V) were eluted by water as well as acetic acid from the column just after two void volumes equaling 2.4 mL. Obviously, they were not retained, as expected, since they were present as anionic arsenite and arsenate species.

Table 4.1. K_d of Various Species on Cation Exchange Resin at Different Conditions

Species	K_d									
	4M ammonia	1M ammonia	0.1M ammonia	0.01M ammonia	H_2O	0.001M HNO_3	0.01M HNO_3	0.1M HNO_3	1M HNO_3	4M HNO_3
AsB	1.0	0.5	1280	1920	2540	1760	800	150	12.4	1.8
DMA	<0.1	<0.1	300	390	460	410	310	90	9.4	1.9
MMA	<1	<1	<1	2	2.5	2	2	2	1.6	<1
As(III)	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
As(V)	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
Sb(III)	1.6	2.8	39	46	48	63	98	45	6	1.4
Sb(V)	<1	<1	<1	1.2	1	2	1.7	2.5	<1	<1
Se(IV)	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
Se(VI)	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
Na^+	80	108	136	120	130	130	100	31	3	<1

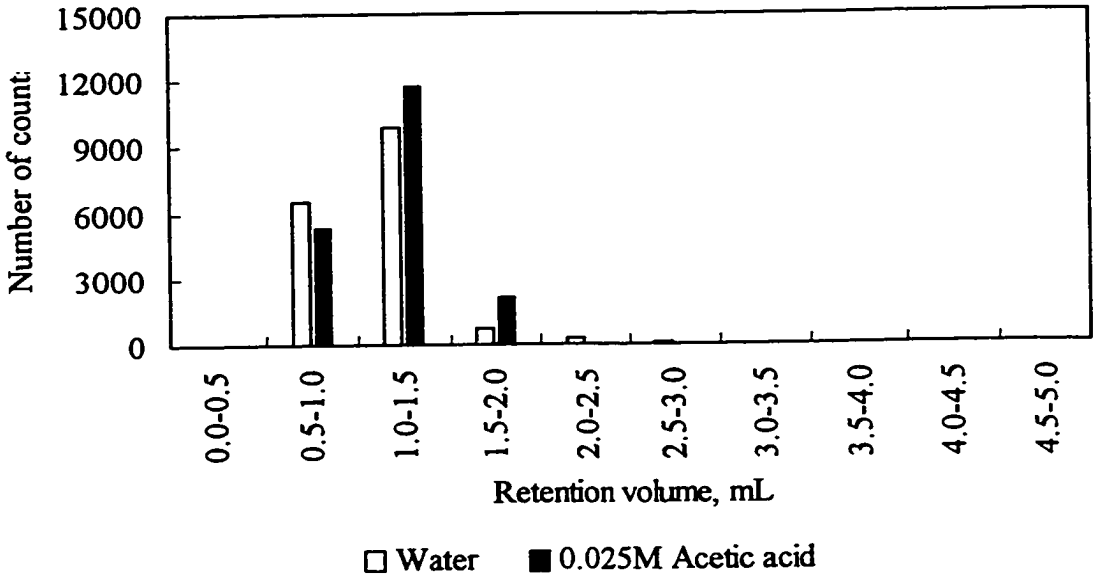

Fig. 4.1 Elution of As(III) on a cation exchange column

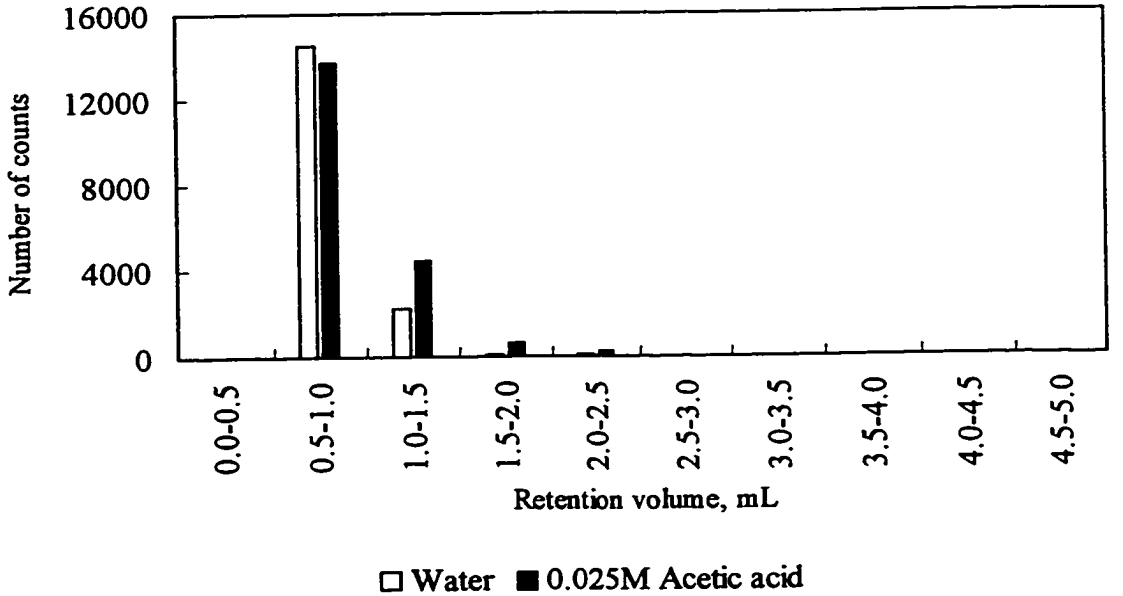

Fig. 4.2 Elution of As(V) on a cation exchange column

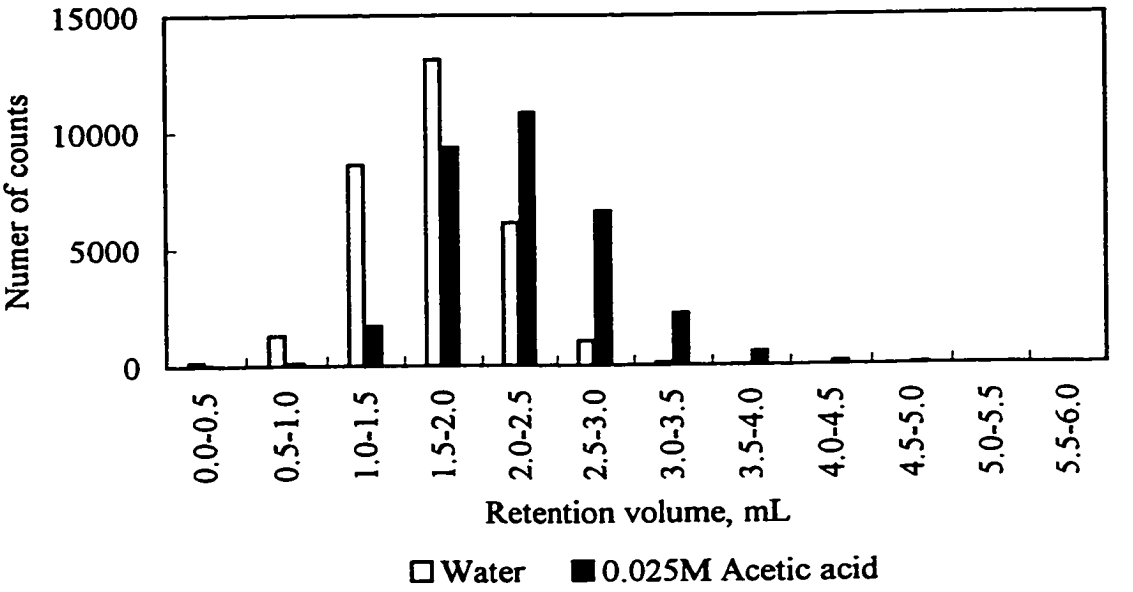

Fig. 4.3. Elution of MMA on a Cation Exchange Column

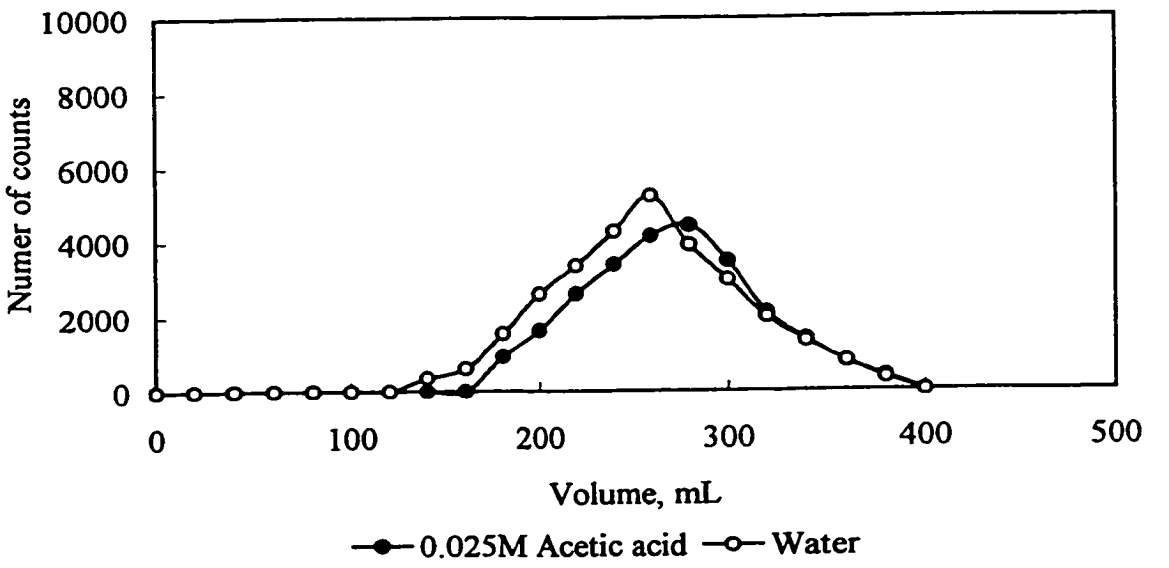

Fig. 4.4. Elution of DMA from a Cation Exchange Column

The MMA peak was eluted from the column shortly after As(III) and As(V) by both eluting agents (Fig. 4.3). For the purpose of separation, the difference in the elution volumes of these three species is too small to be of any practical value. On the other hand, DMA was eluted between 120 and 380 mL of water and between 160 and 400 mL of 0.025M acetic acid (Fig. 4.4). It was observed that AsB was so strongly retained by the column that even after elution with 500 mL of either water or 0.025M acetic acid, it was not detected in the eluate. It is therefore possible to separate either DMA alone or DMA and AsB from As(III), As(V) and MMA. It is also possible to separate DMA from AsB; however, the time required to do this separation would be prohibitively long under these experimental conditions.

4.1.3 Effect of Column Height on the Retention of DMA

A larger retention volume for DMA was obtained by using a longer column as shown in Fig. 4.5. The DMA was retained by a 17-cm long column and up to a volume of 250 mL, which is almost the double of that by a 8.5-cm long column. These results suggest that in an actual column separation procedure, up to 250 mL of water sample can be loaded on to the column to collect the AsB and DMA present in it.

4.1.4 Elution of DMA and AsB from the Cation Exchange Column

As indicated in the static distribution experiment (Section 4.1.1), AsB and DMA species have higher K_d on the cation exchange resin in slightly basic (0.1M ammonia) to slightly acidic (0.01M HNO_3) solutions, and have lower K_d in more concentrated HNO_3 and ammonia solutions. Therefore, more concentrated acid or base solutions could be used for a rapid elution of these species from the column.

Although both 1M ammonia and 4M HNO_3 solutions could elute AsB and DMA from the column effectively, a closer look at the K_d suggests that the 1M ammonia solution should be a better eluting agent because it could also remove sodium ions quantitatively which is advantageous if NAA is the detection technique. The reason for this is quite simple. If the cation exchange column is used to separate AsB and DMA from natural water samples containing high levels of sodium such as seawater, it will

concentrate all of these three cationic species on the column. Then if 4M HNO_3 solution is chosen as the eluting agent, sodium will also be eluted along with AsB and DMA (Fig. 4.6). If, however, 1M ammonia is selected as the eluting agent, only AsB and DMA will be eluted leaving sodium on the column as shown in Fig. 4.7. It is evident that AsB and DMA could be completely eluted by 12 mL of 1M ammonia solution, while the sodium ion was retained on the column.

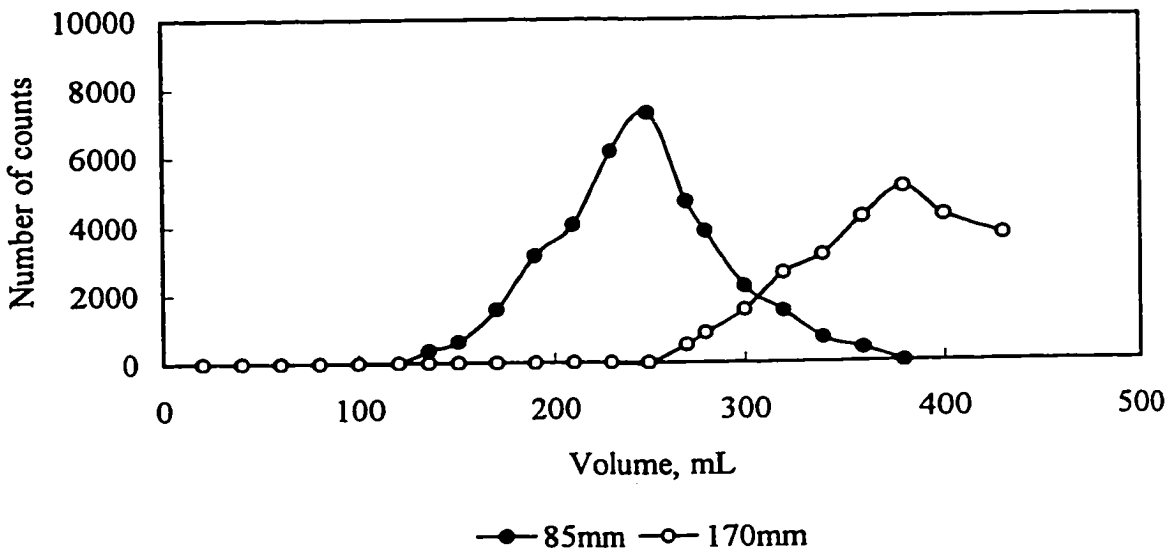

Fig. 4.5. Elution of DMA by DDW from Cation Exchange Columns with Different Resin Bed Heights.

4.1.5 Conclusions

In the past, open-column cation exchange chromatography has mostly been applied to samples containing high levels of arsenic species using large amounts of resins and large volumes of eluting agents [209-211, 216-220]. Consequently, a satisfactory enrichment factor was more difficult to obtain and this technique was not widely used in recent years. However, the selective retention of AsB and DMA by the cation exchange

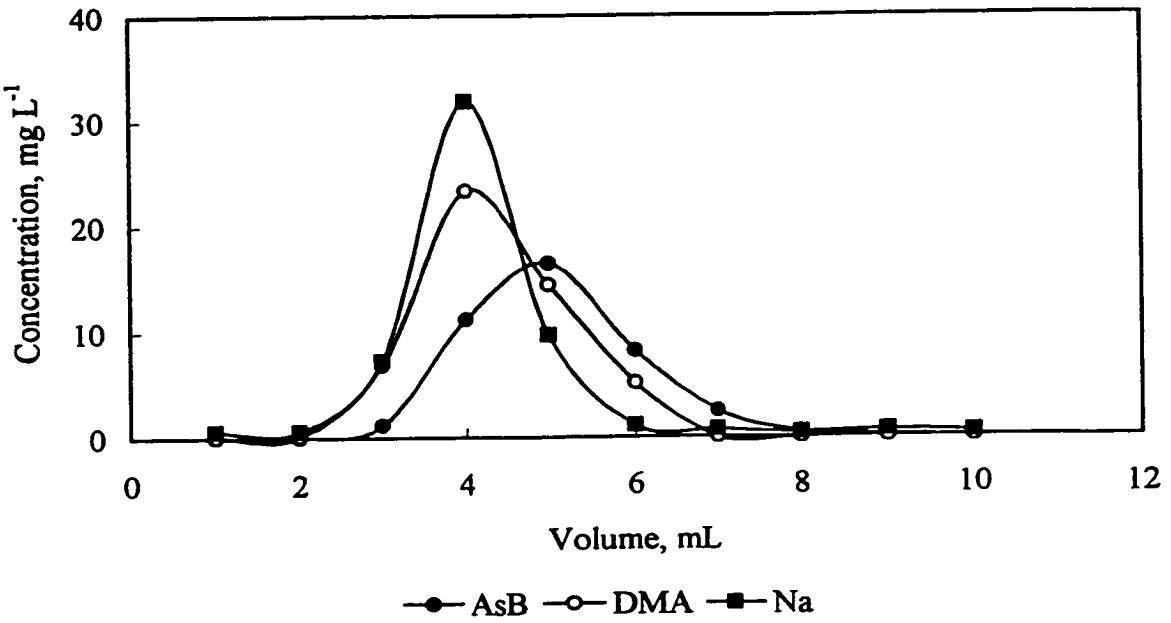

Fig. 4.6. Elution of AsB, DMA and Na^+ by 4M HNO_3 Solution

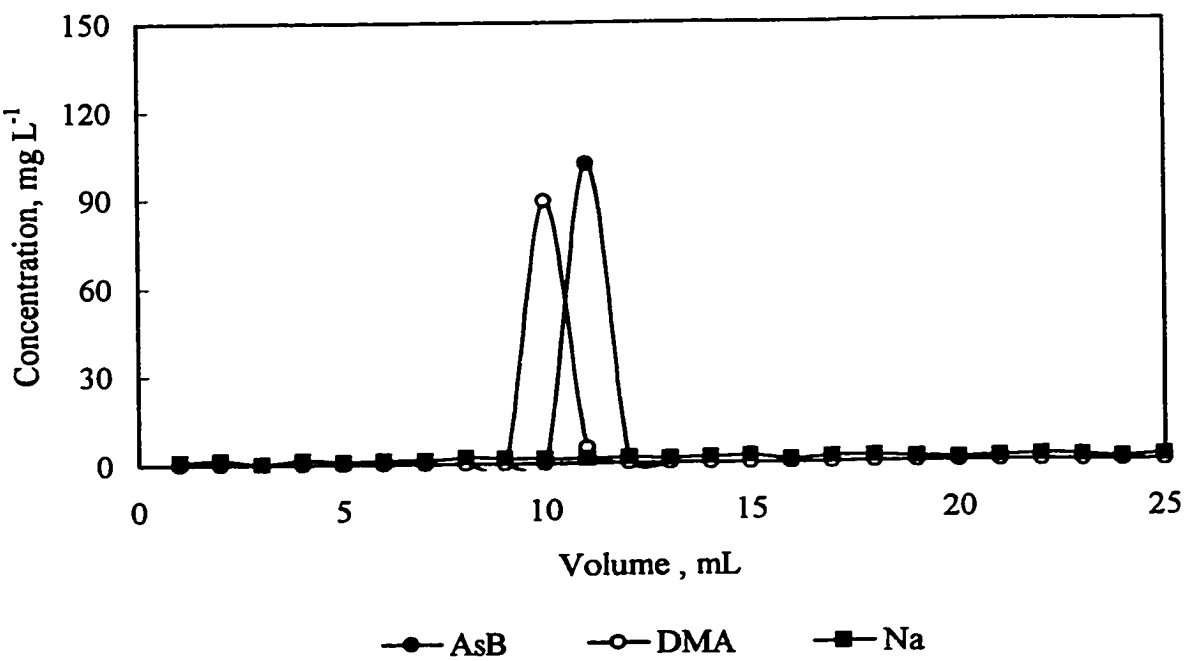

Fig. 4.7. Elution Curves of AsB, DMA and Na^+ by 1M Ammonia Solution

column can be advantageously used as a group separation step for cationic arsenic species only as has been done in this thesis. This method can also be used not only to preconcentrate the species but also to reduce interference from sodium ions, as shown in our work. Moreover, this step eliminates the problem of distinguishing As(III) from AsB peaks which are commonly encountered in HPLC using anionic exchange columns.

4.2 Separation of Arsenic, Antimony and Selenium Species by Solvent Extraction

It is evident from the literature survey (Section 2.1) that solvent extraction is one of the useful techniques for the speciation of arsenic. A solvent extraction method has been developed for the separation of inorganic arsenic, antimony and selenium species. The details of this method development are given below.

4.2.1 Selection of a Solvent

Six solvents, namely chloroform, cyclohexane, DIBK, MIBK, toluene, and *o*-xylene, were evaluated for their suitability for extracting As(III), Sb(III), and Se(IV) species using APDC as the chelating agent at 1:1 volume ratio and pH 5.5. The recoveries of each species together with some of the properties of these solvents are listed in Table 4.2.

Quantitative recoveries (>95%) of all three species were obtained with four solvents, namely chloroform, DIBK, MIBK, and toluene. Although *o*-xylene was found to extract Sb(III) and Se(IV) quantitatively, the recovery for As(III) was only 77% (Table 4.2). Cyclohexane gave very poor recoveries, as expected, due to the non-polar nature of the solvent. Similar very low extraction yield for Sb(III) has been reported by Kamada and Yamamoto [137] using another non-polar solvent, CCl_4. The extraction yields were found to decrease with decreasing values of both dielectric constant and dipole moment as shown in Table 4.2.

Other parameters used for selecting a solvent include density, boiling point, and its solubility in water. Obviously, solvents with higher boiling points, lower solubility in water, and greater difference in density compared to water are preferred. Chloroform has

Table 4.2. Recoveries of As(III), Sb(III) and Se(IV) by using Different Solvents

Solvent	Recovery, %			Density (20°C)* g mL^{-1}	b.p. °C*	Solubility (20 °C)* wt. %,	Dielectric constant (20 °C)*	Dipole moment (20°C)* debye unit
	As(III)	Sb(III)	Se(IV)					
MIBK	103 ± 5	100 ± 6	100 ± 4	0.801	115.7	1.7^{25}	13.11	
Chloroform	104 ± 5	101 ± 6	99 ± 4	1.484	61.7	0.80	4.81	1.1^{25}
DIBK	101 ± 5	100 ± 6	100 ± 2	0.806	165	0.05		
Toluene	101 ± 4	100 ± 6	100 ± 2	0.866	110.6	0.05	2.38	0.45
o-Xylene	4 ± 1	8 ± 2	5.1 ± 0.5	0.880	144		2.57	0.64
Cyclohexane	77 ± 4	97 ± 6	102 ± 2	0.779	80.7	0.01	2.02^{25}	0

* [312, 313]

the highest difference in density with water and should be a very good solvent; however, the chlorine in chloroform becomes highly active on irradiation with neutrons and makes the use of short-lived nuclides, such as ^{77m}Se (half-life = 17.4 s) very difficult if not impossible. The unpleasant smell of DIBK makes it an undesirable solvent for extraction. Both toluene and MIBK would be good solvents for the present study. MIBK was finally selected by an overall consideration of its higher boiling point, lower solubility in water, acceptable density difference with water, not a very unpleasant odor, and no interfering elements.

4.2.2 Selection of a Chelating Agent and pH

Both APDC and DBDC chelating agents were evaluated in conjunction with MIBK for the extractions of As(III), As(V), Sb(III), Sb(V), Se(IV) and Se(VI) as a function of pH (ranging from 1 to 10), and the results are presented in Figs. 4.8 and 4.9, respectively.

As shown in Fig. 4.8, As(III) can be quantitatively extracted by APDC/MIBK in the pH range of 3-9 whereas As(V) is not extracted at all at pH 5-10. The lower recovery of As(III) at pH less than 3 might have been caused by the instability of As(III)-PDC^- complex in MIBK during the phase separation as explained in Section 4.2.6. Up to 25% As(V) can be extracted at pH 2-5. Therefore, these two arsenic species can be separated from each other by APDC/MIBK at pH 5-9.

The quantitative recovery of Sb(III) in the pH range of 1-10 indicates that Sb(III) is more readily extracted than As(III) and Se(IV) by APDC/MIBK. It was found that about 80% of Sb(V) could be extracted into MIBK at pH 1-3, and none above pH 5. Therefore, Sb(III) can be quantitatively separated from Sb(V) at pH 5-10. Other researchers have also reported similar results using APDC/chloroform [132], APDC and a mixed solvent system of chloroform and carbon tetrachloride [134], and APDC/MIBK [137]. The pH range for the quantitative extraction of Se(IV) from Se(VI) was found to be from 1 to 7 using the APDC/MIBK system.

The different effects of pH on the extraction of As(V), Sb(V) and Se(VI) can be explained in terms of their acid dissociation constants. Se(VI) in the form of H_2SeO_4 has a pK_{a2} 1.7, and As(V) in the form of H_3AsO_4 has pK_{a1} 2.2, pK_{a2} 6.9 and pK_{a3} 11.5;

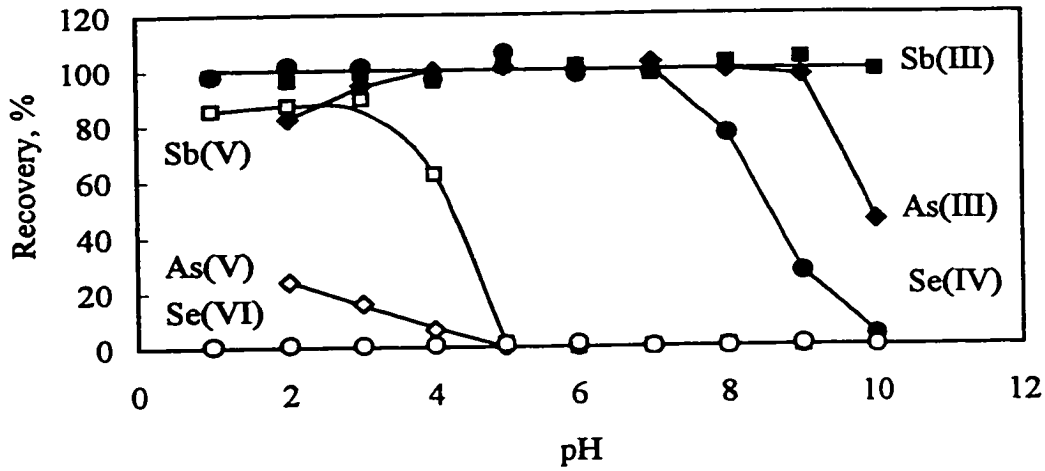

Figure 4.8. Extraction Recoveries of Various Species by APDC/MIBK at Different Aqueous Phase pH

◆ As(III) ◇ As(V) ■ Sb(III) □ Sb(V) ● Se(IV) ○ Se(VI)

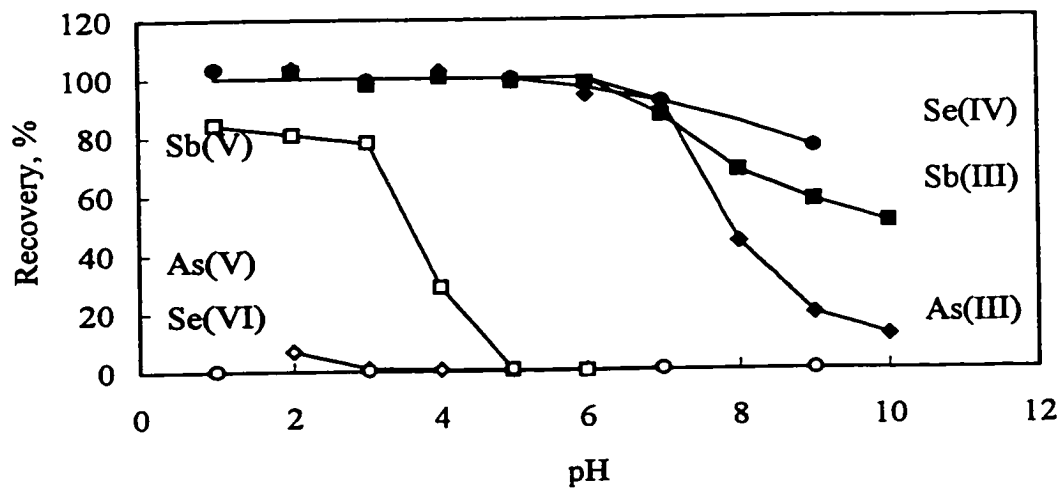

Fig. 4.9. Extraction Recoveries of Various Species by DBDC/MIBK at Different Aqueous pH

◆ As(III) ◇ As(V) ■ Sb(III) □ Sb(V) ● Se(IV) ○ Se(VI)

however, Sb(V) in the $HSb(OH)_6$ form has pK_a 4.4. So Sb(V) is weakest among these three acids and shows a higher partition between the organic phase to the aqueous phase.

In general, The extraction behavior of As(III), Sb(III) and Se(IV) by DBDC/MIBK at pH 1-10 is similar to that by APDC/MIBK. However, APDC was selected as the chelating agent in this work because of its high purity, ease of availability, and high solubility in water.

4.2.3 Effect of pH on the Extraction of MMA, DMA and AsB

Experiments were carried out in order to ensure that MMA, DMA and AsB do not interfere with the extraction of As(III) and As(V) in the selected pH range. The extraction of the above three organic arsenic species by organic solvents has not yet been extensively studied. Hasegawa and coworkers [150] are the only researchers who reported that MMA and DMA could not be extracted with DDTC/chloroform at a pH range of 2 to 10. The results obtained in this thesis using APDC/MIBK are presented in Fig. 4.10. These graphs clearly indicate that AsB remained completely un-extracted in the aqueous phase in the pH range of 2 to 10. No MMA was extracted above pH 3 while no DMA was extracted above pH 4. These results suggest that MMA, DMA and AsB would not interfere with the determination of As(III) above pH 4 by the APDC/MIBK system.

4.2.4 Back Extraction of As(III), Sb(III) and Se(IV)

The use of a back extraction step in a separation procedure has certain advantages [132, 133, 140]. It can remove the organic solvent, as needed for some elemental determination techniques, as well as preconcentrate the analyte further. In the present work, aqueous solutions of 0.1 to 6M HNO_3 were used to back extract As(III), Sb(III) and Se(IV) from MIBK. The results are presented in Table 4.3. It is evident that 4M HNO_3 could be used to quantitatively back extract As(III) and Sb(III). However, only 36% of Se(IV) could be extracted at this acidity, which increased to 66% with 6M HNO_3. This poor recovery of Se(IV) is explained in terms of phase volume ratio which is described in Section 4.2.5.

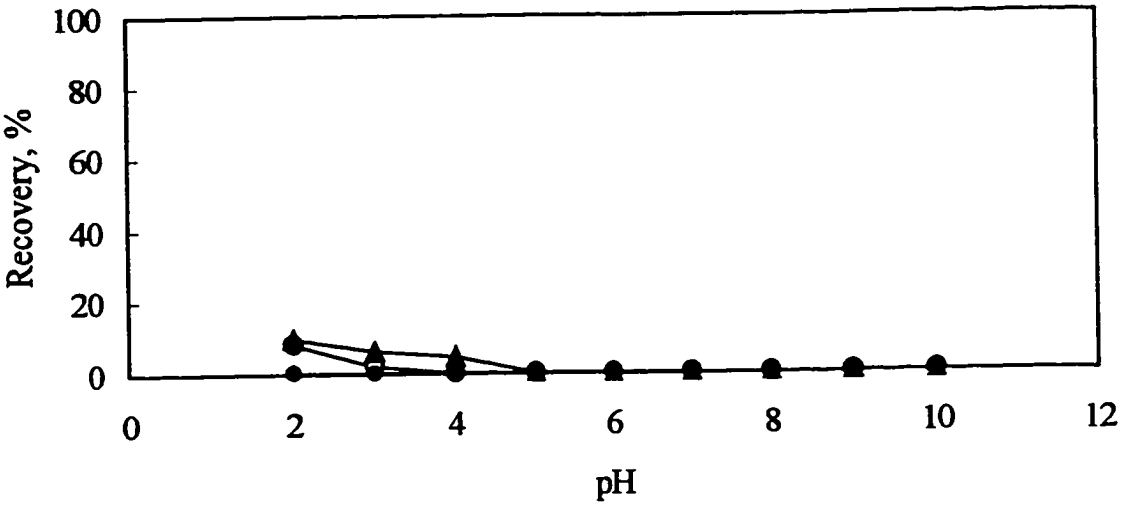

Fig. 4.10. Effect of pH on the Extraction of MMA, DMA and AsB by APDC/MIBK

Table 4.3. Back Extraction Recoveries by Different Concentrations of HNO_3

HNO_3, M	0.1	1	4	6
As(III)	93 ± 4	99 ± 4	99 ± 2	99 ± 1
Sb(III)	82 ± 4	90 ± 3	98 ± 3	100 ± 4
Se(IV)	7 ± 2	8 ± 2	36 ± 2	66 ± 3

In order to achieve detection limits of the order of ppb by NAA using the DUSR facility, the samples need to be irradiated for a relatively long period. Liquid samples, whether in MIBK or HNO_3, cannot be directly irradiated for such a long time because of the possible rupture of the polyethylene irradiation vials due to a pressure build-up. Attempts were made to evaporate MIBK to dryness by heating under an IR lamp for about 3.5 h. The results presented in Table 4.4 show that there is a significant loss, on the average 27%, of As(III). It is possible that the As(III) extracted in MIBK as $As(PDC)_3$ is volatile enough to cause a significant loss during evaporation. Back extraction into an

aqueous phase not only eliminated this loss but also reduced the time required to evaporate the sample to dryness so that it can be directly irradiated.

Table 4.4. Loss of As(III) during Evaporation of MIBK

Trials	1	2	3	4	5	6	7	8	9	Avg.
As(III) loss, %	35	26	28	25	26	20	33	26	25	27 ± 3

4.2.5 Effect of Phase Volume Ratio on Extraction and Back Extraction

The aqueous-to-organic phase volume ratios in both extraction and back extraction steps are related to the enrichment factor of the species. A higher aqueous-to-organic phase volume ratio in the extraction step and a lower value in the back extraction step are obviously preferred. The phase volume ratio is defined by:

$$V_a/V_o \qquad (4.2)$$

Where V_a is the volume of the aqueous phase and V_o is that of the organic phase. Experiments were carried out to measure the recoveries of As(III), Sb(III) and Se(IV) species with different phase volume ratios in both steps. For the extraction experiments, 50 μg of each of As(III), Sb(III) and Se(IV) were added into a mixture of a buffer of pH 5.5, DDW, and 5% APDC solution to obtain total aqueous phase volumes of 2, 4, 10, 20, 40, 100, and 200 mL. These aqueous solutions were extracted with 2 mL of MIBK. After the phase separation, the concentrations of arsenic, antimony and selenium in the MIBK phases were determined by NAA, and the recoveries were calculated and are presented in Fig. 4.11. For the back extraction experiments, 50 μg of each of As(III) and Sb(III) in 50 mL aqueous phase at pH 5.5 was extracted by 50 mL MIBK. Then 20, 10, 5, and 1 mL portions of this MIBK phase were separately back extracted with 1 mL of 4M HNO_3. The concentrations of arsenic and antimony in the 4M HNO_3 solutions were measured by NAA, and the results are presented in Fig. 4.12.

From the results presented in Fig. 4.11, it is evident that nearly 100% recoveries of As(III) and Sb(III) are obtained if the ratio in extraction step is kept between 1 and 10.

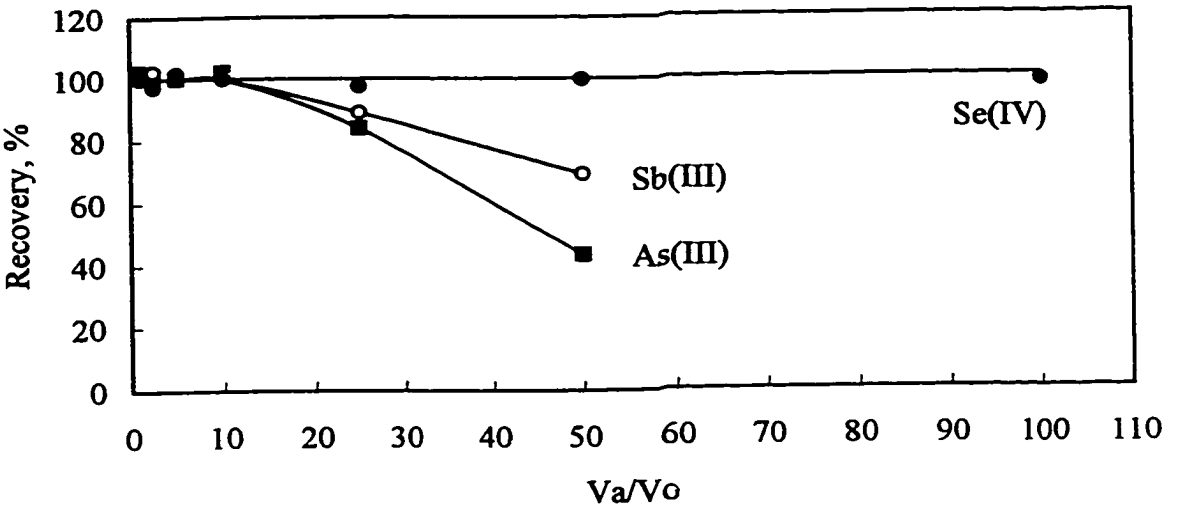

Fig. 4.11. Recoveries of As(III), Sb(III) and Se(IV) at Different Extraction Phase Volume Ratios

■ As(III) o Sb (III) ● Se(IV)

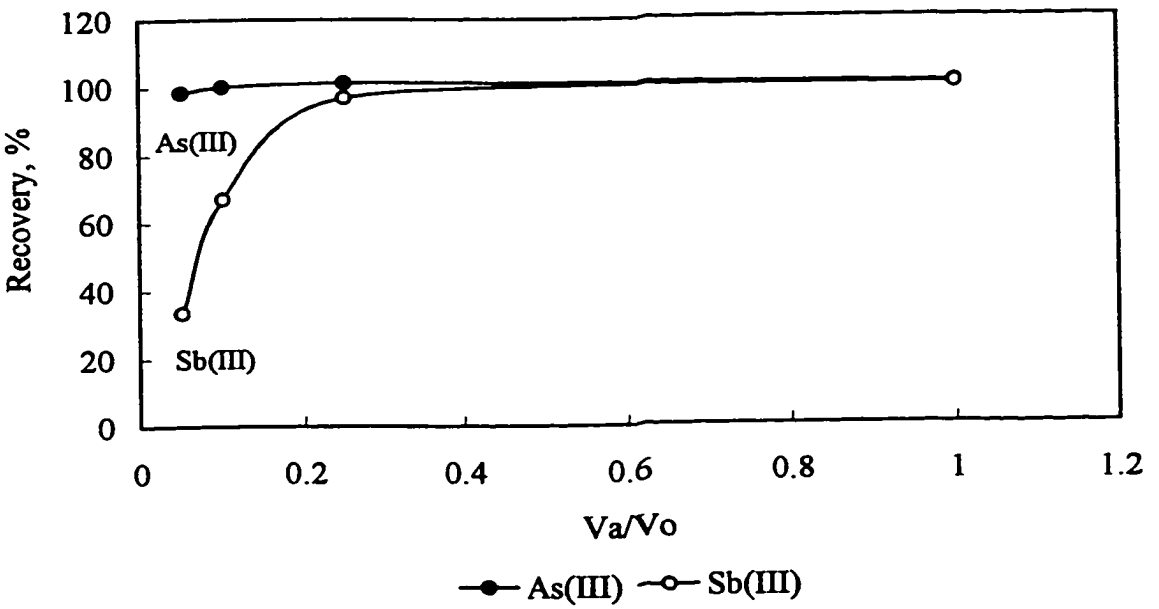

Fig. 4.12. Recivery of As(III) and Sb(III) at Different Back Extraction Phase Volume Ratios

Mok and Wai [132] found that a ratio of 20 or less is desirable for the quantitative extraction of As(III) and Sb(III) using an APDC/chloroform system. For the back extraction step, It was observed that a ratio of 0.25 to 1 gave the best yield for As(III) and Sb(III) as shown in Fig. 4.12.

For the quantitative recovery of Se(IV) in the extraction step, the V_a/V_o ratio can be as high as 100, as indicated in Fig. 4.11. Since selenium was concentrated in the MIBK phase and since it could be determined free from interference through the short-lived nuclide ^{77m}Se by directly irradiating the organic liquid, it was not necessary to go through the back extraction step. Moreover, the back extraction of Se(IV) in 4M HNO_3 was not quantitative as it gave a yield of only 36% at a V_a/V_o ratio of 1 (Table 4.3).

4.2.6 Stability of PDC Complexes of As(III), Sb(III) and Se(IV)

The stability of As(III), Sb(III) and Se(IV) complexes with APDC in MIBK with time was investigated. Experiments were carried out at two pH values (2 and 5.6) using a V_a/V_o ratio of 1 for 326 h from the time of the extraction. At pH 5.6, the concentrations of As(III), Sb(III) and Se(IV) in MIBK were found to be extremely stable for more than 300 h after the extraction as shown in Fig. 4.13. However, at pH 2, the concentrations of the three species decreased with increasing time. This was particularly true for As(III) which decreased significantly within a short time, *viz.* to about 50% of the original value within 5 h. The decomposition of APDC itself at low pH and of the relatively less stable arsenic PDC^- complex could be the main reasons for this observation. Therefore, if the pH of the aqueous phase is kept within 5 and 7, sufficient time (such as overnight) can be allowed for the phase separation without any loss of As(III), Sb(III) and Se(IV).

Kamada and Yomamoto [137] also reported that Sb(III) complex with APDC was stable in MIBK for at least 6 h under pH 5-8. Chung [134] found that the of As(III) and Sb(III) complex with APDC could be stable in the MIBK and carbon tetrachloride mixed solvent for 24 h at pH 5 and that of Se(IV) for 6 h at the same pH.

4.2.7 Reduction of As(V), Sb(V) and Se(VI)

Three experiments were carried out using three different reducing agents, namely L-cysteine (5%), potassium thiosulfate (25%) and potassium iodide (25%), to evaluate

their ability to reduce of As(V), Sb(V), and Se(VI) to As(III), Sb(III), and Se(IV), respectively. About 1 mL of each of the reducing agents was used at two pH values, namely 1 and 4. The expected reduced species, namely As(III), Sb(III) and Se(IV), were extracted by APDC/MIBK at pH 5.5 by the procedure described in Section 3.6.

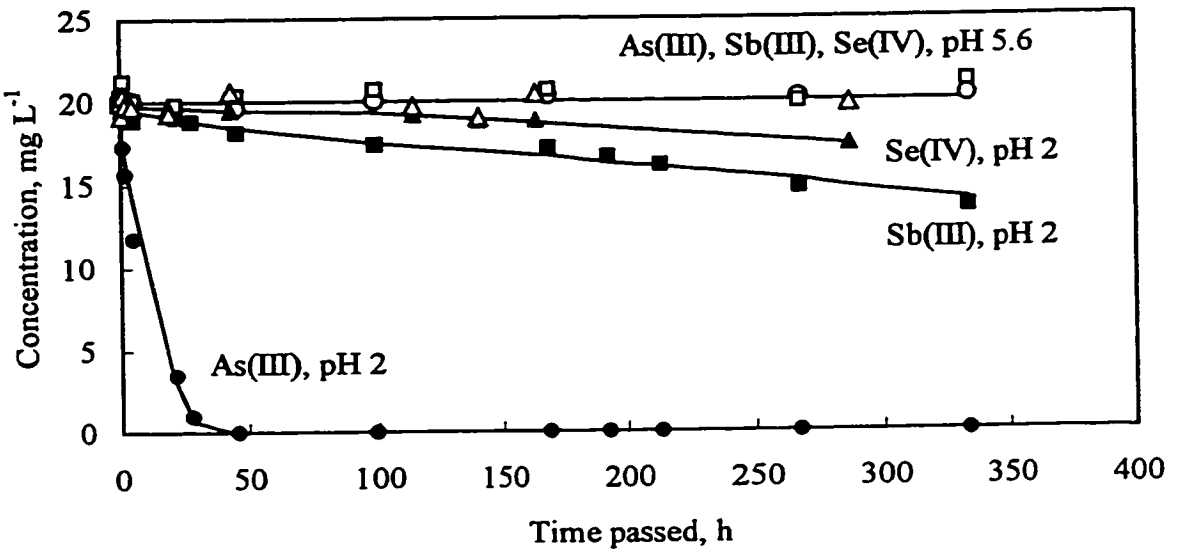

Fig. 4.13. Concentrations of As(III), Sb(III) and Se(IV) at Different Times after been Extracted into MIBK

● As(III), pH2 ■ Sb(III), pH2 ○ As(III), pH5.6
□ Sb(III), pH5.6 ▲ Se(IV), pH2 △ Se(IV), pH5.6

The degree of reduction for As(V), Sb(V) and Se(VI) by the three reducing reagents at pH 1 and pH 4 are shown in Fig 4.14 and Fig 4.15, respectively. It is clear that about 1 mL of L-cysteine can quantitatively reduce the As(V) and Sb(V) to As(III) and Sb(III), respectively, at both pH 1 and 4. However, the yield for Se(VI) to Se(IV) was found to be very low, *viz.* less then 0.2% at both pH values. The reduction of As(V) and Sb(V) by potassium thiosulfate was found to be quantitative at pH 1 (Fig. 4.14). However, at pH 4, the yield of As(III) was about 33% and that of Sb(III) was even lower, less then 2% (Fig. 4.15). Moreover, potassium thiosulfate gave a precipitate at both pH values. Again, the reduction of Se(VI) to Se(IV), at both pH 1 and 4, was found to be negligible by potassium thiosulfate.

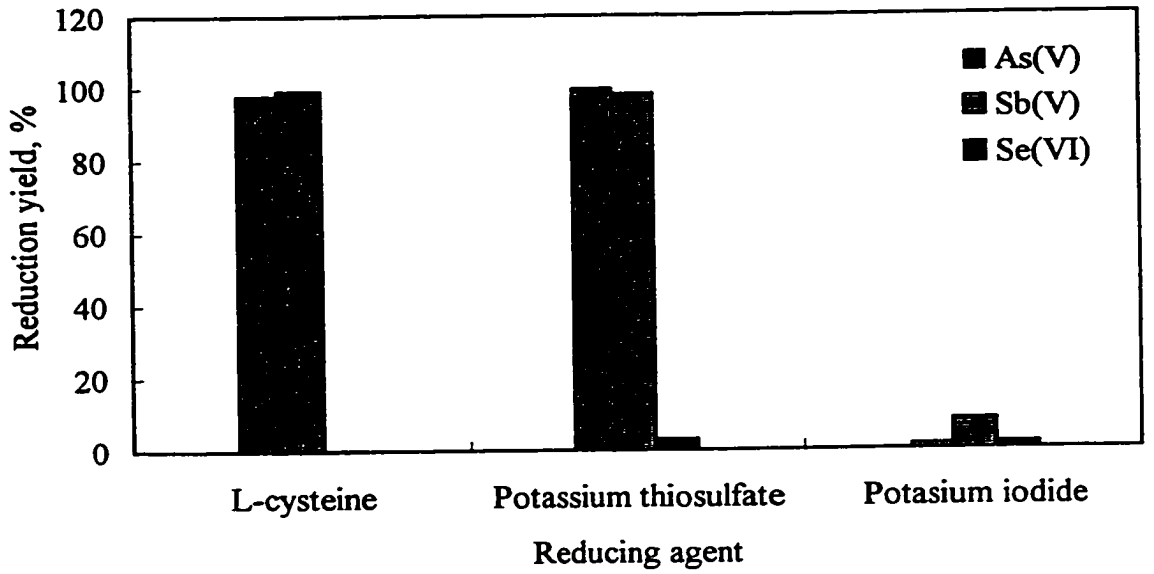

Fig 4.14. Reduction Yields of As(V), Sb(V) and Se(VI) by Different Reducing Agent at pH 1

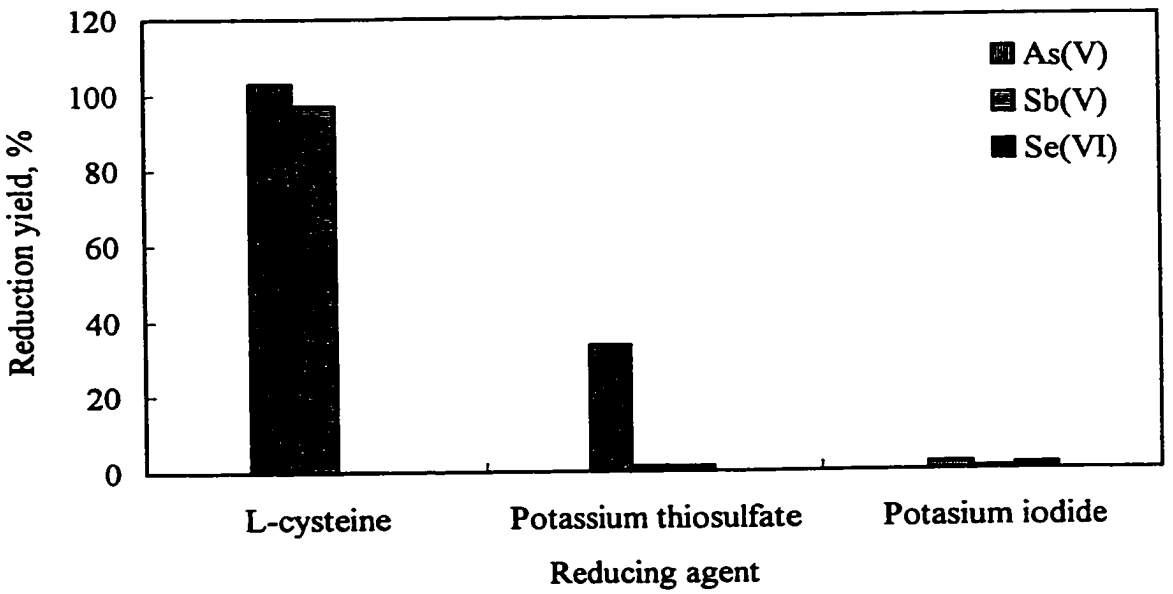

Fig. 4.15. Reduction Yields of As(V), Sb(V) and Se(VI) by Different Reducing Agents at pH 4

The use of potassium iodide for the reduction of As(V) and Sb(V) at pH 1 was found to be interesting. The reduction reaction took place at pH 1 as indicated by the color change due to the formation of iodine (*i.e.* I_2). However, the color disappeared after the pH of the mixture was adjusted to 5.5, as required for the solvent extraction method. It appears that the direction of the reaction is reversed at pH 5.5. Very low yields of As(III) and Sb(III) were obtained. Potassium iodide at pH 4, on the other hand, did not reduce As(V) and Sb(V) as there was no color change. The reduction yield of Se(VI) to Se(IV) by potassium iodide was found to be very low at both pH 1 and 4 as shown in Figs. 4.14 and 4.15, respectively.

The reduction of As(V), Sb(V) and Se(VI) by L-cysteine was further studied for a wider pH range of 1 to 7. As shown in Table 4.5, the reduction of As(V) and Sb(V) were found to be complete between pH 1 to 6. A 5% L-cysteine solution was selected as the reducing reagent for this work. The pH chosen was 5.5 which also happened to be the pH for the solvent extraction procedure so that no pH adjustment was necessary between the reduction and the extraction steps. In addition, L-cysteine did not form any precipitate which made the phase separation step in the solvent extraction method easier than potassium thiosulfate which tended to give a precipitate.

Unfortunately, the reduction of Se(VI) to Se(IV) by L-cysteine, potassium thiosulphate and potassium iodide were found to be unsuccessful. The reason could be that these agents reduced Se(VI) to Se(0) which tended to remain as a precipitate and not extracted by APDC/MIBK.

Table 4.5. Reduction Yields of As(V), Sb(V) and Se(VI) by 5% L-Cysteine Solution at Different pH

Species	pH						
	1	2	3	4	5	6	7
As(V), %	100 ± 2	98 ± 2	98 ± 2	102 ± 2	101 ± 2	99 ± 2	63 ± 2
Sb(V), %	100 ± 2	100 ± 2	101 ± 2	99 ± 2	102 ± 2	103 ± 3	98 ± 2
Se(VI), %	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1

4.2.8 Reduction and Extraction of MMA, DMA and AsB and their Potential Interferences to the Determination of As(V) Species

It is evident from Section 4.2.3 that MMA, DMA and AsB do not interfere with the determination of As(III) and As(V) at pH 5.5 by the APDC/MIBK solvent extraction method developed here. In this method, As(V) is determined after its reduction to As(III). It is therefore necessary to investigate any interference that may arise from the reduction of MMA, DMA and AsB.

Experiments were carried out to evaluate the effects of reduction of MMA, DMA and AsB at pH 5.5 by L-cysteine. Each of MMA, DMA and AsB solutions was separately mixed with 5% L-cysteine solution, allowed to react for 30 min, and then the mixture was subjected to APDC/MIBK extraction. The yields of extractable arsenic species are given in Table 4.6. It is evident that essentially no arsenic (<0.1%) was extracted from AsB. However, about 45% of arsenic in MMA and 53% of arsenic in DMA were extracted by APDC/MIBK after their reduction, respectively. Therefore, there exists a potential interference from MMA and DMA to the determination of As(V) by this method.

In the speciation analysis of real samples, the interference from DMA can be easily eliminated by the cation exchange chromatographic group separation method described in Section 4.1, where AsB and DMA are separated from As(III), As(V) and MMA species before solvent extraction. This group separation method cannot eliminate the potential interference from MMA. It should be noted here that MMA and DMA have been found to be the minor species in most natural water samples; in addition, the concentration of MMA is lower than that of DMA. So the potential interference from MMA to the determination of As(V) can be considered negligible.

The reduction products from MMA and DMA by L-cysteine are not presently known. Hasegawa and coworkers [150] reported that MMA(III) and DMA(III) could be produced from MMA and DMA by hydrogen sulfide; the trivalent species were extracted by DDDC/chloroform, which is also a dithiocarbamate chelating extraction system. These APDC/MIBK extractable products observed in our work might also be MMA(III) and DMA(III).

Table 4.6. Yields of Extractable Reduction Products of MMA, DMA and AsB

Species	MMA	DMA	AsB
Reducing reagent	L-cysteine	L-cysteine	L-cysteine
pH	5.5	5.5	5.5
Yield of extractable reduction product, %	45 ± 11	53 ± 21	<0.1

Experiments were also carried out to examine the reduction of MMA, DMA and AsB by potassium thiosulfate at pH 1 to 6. The results are shown in Fig. 4.16. It was found that the reduction was dependent on the pH. The reduction yields for both MMA and DMA were very low at pH 6, and increased with decreasing pH. MMA was more readily reduced than DMA by potassium thiosulfate. MMA was 100% reduced at pH 1 to 2.5 while 2.2-7.3% of DMA was reduced at the same pH interval. It is also evident from Fig. 4.16 that potassium thiosulfate did not reduce AsB at all.

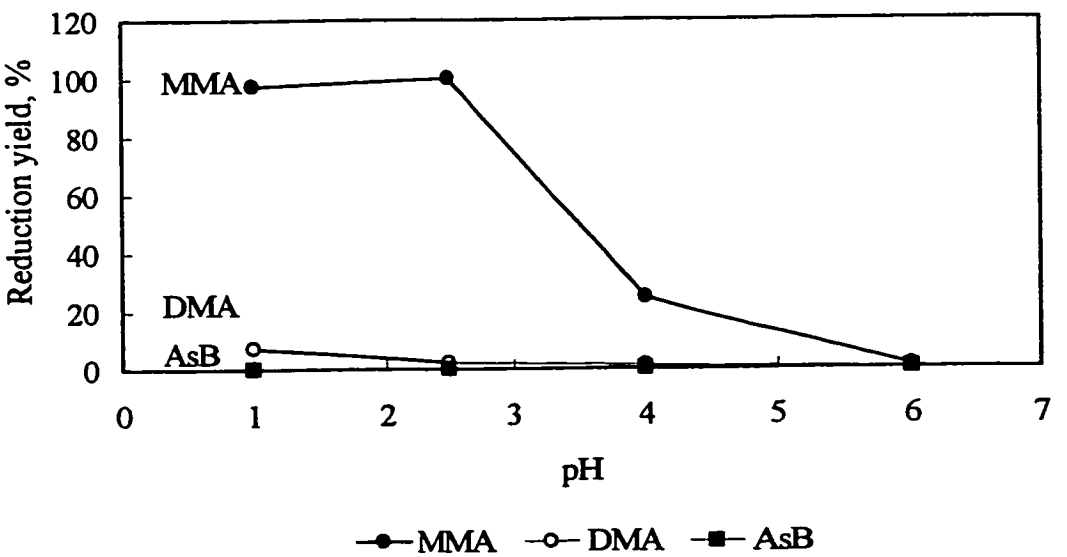

Fig. 4.16. Reduction Yields of MMA, DMA and AsB by $K_2S_2O_3$ at Different pH

4.2.9 Conclusions

The APDC/MIBK solvent extraction method developed in this work can be used to selectively extract As(III), Sb(III) and Se(IV) species in the pH range of 5 to 7. Within this pH range, the potential interference for As(III) from organic arsenic species, such as MMA and DMA, can be eliminated. The determination of Se(IV) species can be performed by direct irradiation of the organic solvent without any back extraction and using its short-lived nuclide, ^{77m}Se. The determinations of arsenic and antimony species are more conveniently done using a back extraction step and irradiation of the dried solid sample. L-cysteine has been found to be an efficient reducing agent for As(V) and Sb(V) species; however, none of the three reducing agents used was able to reduce Se(VI) to Se(IV). The APDC/MIBK solvent extraction method has been observed to give high enrichment factors for arsenic, antimony and selenium species, which allows the determination of ultra low levels.

4.3 Separation of Arsenic, Antimony and Selenium Species by Solid-phase Extraction

It is evident from the literature survey (Section 2.4) that SPE is a more suitable separation technique for elemental determination by NAA. We have developed a SPE method for the separation of inorganic arsenic, antimony and selenium species in water samples. This method involves the preparation of the HDBDC/XAD-4 chelating reagent impregnated resin, the selective extraction of the As(III), Sb(III) and Se(IV) species, and the determination using NAA. The details of the method are given below.

4.3.1 Preparation of Chelating Agent Impregnated Resin

The two basic components of a chelating agent impregnated resin are the chelating agent and the solid support. The chelating agent needs to be selective for the species of interest, while the solid support should be able to strongly "hold" the chelating agent as well as its complex. So far, C_{18} bound silica gel [157], silica gel [158], polyurethane foam (PU) [160], and silanized diatomite (Chromaton N-AW-HMDS) [159] have been used for this purpose. There has been no report on the use of Amberlite XAD resin as the solid support for arsenic speciation. Its styrene divinylbenzene copolymer

nature with high affinity for non-ionic materials makes it quite promising as an inert support [314-318], in particular, the XAD-4 resin with a surface area of 725 m^2g^{-1} and an average pore diameter of 40 Å. The two dithiocarbamate (DTC) chelating agents, namely APDC and DBDC, which have been used in the solvent extraction methods described above, were also used in SPE to coat the XAD-4 resin. The adsorption rate, isotherms and the use of ion-pairing reagents to improve the coating have been studied.

4.3.1.1 Spectrophotometric Determination of Chelating Agents

Spectrophotometric methods were used for determining the concentrations of APDC and DBDC wherever needed. The two maximum absorption wavelengths of APDC were found at 254 nm and 278 nm, and those of DBDC at 262 nm and 292 nm. The molar absorptivities of APDC and DBDC were calculated and found to be quite similar, *viz.* in the range of 5×10^3 to 6×10^3 M^{-1} cm^{-1}. The calibration curves are shown in Fig. 4.17.

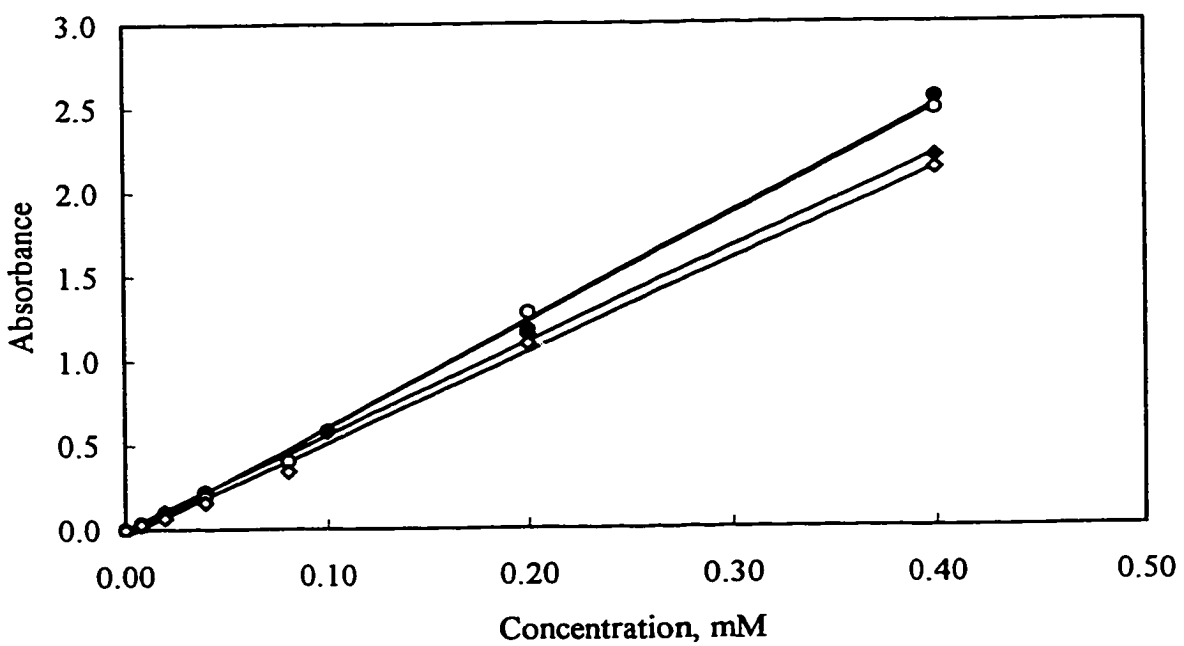

Fig. 4.17. Calibration Curves of Spectrophotometric Determination of DTC (solvent: 75% methanol)

● APDC@254nm ◆ APDC@278nm ○ DBDC@262nm ◇ DBDC@292 nm

4.3.1.2 Adsorption of Various Forms of Chelating Agents

Experiments were done for selecting the most suitable chelating agent and its form for SPE of As(III), Sb(III) and Se(IV). The coatings of XAD-4 with APDC and with DBDC in their acid forms (HPDC and HDBDC), their anionic forms (PDC^- and $DBDC^-$), and the ion-paired forms with counter charged ion-pairing agents, namely CTA^+ and $TMAm^+$, were carried out. The coating performance was evaluated by two parameters, namely the concentration of DTC on the resin phase ($[DTC]_r$) and the solid-to-aqueous concentration ratio, $[DTC]_r/[DTC]_a$. The values obtained using different forms of APDC and DBDC and different methanol concentrations are presented in Table 4.7. It was not possible to do adsorption experiments of HDBDC on XAD-4 at <75% methanol concentrations because of the solubility problem.

The methanol concentration in the solvent was found to significantly affect the adsorption of DTC. As shown in the Table 4.7, the value of $[DTC]_r/[DTC]_a$ for PDC^- on XAD-4 decreased from 0.0061 to 0.00001 when the methanol concentration increased from 20% to 80%. Similarly, the value of $[DTC]_r/[DTC]_a$ for HPDC on XAD-4 decreased from 0.021 to 0.0053 with methanol concentration increasing from 20% to 50%. The values of $[DTC]_r/[DTC]_a$ for HDBDC on XAD-4 was found to be 0.076, 0.043 and 0.012 when the methanol concentration was 75%, 80%, and 88%, respectively. Therefore, it can be concluded that for all three forms of DTC the lower the methanol concentration the higher is the adsorption of DTC on XAD-4.

It is also clear from Table 4.7 that DBDC is more readily adsorbed on XAD-4 than APDC in both anionic and acidic form. For example, for $[DTC]_r/[DTC]_a$, a value of 0.0034 was obtained for $DBDC^-$ in 80% methanol compared to only 0.00001 for PDC^- in 80% methanol. Values of $[DTC]_r/[DTC]_a$ of 0.076 and 0.043 were obtained for HDBDC in 75% and 80% methanol systems, respectively. However, values of only 0.021 and 0.0053 were obtained for HPDC in 20% and 50% methanol, respectively. Both APDC and DBDC in their acid forms gave higher adsorption than their anionic forms as shown in Table 4.7.

Experiments were also carried out to examine the effects, if any, of ion pairing reagents have on the adsorption of DBDC on XAD-4. Due to the solubility problems, unfortunately all reagents could not be prepared at the same low methanol concentration

and no direct comparisons can thus be made. It appears from Table 4.7 that the two ion pair reagents, namely CTA^+ and $TMAm^+$, did not show any significant difference in the adsorption of $DBDC^-$ by XAD-4.

In conclusion, DBDC in its acid form or in its ion-pairing form with CTA^+ or $TMAm^+$ was found to be a better chelating agent than APDC. Therefore, DBDC was selected in this thesis for the preparation of impregnated resin for SPE of As(III), Sb(III) and Se(IV).

Table 4.7. Adsorption of Dithiocarbamate on XAD-4 Resin

Form	% Methanol in water	$[DTC]_i$ mM	$[DTC]_r$ mmol g^{-1}	$[DTC]_r/[DTC]_s$ L g^{-1}
PDC^-	20	20	0.10	0.0061
PDC^-	80	20	0.00023	0.00001
HPDC	20	20	0.24	0.021
HPDC	50	20	0.084	0.0053
$DBDC^-$	80	20	0.057	0.0034
HDBDC	75	20	0.32	0.076
HDBDC	80	20	0.27	0.043
HDBDC	88	20	0.24	0.012
$TMAm^+$-$DBDC^-$	60	10	0.27	0.12
CTA^+-$DBDC^-$	70	10	0.28	0.11

$[DTC]_i$ = initial concentration of DTC

4.3.1.3 Rate of Adsorption

Experiments were carried out to measure the rate of adsorption of DBDC on the XAD-4 resin. About 90 mL of 10mM HDBDC in 80% methanol was shaken with 5 g of the resin in a mechanical shaker at 21°C for various lengths of time. The concentrations

of HDBDC in the aqueous phase were measured spectrophotometrically at 262 nm. The concentrations of HDBDC on the resin phase, $[HDBDC]_r$, were calculated and plotted against the shaking time as shown in Fig. 4.18. The adsorption rate of HDBDC on XAD-4 was found to be rapid at the beginning and then reached a plateau at about 3 h. Therefore, an equilibration time of 3 h with shaking was selected in the experiments that followed.

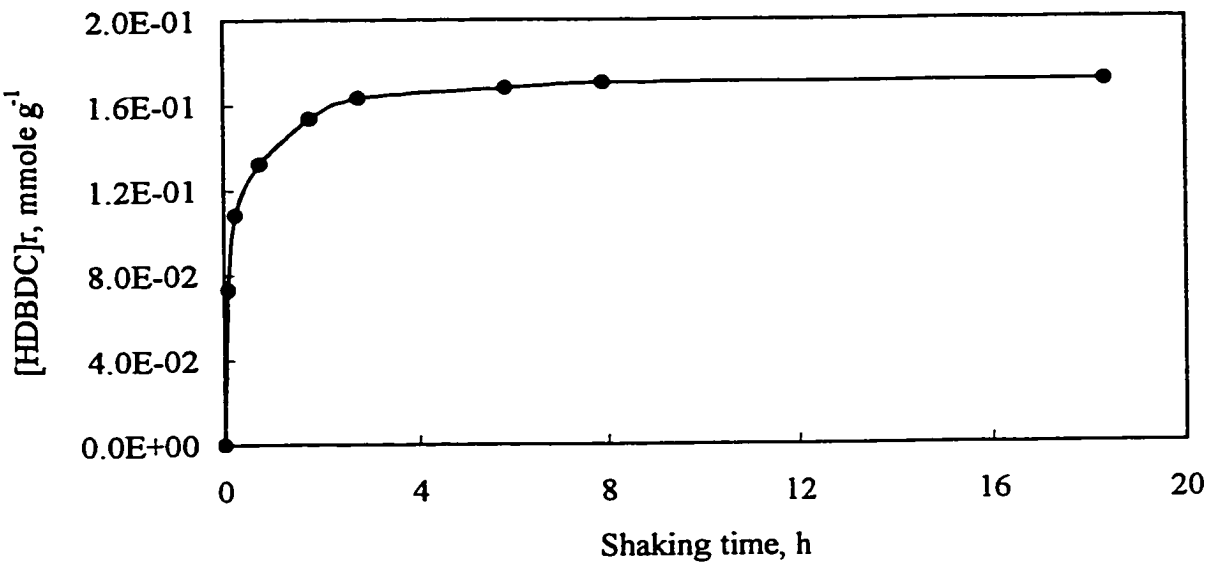

Fig. 4.18. The Adsorption Rate of HDBDC on XAD-4 Resin
HDBDC solution: 10 mM HDBDCin 80% methanol;
Temperature: 21°C.

4.3.1.4 Adsorption Isotherm

The adsorption isotherms for HDBDC on XAD-4 resin were determined at 21°C using 88% and 95% methanol-water mixtures. The treatment of the data by Freundlich adsorption model gives a linear relationship between log $[HDBDC]_r$ and log $[HDBDC]_a$. The results are shown in Fig. 4.19; the values of log K and n are given in Table 4.8. The graphs show that the extent of adsorption decreases with increasing concentration of methanol, as described earlier.

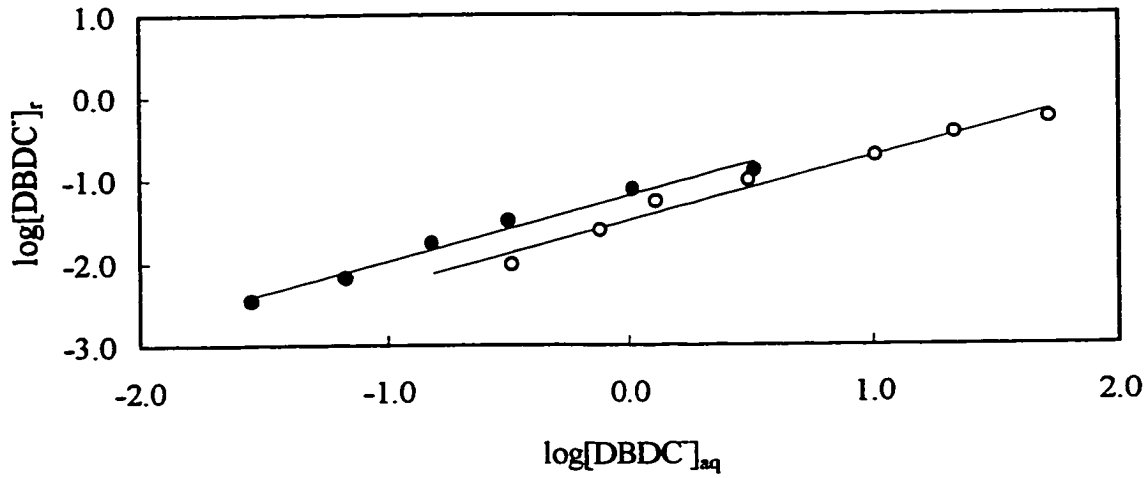

Fig 4.19. Adsorption Isotherms for DBDC on XAD-4 at Different Methanol Concentrations

● 88% MeOH ○ 95% MeOH

Table 4.8. Values for the Freundlich Equation Constants

Solvent	Log K	n
88% methanol	-1.49	0.77
95% methanol	-1.20	0.78

4.3.2 Separation of Arsenic, Antimony and Selenium Species by SPE

Experiments on SPE of As(III), As(V), Sb(III), Sb(V), Se(IV) and Se(VI) were carried out using DBDC/XAD-4 and the procedure described in Section 3.7.2. The pH of the sample solution was buffered at 5.5 as required in the solvent extraction method. The recoveries obtained for each of the species are shown in Table 4.9. It is evident that DBDC/XAD-4 resin quantitatively extracted As(III) and Sb(III) species with a sample volume of 100 mL or less compared to <4% of As(V) and Sb(V). A larger sample

volume, such as 200 mL, caused a decrease of the extraction of As(III) and Sb(III). It was found that Se(IV) could be quantitatively extracted by the column for up to 200 mL of the sample along with 22-35% of Se(VI). This poor selectivity for selenium species limited the use of this column for the speciation analysis of inorganic selenium.

Table 4.9. SPE Yields of Various Species by DBDC/XAD-4

Sample volume, mL	Recovery, %					
	As(III)	Sb(III)	Se(IV)	As(V)	Sb(V)	Se(VI)
50	102 ± 4	97 ± 4	103 ± 4	1.0 ± 0.3	3.4 ± 3	35 ± 6
100	96 ± 3	99 ± 3	100 ± 3	1.2 ± 0.4	2.2 ± 2	32 ± 5
200	88 ± 2	83 ± 2	100 ± 2	2.1 ± 0.3	2.6 ± 0.7	22 ± 7

Experiments on SPE of As(III) and Sb(III) at pH 5.5 using CTA^+-$DBDC^-$/XAD-4 were also carried out. The recoveries are listed in Table 4.10. The results indicate that As(III) and Sb(III) could be simultaneously as well as quantitatively extracted from up to 100 mL of water samples.

Table 4.10. Recovery of As(III) and Sb(III) by SPE using CTA^+-$DBDC^-$/XAD-4

Sample volume, mL	Recovery, %	
	As(III)	Sb(III)
50	102 ± 4	103 ± 4
100	96 ± 4	100 ± 4
200	53 ± 6	101 ± 6

4.3.3 Conclusions

The chelating agent DBDC was successfully coated onto the non-polar co-polymeric adsorbent XAD-4 for the analysis of inorganic arsenic and antimony species. As in the APDC/MIBK solvent extraction method described before, the SPE method using DBDC/XAD-4 resin was also found to be selective for As(III) and Sb(III) in presence of As(V) and Sb(V). However, the extraction selectivity between Se(IV) and Se(VI) species was found to be poor. The degree of coating of DBDC on XAD-4 was found to be dependent on the methanol concentration, the chelating agent and its form in the solution. The SPE of As(III) and Sb(III) by HDBDC/XAD-4 and by CTA^{+}-$DBDC^{-}$ /XAD-4 was found to be similar. The column packed with only 1.2 g of the prepared HDBDC/XAD-4 resin can selectively extract As(III) and Sb(III) species for up to 100 mL of water samples, which gives a fairly high enrichment factor. The SPE method was found to be a convenient preconcentration and separation method for speciation analysis when NAA was the determination method. This procedure involves only the drying of the resin before irradiation with neutrons while other methods require elution of the species from the resin prior to the determination.

4.4 Separation of Arsenic Species by HPLC

It is evident from the literature survey (Section 2.5) that HPLC is becoming more and more important as a separation technique for arsenic species. We have developed a HPLC method using an anion exchange column for the separation of five arsenic species, namely As(III), As(V), MMA, DMA and AsB. The details of method development are given below.

4.4.1 General Considerations for Coupling HPLC with NAA

For the speciation of arsenic by HPLC, NAA techniques have a few special requirements compared to the conventional detection techniques such as UV and conductivity. In HPLC-NAA, the absence of some elements in the mobile phase, which can produce highly radioactive nuclides and interfere with the determination of the elements of interest, is required. For example, sodium in the mobile phase can cause problems if present in high concentrations as well as after long irradiations in the

determination of arsenic. A long decay time does not reduce the interference very effectively because the half-lives of ^{24}Na (14.96 h) and ^{76}As (26.32 h) are not that different. In addition, it increases the Compton background leading to a poorer detection limit for arsenic. Bromine interferes in a slightly different way. The 554-keV gamma-ray of ^{82}Br can seriously interfere with the 559-keV gamma-ray of ^{76}As if a high-resolution Ge(Li) detector is not used. Also high activities of ^{82}Br can mask the ^{76}As photopeak. Again the half-life of ^{82}Br (35.30 h) is similar to that of ^{76}As. Although the presence of chlorine in the mobile phase is not a problem for assaying arsenic due to the much shorter half-life of ^{38}Cl (37.24 min), the high activity can cause radiation hazards while handling the irradiated samples. Therefore, the mobile phase in HPLC-NAA ideally should not include any of these three elements. Elements such as carbon, nitrogen, oxygen, hydrogen, phosphorus, sulfur, and potassium, in the mobile phase are not expected to interfere seriously with the determination of arsenic by NAA.

It is evident from the literature survey (Section 2.5) that an anion exchange column is a good candidate for the stationary phase in HPLC for the separation of arsenic species. In this thesis, a Hamilton PRP-X100 polymeric bound anion exchange column was used. A mobile phase of phosphate buffer ($(NH_4)_2HPO_4/NH_4H_2PO_4$) was selected here because of its good separation performance and purity with respect to elemental contaminants.

4.4.2 Separation of As(III) from AsB by HPLC

Many authors reported that the HPLC separation of As(III) from AsB using the PRP-X100 anion exchange column is not very satisfactory [59, 67, 231, 235, 238]. The reason for this poor separation could be the pH selected for the separation, generally pH 5-7, where As(III) tends to remain mostly as neutral (pK_a=9.2) and AsB as either a cation or a zwitterion. As a result, the difference on the retention volume of these two species is small. Furthermore, the levels of As(III) are generally much lower than that of AsB in seafoods and marine organisms making their separation even harder.

The As(III) species can be ionized using a phosphate solution of higher pH and can possibly be retained by PRP-X100. An attempt was made in this work to separate As(III) from AsB using a 5mM $(NH_4)_2HPO_4$ solution of pH of 8.8. Fractions of about

0.13 mL were collected, and the chromatogram is shown in Fig. 4.20. A higher retention volume for As(III) than for AsB was observed, as expected. However, the resolution of these two peaks was still not satisfactory for a reliable separation of AsB from As(III) by HPLC.

This problem, however, can be conveniently solved by using a pre-separation step before the HPLC. The open-column cation exchange group separation method described in Section 4.1 can be used for this purpose. The five arsenic species can be separated into two groups with one group of As(III), As(V) and MMA and the other of AsB and DMA. These two groups can then be separated into individual species by using the HPLC method described above.

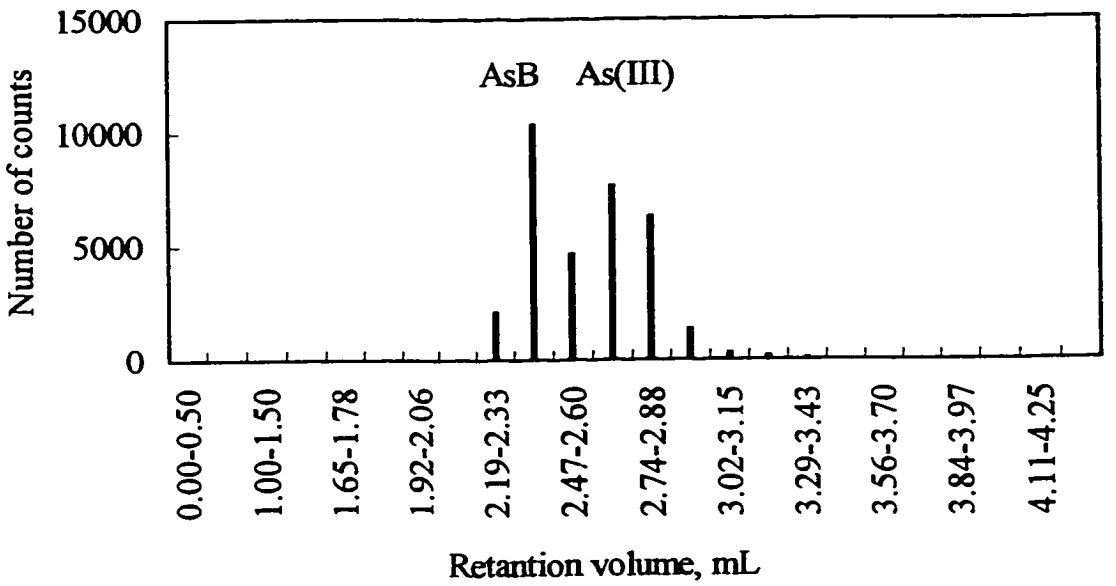

Fig. 4.20. HPLC-NAA Chromatogram of AsB and As(III)
Column: PRP-X100; Mobile phase: 5mM phosphate buffer (pH 8.8); NAA: t_i-t_d-t_c=30 min-2 h-5 min.

4.4.3 Separation of AsB, DMA and MMA by HPLC

The pH and the concentration of the mobile phase are two important factors for the separation of arsenic species by HPLC. Experiments were first carried out to test the ability of the phosphate buffer to separate MMA, DMA and AsB at a constant pH of 6.5

but different concentrations of 5, 10 and 20 mM. About 10 μg of each species was injected and the eluate was collected in 0.25 mL fractions directly in the irradiation vials. Vials were heat-sealed and irradiated for 30 min at a neutron flux of 5×10^{11} cm^{-2} s^{-1}. All samples were counted for 5 to 10 min after a decay period of 2 to 5 h. The chromatogram was constructed by plotting the number of counts in the 559-keV photopeak area of ^{76}As against the retention volume. The chromatograms of AsB, DMA and MMA using phosphate buffers at 5, 10 and 20 mM are presented in Figs. 4.21 to 4.23, respectively. It is evident that the best separations of AsB, DMA and MMA were obtained by using 5 and 10 mM buffers at pH 6.5. The 10-mM buffer is perhaps preferable for HPLC-NAA because it requires the collection of only 25 fractions compared to 30 fractions using the 5-mM buffer. The 10-mM buffer was selected for further studies. If only AsB is to be separated from DMA, then any buffer concentrations between 5 to 20 mM are satisfactory (Fig. 4.23). The effect of the concentration of the buffer on the retention volumes of the three arsenic species is summarized in Fig. 4.24 which shows that more concentrated the buffer the stronger is its elution ability.

The effect of pH (5.4, 6.5 and 7.4) on the retention of AsB, MMA, and DMA at the 10-mM buffer concentration was then investigated. The pH influences the ionization of the analyte and the mobile phase simultaneously. The individual chromatograms are shown in Figs. 4.25, 4.22 and 4.26, respectively. It was found that the AsB and DMA co-eluted from the chromatogram at pH 5.4 (Fig. 4.25). At a higher pH of 7.4, the separation of AsB from DMA was good but that of DMA from MMA deteriorated (Fig. 4.26). When the pH of the mobile phase was set to 6.5, the separation of all three species was found to be quite satisfactory (Fig. 4.22). These results are summarized in Fig. 4.27. A 10-mM phosphate buffer at pH 6.5 was selected as the mobile phase for the separation of AsB and DMA species.

4.4.4 Separation of As(III), As(V) and MMA by HPLC

Since As(V) and MMA have lower pK_a (Table 2.4) than other arsenic species, they are expected to have higher retention volumes. A high concentration of phosphate buffer therefore is needed to elute them. Experiments were carried out to investigate

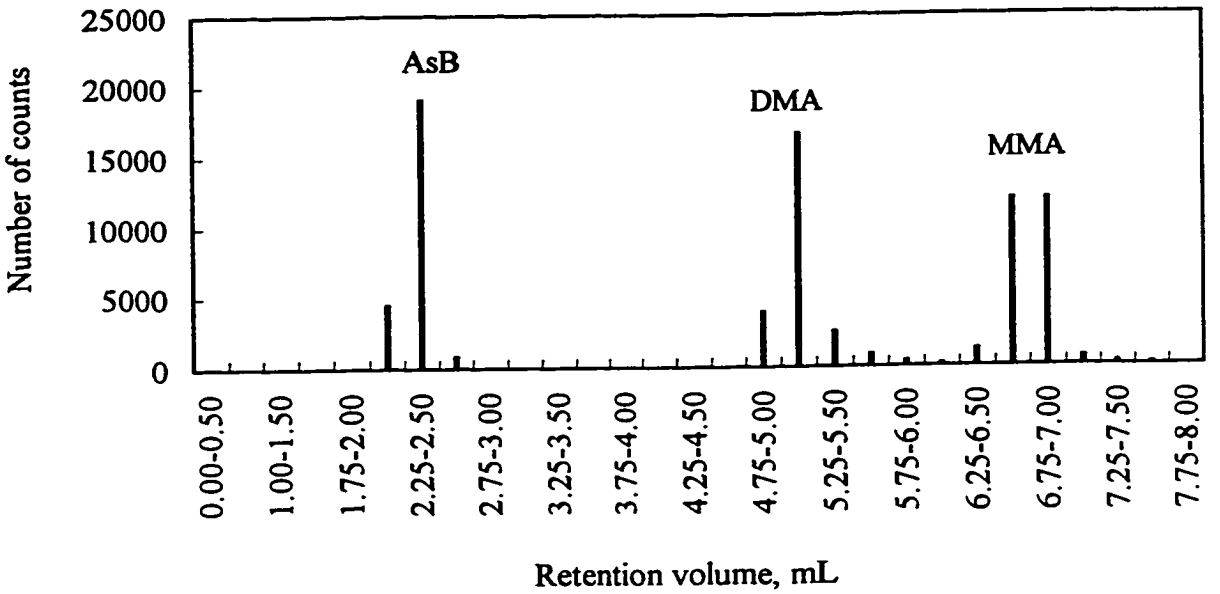

Fig. 4.21. Chromatogram of AsB, DMA and MMA
Column: PRP-X100; Mobile phase: 5mM phosphate buffer (pH6.5); NAA: t_i-t_d-t_c = 30 m - 2 h - 5 min.

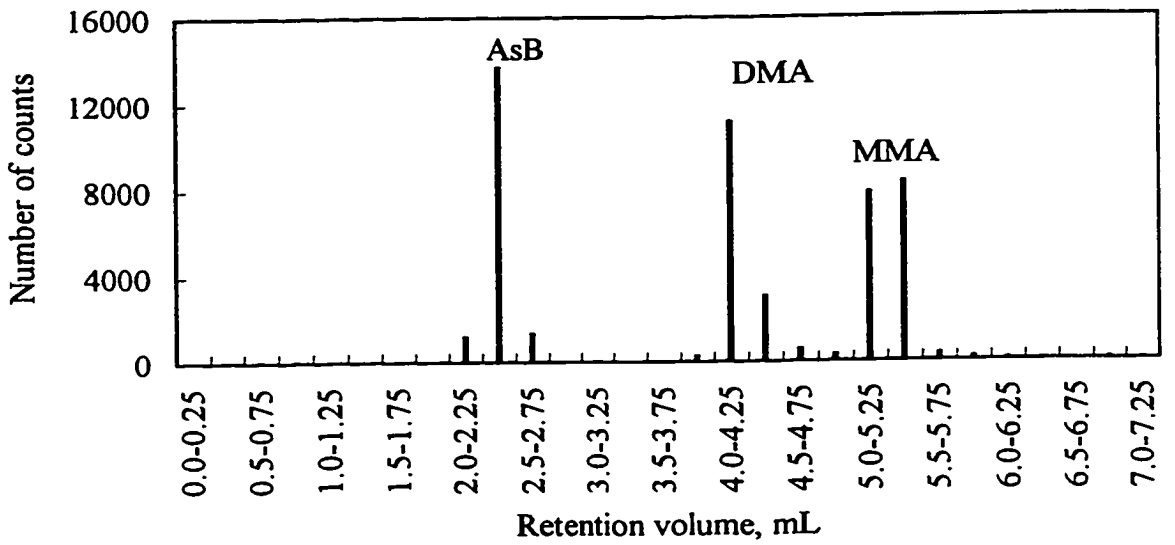

Fig. 2.22. HPLC-NAA Chromatogram of AsB, DMA and MMA
Column: PRP-X100; Mobile phase: 10mM phosphate buffer (pH 6.5); NAA: t_i-t_d-t_c = 30 min - 2 h- 10 min.

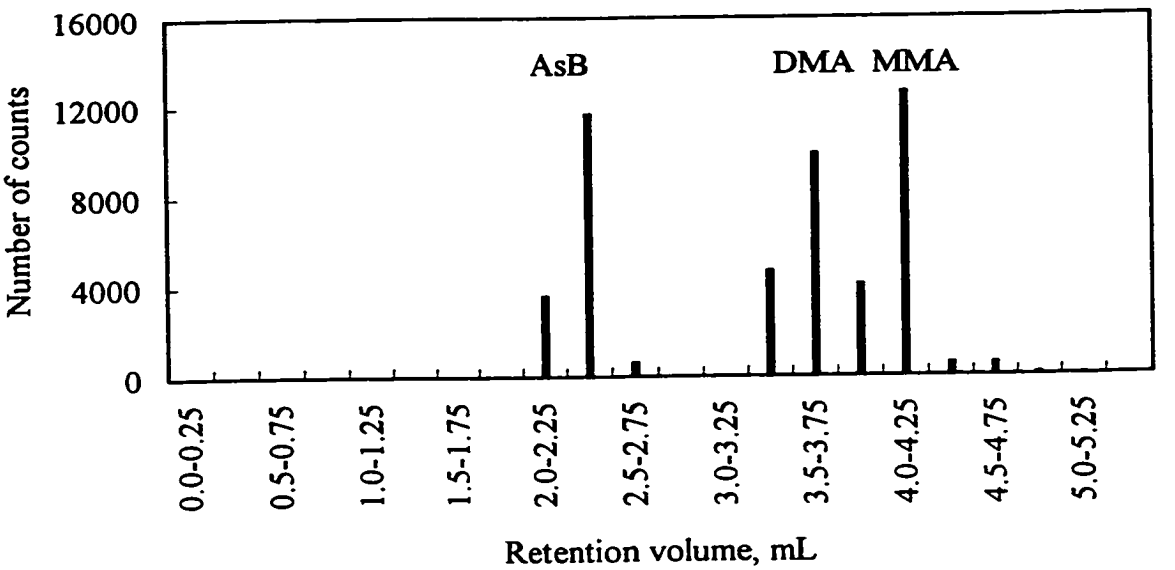

Fig. 4.23. HPLC-NAA Chromatogram of AsB, DMA and MMA Column: PRP-X100; Mobile phase: 20mM phosphate buffer (pH 6.5); NAA: t_i-t_d-t_c = 30 min - 2 h -10 min.

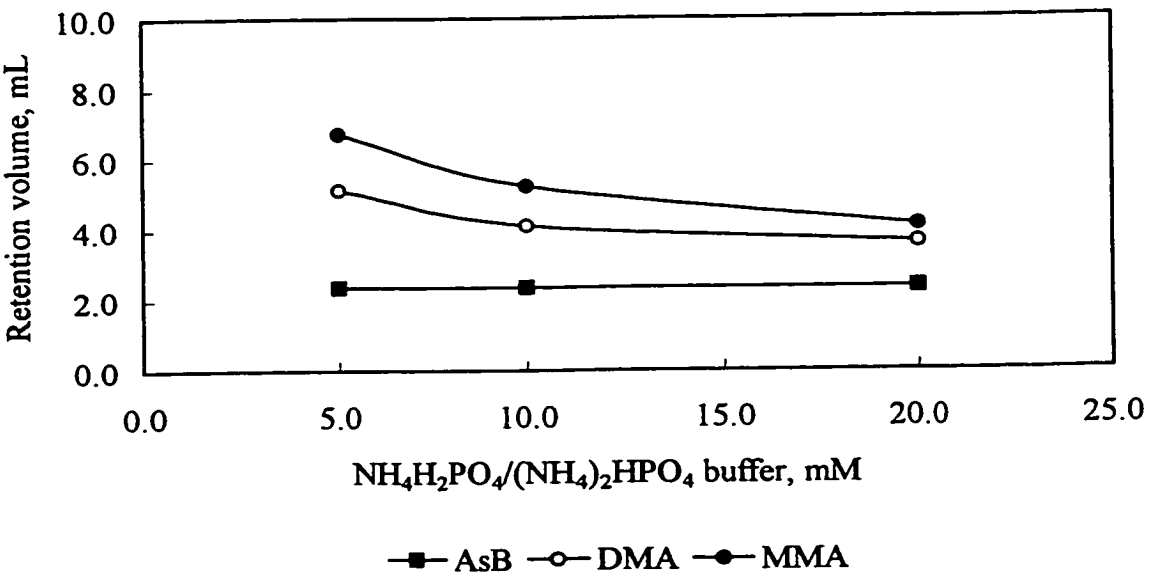

Fig. 4.24. Retention Volumes of AsB, MMA and DMA by Different Buffer Concentrations at pH 6.5

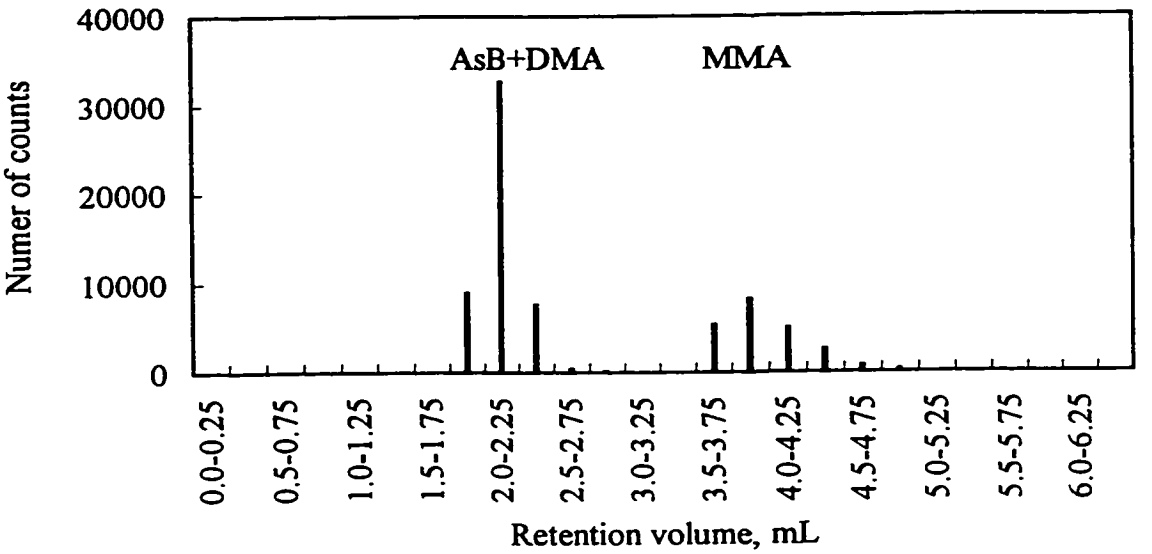

Fig. 4.25. HPLC-NAA Chromatogram of AsB, DMA and MMA Column: PRP-X100; Mobile phase: 10mM phosphate buffer (pH5.4); NAA: t_i-t_d-t_c = 30 min-2 h-10 min.

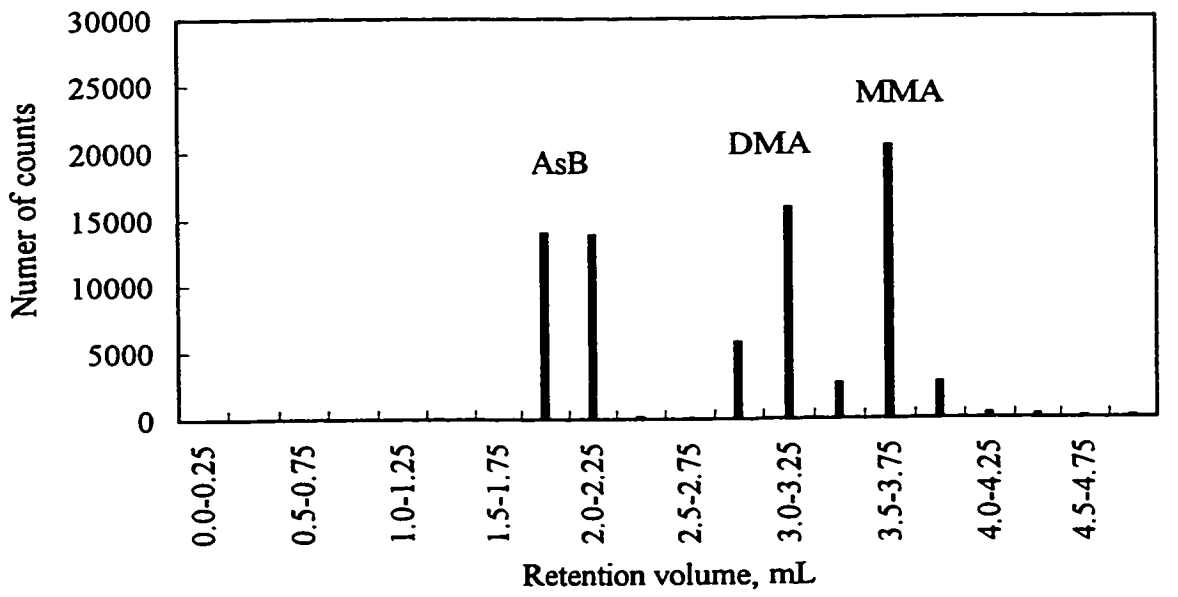

Fig. 4.26. HPLC-NAA Chromatogram of AsB, DMA and MMA Column: PRP-X100; Mobile phase: 10 mM phosphate buffer (pH 7.35); NAA: ti-td-tc = 30 min - 2 h-10 min

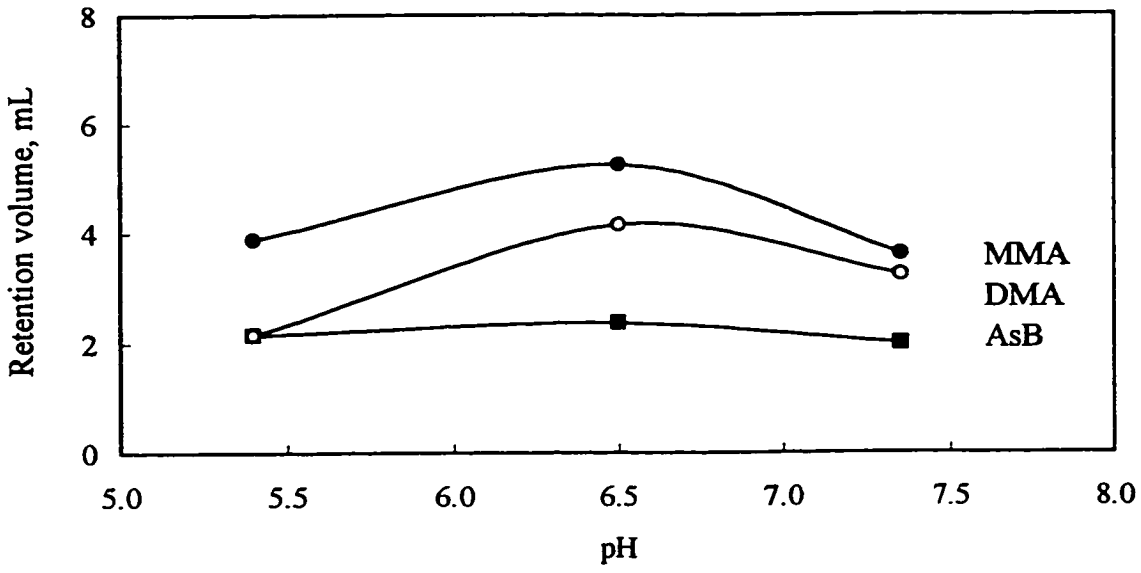

Fig. 4.27. Retention Volumes of AsB, DMA and MMA using 10 mM Phosphate Buffer at Different pH

—■— AsB —○— DMA —●— MMA

the separation of As(III), As(V) and MMA using 75 mM phosphate buffer at pH 5.5 and 6.5. The chromatograms are shown in Fig. 4.28 and 4.29. It is clear that 75 mM buffer at pH 5.5 is a better mobile phase for the complete separation of the above three species. The separation of As(III) from MMA was not satisfactory at pH 6.5.

At pH 5.5, buffer concentrations of 40 and 75 mM were tested for the separation of As(III), As(V) and MMA species. The chromatograms are shown in Fig. 4.30 and 4.29, respectively. Both buffers gave not only a satisfactory separation among the three species but also quite similar retention volumes. The phosphate buffer at pH of 5.5 and at 75 mM concentration were selected in this work for the separation among As(III), As(V) and MMA by HPLC.

4.4.5 Selection of a Fraction Volume

One of the reasons for using NAA in conjunction with HPLC separation for the speciation of arsenic is the very high sensitivity and low detection limit that can be

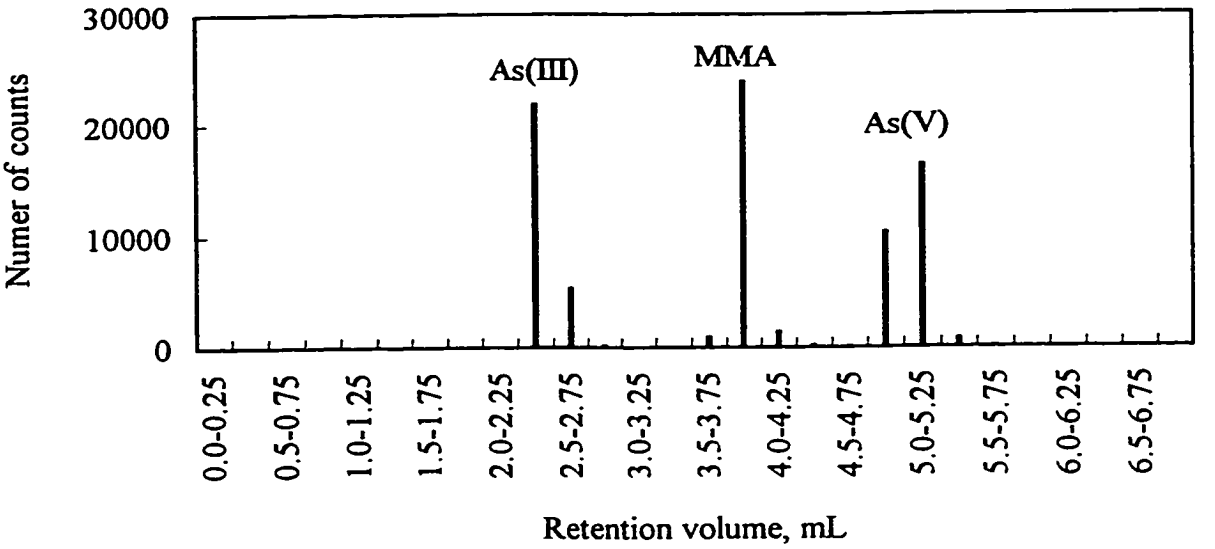

Fig. 4.28. HPLC-NAA Chromatogram of As(III), MMA and As(V) Column: PRP-X100; Mobile phase: 75 mM phosphate buffer (pH5.5); NAA: t_i-t_d-t_c = 30 min -2 h - 5 min.

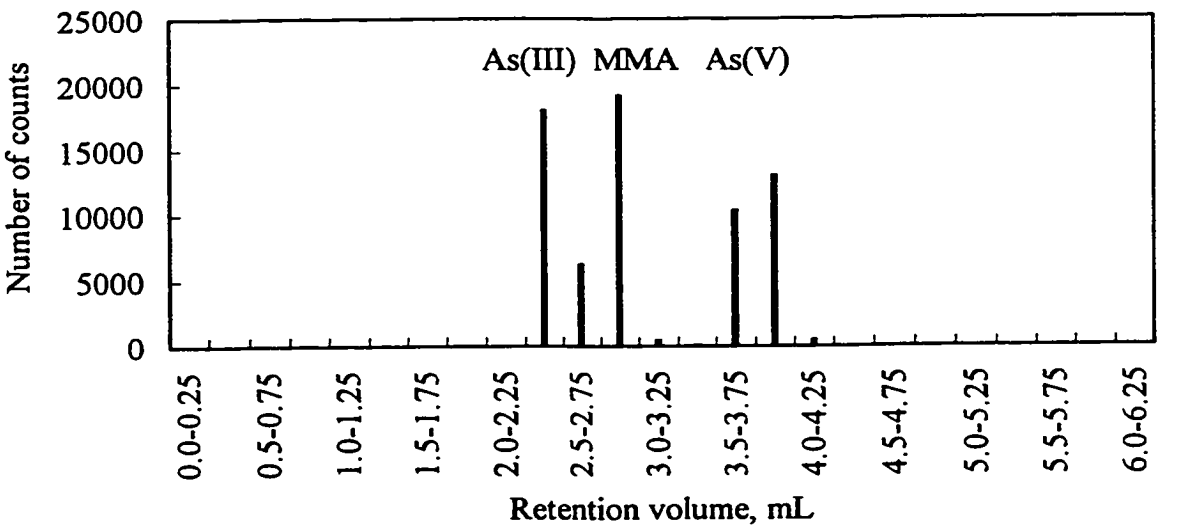

Fig. 4.29. HPLC-NAA Chromatogram of As(III), MMA and As(V) Column: Hamilton PRP-X100; Mobile phase: 75mM phosphate buffer (pH6.5); NAA: t_i-t_d-t_c=30min-2h-10min

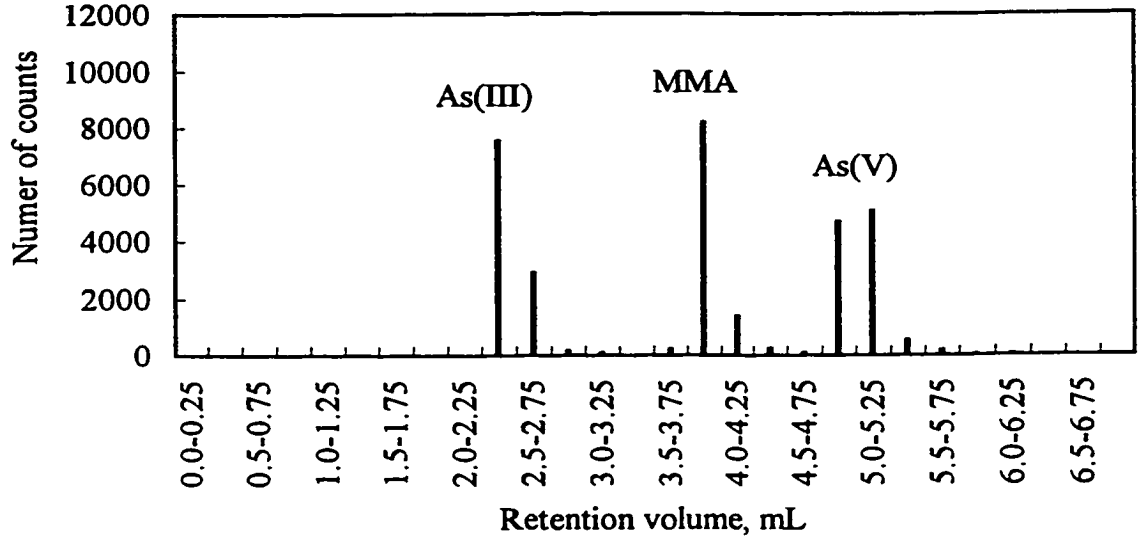

Fig. 4.30. Chromatogram of As(III), MMA and As(V)
Column: PRP-X100; Mobile phase: 40 mM phosphate buffer (pH5.5); NAA: t_i-t_d-t_c = 15 min - 2 h -5 min.

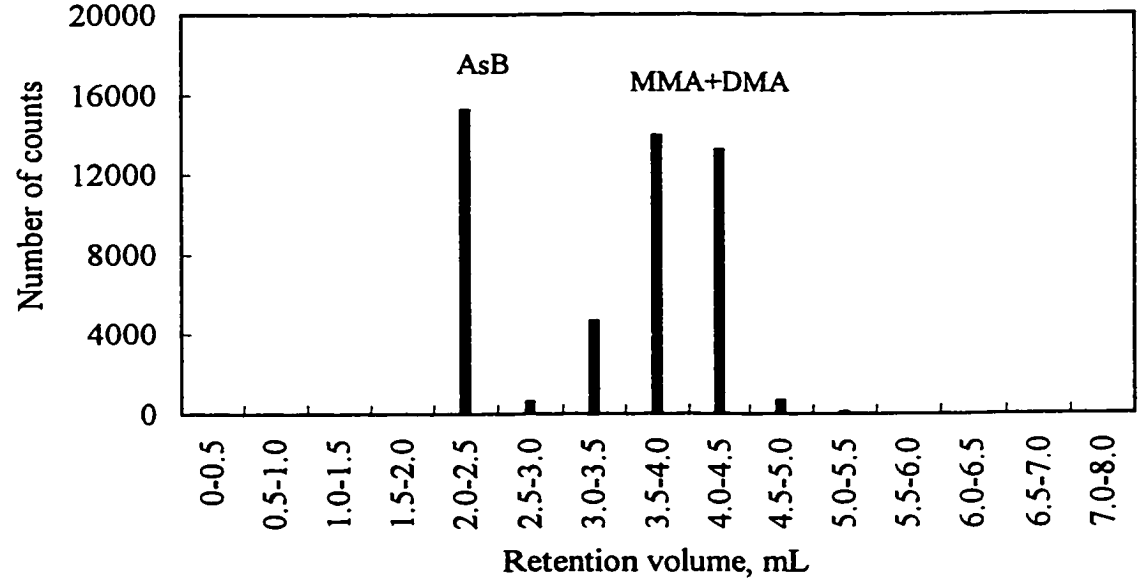

Fig. 4.31. HPLC-NAA Chromatogram of AsB, DMA and MMA by Collecting 0.5 mL Fractions

achieved by NAA. The selection of a suitable fraction volume is important if one wishes to observe a good separation of the peaks of various arsenic species. It is obvious that a smaller fraction volume is preferred. However, one finds that the number of fractions need to be collected becomes larger and larger, which limits the analytical throughput, especially for NAA using long-lived nuclides such as ^{76}As (half-life = 26.32 h). Two fraction volumes, namely 0.25 and 0.5 mL, were examined for the separation of AsB, DMA and MMA using 10 mM phosphate buffer at pH 6.5. The 0.25-mL fractions gave a very good separation of all three arsenic species as shown in Fig. 4.22, compared to the 0.5-mL fractions where MMA and DMA eluted very close to each other as evident from Fig. 4.31. In general, fractions of 0.25 mL were collected for NAA except for the separation of As(III) from AsB using 5 mM phosphate buffer at pH 8.8 where 0.125-mL fractions were collected due to the close proximity of the two peaks as shown in Fig. 4.20.

4.4.6 Potential Multielement Speciation Analysis by HPLC-NAA

It is well known that NAA is a multielement determination technique. It can perhaps be advantageously used for the simultaneous multielement speciation using HPLC. Preliminary experiments were carried out to evaluate the possibility of separating eight species of arsenic, antimony and selenium on the PRP-X100 column by gradient elution using 20 to 75 mM phosphate buffer at pH 6.0 and a methanol-6M HNO_3 solution (99:1 v/v). The chromatogram is shown in Fig. 4.32. It can be seen that a good separation of four arsenic species, namely As(III), As(V), MMA and DMA, was achieved, as expected. The conditions used here were also found to be good for the separation of Se(IV) and Se(VI). The Se(VI) species was found to the most strongly retained among the eight species studied. Obviously, the selected chromatographic conditions were not suitable for the separation of Sb(III) from Sb(V). No further experiments were done along this line because the emphasis was placed in this thesis on the determination of arsenic species.

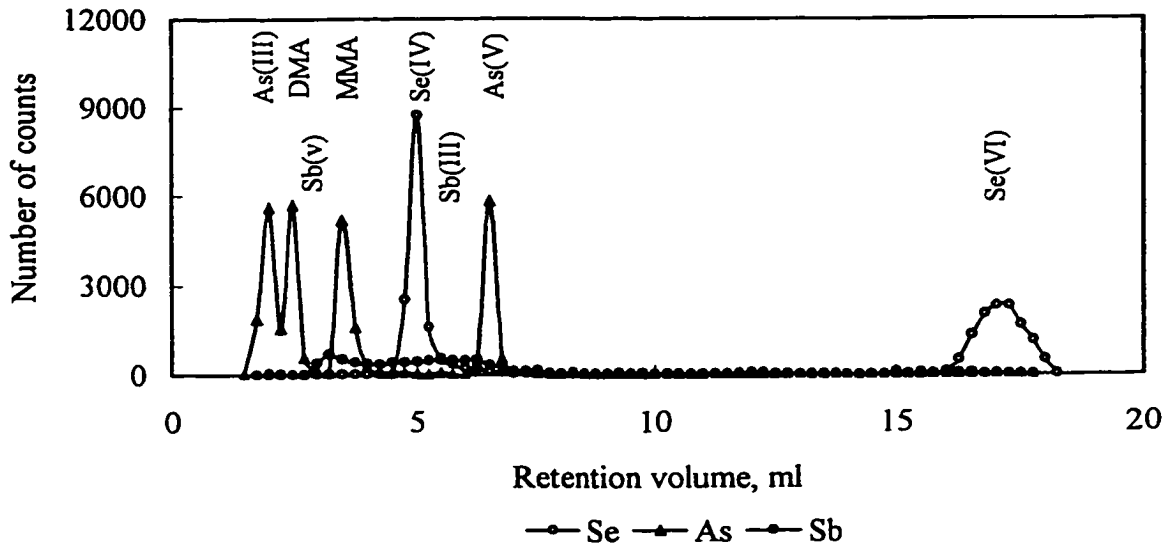

Fig 4.32. HPLC-NAA Chromatogram for Some Arsenic, Antimomy and Selenium Species
gradient program: 0-2.0 mL: 20mM; 2.0-4.5 mL: 75 mM; 4.5-14.5 mL: 20 mM; 14.5-18.5 mL: 1% 6M HNO_3/MeOH.

4.4.7 Conclusions

With a prior group separation of arsenic species, the HPLC method using an anion exchange column as the stationary phase and a phosphate buffer as the mobile phase was found to be effective for the separation of As(III), As(V), MMA, DMA and AsB. A phosphate buffer at 10 mM and pH 6.5 was selected for the separation of AsB and DMA, and a 75 mM buffer at pH 5.5 was chosen for the separation of As(III), As(V) and MMA. The selection of 0.25 mL fractions of the eluate was found to give adequate resolution for these species. The determination of arsenic in these fractions by NAA was found to be very convenient. The matrix elements in the buffer neither caused any high activity nor contributed to the high background counts in the gamma-ray spectra even after a long period of irradiation.

5. SPECIATION OF ARSENIC, ANTIMONY AND SELENIUM IN NATURAL WATERS

This chapter describes the determination of arsenic, antimony and selenium species, namely As(III), As(V), AsB, OBAs, MMA, DMA, Sb(III), Sb(V), and Se(IV), in natural water samples by the methods developed in this thesis. The concentrations of inorganic arsenic, antimony and selenium species were measured by solvent extraction using APDC/MIBK and NAA. Organic arsenic species were determined by a combination of SPE, cation exchange chromatography, and HPLC methods in conjunction with NAA. The sensitivities and the detection limits of the various methods are discussed below. The accuracy of the methods was evaluated by analyzing a Riverine Water CRM. The details of the work done are also described here.

5.1 Solvent Extraction of Inorganic Arsenic, Antimony and Selenium Species

5.1.1 Sensitivities and Detection Limits for Arsenic and Antimony

For instrumental analytical methods, sensitivity is defined by IUPAC as the slope of the calibration curve at the concentration of interest [319]. Sensitivity in NAA is generally expressed as the number of counts obtained per micro- or nanogram of the analyte under the experimental conditions used [320]. The sensitivity in NAA depends on various factors which are included in the activation analysis equation [23, 24]. The sensitivity for arsenic, antimony and selenium were optimized by selecting factors such as irradiation time, decay time, counting time, physical state of the sample, and efficiency of the semiconductor detectors.

According to IUPAC, the limit of detection, expressed as a concentration c_L (or amount, q_L), is derived from the smallest measure, x_L, which can be detected with reasonable certainty for a given analytical procedure [321]. In defining c_L, IUPAC states that

$$x_L = x_B + k\, s_B \qquad (5.1)$$

where, x_B and s_B are the mean of blank and the standard deviation of the blank, respectively. The relationship between x and c is linear with a slope as the sensitivity (s), and the intercept as the blank signal, c_L, which can be calculated as

$$c_L = (x_L - x_B) / s \quad (5.2)$$

A combination of the above two equations gives:

$$c_L = k s_B / s \quad (5.3)$$

A value of k=3 has been strongly recommended by IUPAC for reporting the detection limit for comparison purposes [319, 322]. For calculating detection limits, a number of blank measurements are normally taken in order to obtain the mean of the blank and its standard deviation.

The nuclear analytical chemists and radiochemists tend to use a more specific expression of qualitative detection limit (L_D) put forward by Currie [323, 324]:

$$L_D = (2.71 + 4.65\sqrt{\mu_B}) / s \quad (5.4)$$

where μ_B stands for the blank counts. Since the counts obtained from NAA follows Poisson distribution, the square root of μ_B is approximately equal to the standard deviation of the blank. The difference between the definitions given by IUPAC and by Currie lies in the value for k. The L_D calculated from Equation 5.4 has almost always been used throughout this thesis. Wherever necessary, the detection limit according to the IUPAC definition has also been reported for comparison purposes.

The L_D for the determination of Se(IV) by solvent extraction in conjunction with NAA and CINAA will be described in the following section. The L_D and sensitivities obtained for determination of arsenic and antimony in natural water samples by solvent extraction in combination with conventional NAA are shown in Table 5.1. Due to the problem of pressure build-up inside the irradiation vial, the irradiation time for liquid samples at DUSR was set less than 1 h (30 min is generally recommended).

It is evident from Table 5.1 that high detection limits and low sensitivities for arsenic and antimony were obtained for liquid samples using the gamma-ray spectrometry system #1. A comparison of Expt. #1 and #4 shows that both sensitivities and detection limits were improved using the higher efficiency gamma-ray spectrometry system #2. The lowest detection limits of 0.067 and 0.032 ng mL^{-1} for arsenic and antimony, respectively, in water samples could be obtained using a t_i-t_d-t_c of 1-50-10 h and the system #2 (Expt. #6). The detection limits for arsenic and antimony were further

lowered to 0.026 and 0.010 ng mL^{-1}, respectively, and the sensitivity improved for solid samples using a longer irradiation time and the system #2 (Expt. #11).

Table 5.1. Sensitivity and Detection Limits for Arsenic and Antimony Species in Water Samples by Solvent Extraction-NAA

Expt. No.	t_i-t_d-t_c, h	Detection system	Physical form	Sample type	L_D, ng mL^{-1}		Sensitivity, counts ng^{-1}	
					As	Sb	As	Sb
1	0.5-5-2	System #1	liquid	NASS-1	0.46	0.60	20	13
2	1-10-5	System #1	liquid	SLRS-4	0.20	0.28	87	44
3	1-30-5	System #1	liquid	SLRS-4	0.18	0.24	51	35
4	0.5-5-2	System #2	liquid	NASS-1	0.24	0.40	36	22
5	1-10-5	System #2	liquid	SLRS-4	0.10	0.12	200	120
6	1-50-10	System #2	liquid	SLRS-4	0.067	0.032	131	152
7	2-50-4	System #2	solid	NASS-1	0.056	0.044	142	188
8	2-50-8	System #2	solid	NASS-1	0.040	0.029	270	367
9	2-50-12	System #2	solid	tap water	0.027	0.020	385	540
10	2-95-12	System #2	solid	SLRS-4	0.038	0.013	118	334
11	2-76-24	System #2	solid	seawater	0.026	0.010	336	768

5.1.2 Sensitivities and Detection Limits for Selenium

Selenium was determined in this thesis using a CINAA method which has been described in Section 3.6. The sensitivity and detection limit in CINAA depend on the number of cycles in addition to other parameters affecting conventional NAA. The sensitivities and detection limits for selenium in a seawater sample and a Riverine Water CRM SLRS-4 using solvent extraction in combination with CINAA are presented in

Tables 5.2 and 5.3, respectively. It can be seen that the sensitivity increases with the increasing of cycles when other factors are kept constant. The detection limits decrease with the increasing number of cycles. The improvement of the detection limits in SLRS-4 from 0.19 ng mL^{-1} using one cycle to 0.12 ng mL^{-1} using 5 cycles allowed the quantitative measurement of selenium at 0.16 ng mL^{-1} (Table 5.3). On the other hand, the detection limit was lowered from 0.30 ng mL^{-1} using one cycle to 0.16 ng mL^{-1} using 5 cycles for the seawater sample, the concentration of selenium in it was even lower and could not be measured. Although the detection limit generally decreases with increasing number of cycles, the rate of decrease slows down considerably after a few cycles. Therefore, a proper selection of the number of cycles must be made for optimum results.

Table 5.2. Evaluation of Solvent Extraction-CINAA for Se(IV) in a Seawater Sample

t_i-t_d-t_c, s	Number of cycles	Sensitivity, counts ng^{-1}	L_D, ng mL^{-1}	Se(IV) in sample, ng mL^{-1}
30-10-30	1	1.12	0.30	<0.30
30-10-30	2	2.22	0.21	<0.21
30-10-30	3	3.31	0.22	<0.22
30-10-30	4	4.46	0.19	<0.19
30-10-30	5	5.61	0.16	<0.16

5.1.3 Determination of Inorganic Arsenic, Antimony and Selenium

Five different types of natural water samples were analyzed using the solvent extraction method described in Section 4.2. The levels of the inorganic arsenic, antimony and selenium species are presented in Table 5.4. The uncertainties are expressed at 95% confidential interval. The As(V) species was found to be the predominant species in all water samples. The total concentration of As(III) and As(V) in all the water samples were found to be <1 ng mL^{-1} except the seawater which had a value of 1.57 ng mL^{-1} which is

within the normal range of 1-2 ng mL^{-1} for seawater [131-133, 141]. The level of As(III) in the lake water sample was below the detection limit of 0.06 ng mL^{-1}.

Table 5.3. Evaluation of Solvent Extraction-CINAA for Se(IV) in SLRS-4

t_i-t_d-t_c, s	Number of cycles	Sensitivity, counts ng^{-1}	L_D, ng mL^{-1}	Se(IV) in sample, ng mL^{-1}
30-10-30	1	1.12	0.19	<0.19
30-10-30	2	2.22	0.17	<0.17
30-10-30	3	3.31	0.14	0.14
30-10-30	4	4.46	0.13	0.17
30-10-30	5	5.61	0.12	0.16

Table 5.4. Levels of Inorganic Arsenic, Antimony and Selenium Species in Selected Natural Water Samples (ng mL^{-1})

Species	Seawater	Tap water	Rain water	Spring water	Lake water
As(III)	0.31±0.05	0.13±0.04	0.10±0.02	0.030±0.005	<0.06
As(V)	1.26±0.09	0.37±0.10	0.16±0.03	0.060±0.006	0.09±0.01
Sb(III)	0.060±0.008	<0.10	<0.10	0.021±0.003	<0.10
Sb(V)	0.065±0.004	<0.10	0.12±0.02	0.040±0.006	<0.10
Se(IV)	<0.16	<0.30	<0.30	<0.30	<0.30

Very small amounts of Sb(III) and Sb(V) were found in the seawater and the spring water samples. Only Sb(V) could be quantitatively measured in the rain water samples. Both antimony species were found to be below the detection limits in the tap

water sample. Concentrations of Se(IV) in all water samples was also below the detection limits.

5.1.4 Inorganic Arsenic, Antimony and Selenium in CRMs

Although a number of water CRMs with certified values for the total elemental concentrations are available, no CRM with information on arsenic, antimony and selenium species can be found. We have analyzed two CRMs, namely NRCC Seawater NASS-1 and Riverine Water SLRS-4, for As(III), As(V), Sb(III), Sb(V), and Se(IV) using the solvent extraction method coupled to NAA. The results are given in Table 5.5. It appears that the inorganic arsenic species account for about 93% of the total elemental concentration in NASS-1, and about 79% in SLRS-4. The sum of Sb(III) and Sb(V) in SLRS-4 was found to be 0.22 ng mL^{-1}, which accounts for about 96% of the total antimony concentration of 0.23 ng mL^{-1}. A higher level of Sb(V) was found in NASS-1 than in SLRS-4. The Se(IV) content in SLRS-4 was found to be 0.16±0.02 ng mL^{-1} and that in NASS-2 was below the detection limit. Neither certified nor information values are provided for total selenium in both CRMs.

Table 5.5. Levels of Inorganic Arsenic, Antimony and Selenium Species in CRMs (ng mL^{-1})

Sample	SLRS-4	NASS-1
As(III)	0.22±0.04	0.21±0.03
As(V)	0.32±0.08	1.33±0.09
Sb(III)	0.010±0.004	<0.10
Sb(V)	0.21±0.04	0.31±0.05
Se(IV)	0.16±0.02	<0.16
Certified Total arsenic	0.68±0.06	1.65±0.19
Certified Total antimony	0.23±0.04	

5.2 Determination of Inorganic and Organic Arsenic in Natural Waters

5.2.1 Recommended Scheme for Speciation of Arsenic in Natural Waters

The overall scheme for the separation of inorganic and organic arsenic species is shown in Fig. 5.1. About 200 mL of the filtered water sample was used to start with. Organically bound arsenic (OBAs) was the first arsenic species to be separated by a solid phase extraction method using a C_{18}-bonded silica column. The water sample was allowed to pass through the column first, followed by 10 mL of DDW. The column was then eluted with 7 mL of acetonitrile. The eluate was collected in a medium-size irradiation vial packed with a single layer of a filter paper (Whatman, ashless), dried under an infrared lamp, and analyzed for arsenic by NAA. The irradiation time for the determination of OBAs was 5 to 7 h in the inner sites of DUSR. The samples were counted for 5 to 24 h after a decay of a minimum of 24 h.

The second series of steps involved the separation of DMA and AsB species using a cation exchange column, which was connected to the C_{18} column in series. The cation exchange column was eluted with 15 mL of 1M ammonia and the eluate was collected in a 25-mL flask; and it was evaporated to dryness using a rotary evaporator. The dried residue was dissolved in 2 mL DDW. Exactly 200 μL of this solution was injected into the HPLC system for the separation of AsB species from DMA. The HPLC mobile phase was 10-mM $NH_4H_2PO_4/(NH_4)_2HPO_4$ buffer at pH 6.5, and the flow rate was about 1 mL min^{-1}. The fractions containing the arsenic species were collected, placed in small-size irradiation vials, dried under an infrared lamp, and analyzed for arsenic in the AsB species and in DMA by NAA. Blank experiments were done by injecting 200 μL of DDW using the same HPLC procedure.

The water samples, after passing through the C_{18} and cation exchange columns, were split into three parts. About 21 mL of the water sample was evaporated to dryness, and then dissolved in 1 mL DDW. The As(III), As(V) and MMA species were further separated by HPLC into individuals using the same column but a different mobile phase which was a 75mM $NH_4H_2PO_4/(NH_4)_2HPO_4$ buffer at pH 5.5. The arsenic content of As(III), As(V) and MMA were determined by NAA.

The remaining 189 mL of the water sample was further split into two portions of about 94 mL each for the separation of As(III) and As(V) by solvent extraction. To the

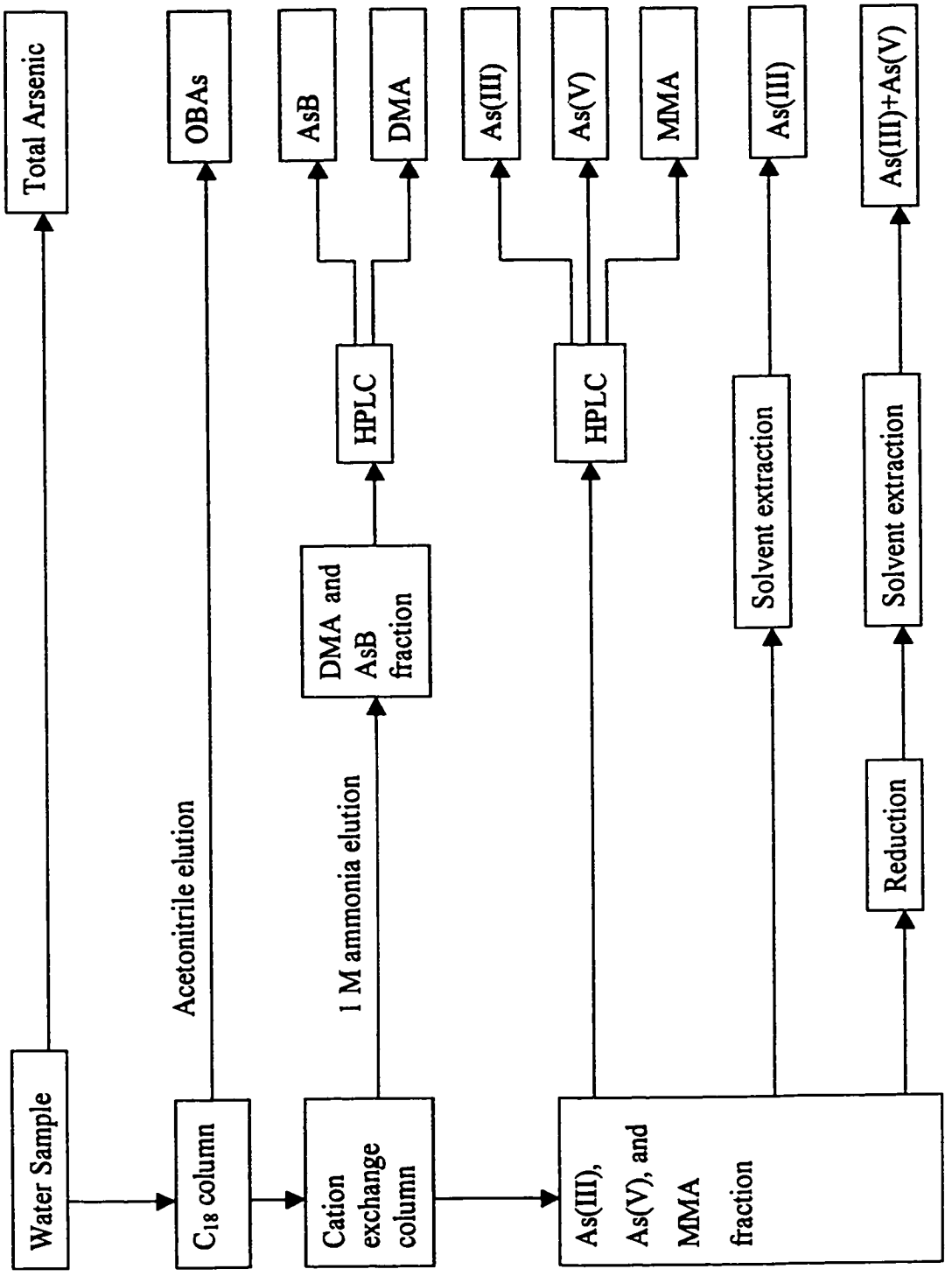

Fig. 5.1. Speciation Scheme for Arsenic in the Riverine Water CRM SLRS-4

first 94-mL of the water sample the following solutions were added in sequence: 15 mL of 0.05M acetic acid-ammonium acetate buffer of pH 5.5, 8 mL of 6% EDTA solution, and 2 mL of 5% APDC. The As(III) species was extracted from this solution by vigorously shaking it with 10 mL of MIBK for 10 min on a wrist action shaker. After the phase separation, 18 mL of the MIBK was back extracted with 2 mL of 4M HNO_3 solution. After the phase separation, this HNO_3 solution was transferred into a medium-size irradiation vial, dried under an IR lamp, and heat-sealed for NAA.

The second 94-mL portion of the water sample was adjusted to about pH 4, and 5 mL of 5% L-cysteine solution were added to it, and was allowed to react for 30 min. After this reduction step, the pH of the solution was checked and adjusted to 5-6 if needed, and the extraction of As(III)+As(V) was carried out using the same procedure as that described for As(III) alone.

5.2.2 Speciation of Arsenic in a Riverine Water CRM

Speciation of arsenic in the Riverine water CRM (NRCC SLRS-4) was carried out using the recommended procedure described above. Concentrations of six arsenic species in this CRM are shown in Table 5.6. The concentrations of As(V) and DMA by HPLC-NAA were 0.46±0.1 and 0.06±0.02 ng mL^{-1}, respectively. The levels of AsB, As(III) and MMA were below their detection limits by HPLC-NAA. The levels of As(III) and As(V) were also determined by the solvent extraction NAA method and found to be 0.22±0.04 and 0.32±0.08 ng mL^{-1}, respectively. It is obvious (Table 5.6) that the detection limits for As(III) and As(V) by solvent extraction NAA are much lower than that by HPLC-NAA. It is also evident that As(V) and As(III) were found to be the predominant species in this water sample. The DMA concentration accounts for about 11% of the total arsenic measured, and about 9% of the certified total arsenic concentration. The sum of the detected species accounts for about 76% and 79% of the total arsenic by the HPLC-NAA method and the solvent extraction NAA method, respectively. The HPLC-NAA chromatograms of AsB and DMA, as well as As(III), MMA and As(V) in a spiked sample are shown in Figs. 5.2 and 5.3. The chromatograms for the SLRS-4 sample are given Fig. 5.4 and 5.5.

Table 5.6. Concentrations of Arsenic Species in Selected Riverine Water CRMs (ng mL^{-1})

Sample	SLRS-4*	SLRS-4*	SLRS-1 [46]	SLRS-1 [290]	SLRS-2 [258]	SLRS-3 [290]
Method	HPLC-NAA	Solvent extraction-NAA	Chemical separations-AAS	HPLC-HG-ICP-MS	HPLC-HG-ICP-MS	HPLC-HG-ICP-MS
As(V)	0.46 ± 0.10	0.32 ± 0.08	0.18 ± 0.02	0.30 ± 0.014	0.44 ± 0.02	0.49 ± 0.031
As(III)	<0.21	0.22 ± 0.04	0.16 ± 0.01	<0.001	0.05 ± 0.01	<0.001
AsB	<0.038					
MMA	<0.21		<0.02	0.05 ± 0.008	0.10 ± 0.01	0.08 ± 0.01
DMA	0.06 ± 0.02		0.05 ± 0.01		0.13 ± 0.01	
OBAs	<0.005		<0.01			
Unreactive**			0.12 ± 0.02			
Sum	0.52 ± 0.10	0.54 ± 0.09	0.52 ± 0.06	0.35 ± 0.024	0.72 ± 0.04	0.57 ± 0.041
Total As	0.71 ± 0.07		0.54 ± 0.05			
Certified Total arsenic	0.68 ± 0.06	0.68 ± 0.06	0.55 ± 0.08	0.55 ± 0.08	0.77 ± 0.09	0.72 ± 0.05

* This work

** Fraction of arsenic that was unreactive towards $NaBH_4$.

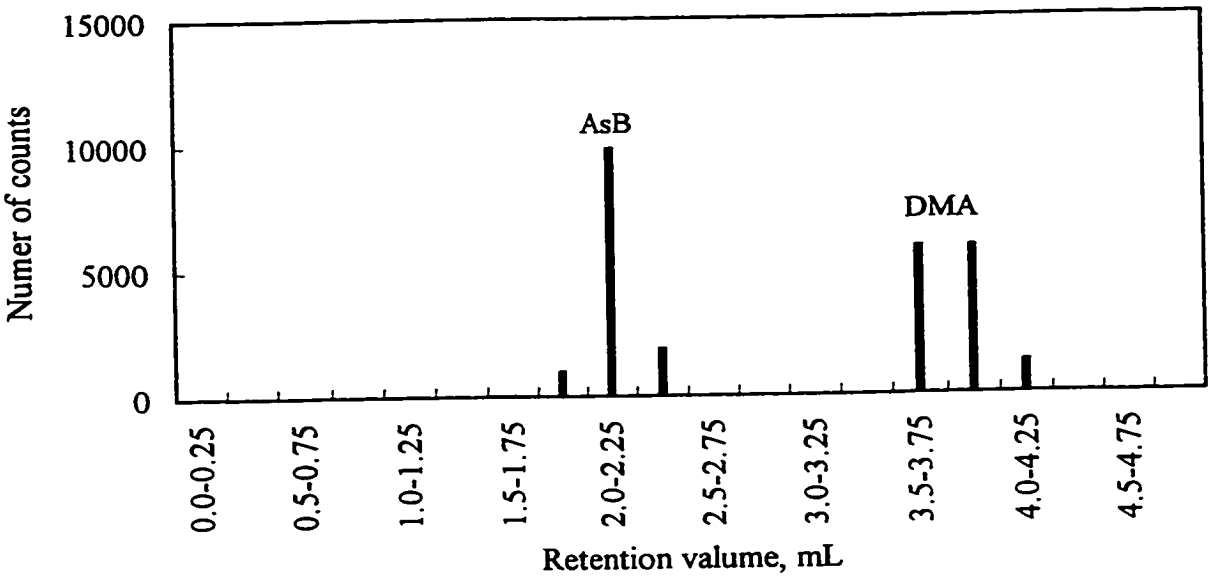

Fig. 5.2. Chromatogram of AsB and DMA in SLRS-4 Spiked with 1.25 ng mL^{-1} of Each Species
NAA: flux=$5X10^{11}$n $cm^{-2}s^{-1}$; t_i-t_d-t_c=2-48-5 h

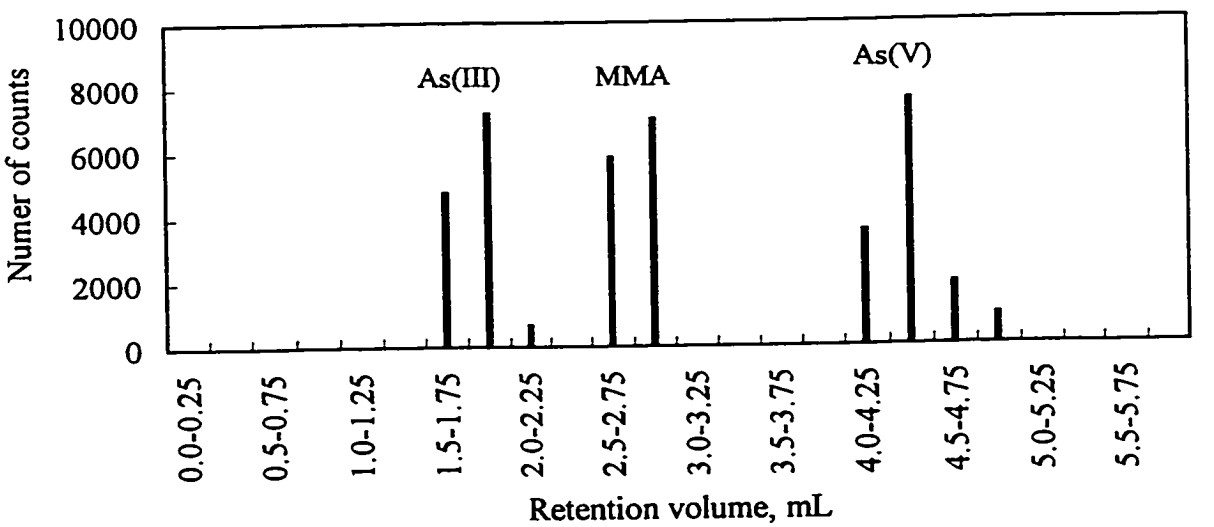

Fig. 5.3. Chromatogram of As(III), MMA and As(V) in SLRS-4 Spiked with 1.25 ng mL^{-1} of Each Species
NAA: flux=$5X10^{11}$ n cm^{-2} s^{-1}; t_i-t_d-t_c=2-20-2 h.

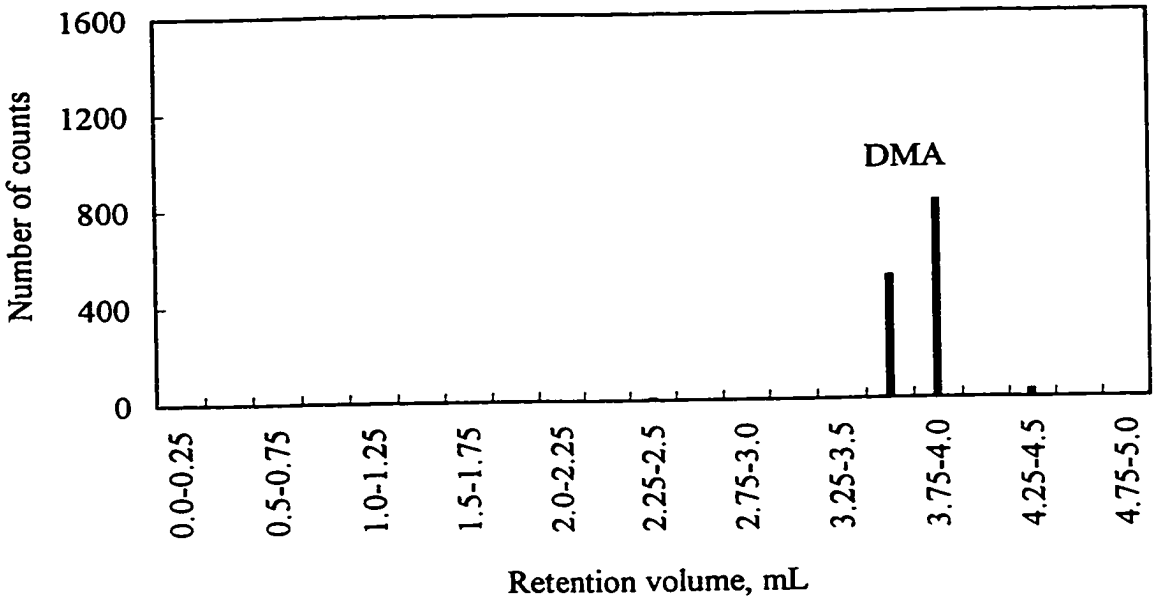

Fig. 5.4. Chromatogram of AsB and DMA in SLRS-4
NAA: flux=1X10^{11}n cm^{-2} s^{-2}; t_i-t_d-t_c=5-20-5 h

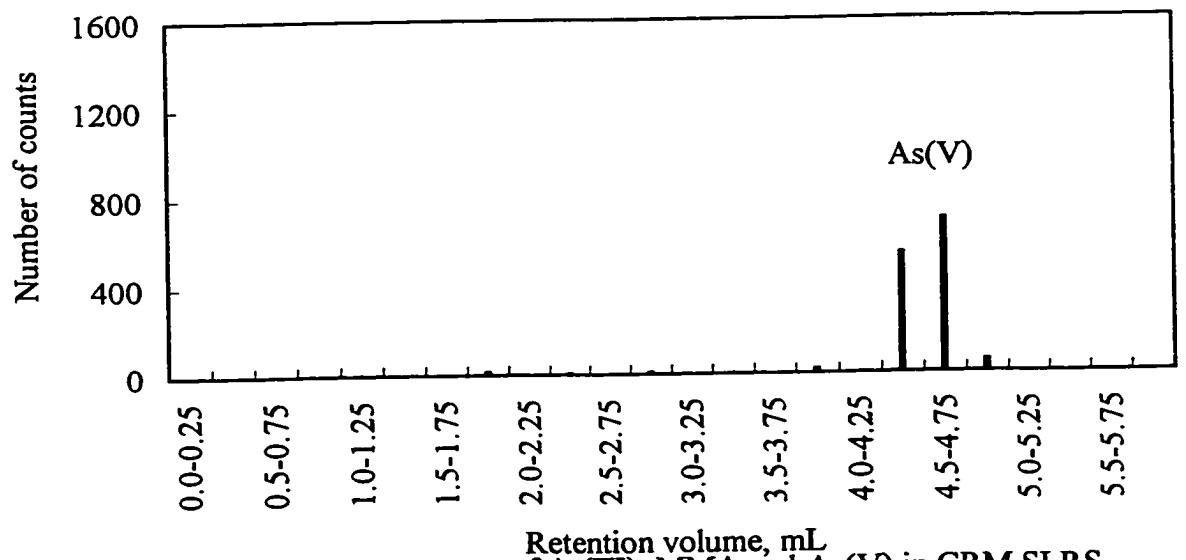

Fig. 5.5. Chromatogram of As(III), MMA and As(V) in CRM SLRS-4

NAA: flux=1X10^{11}n cm^{-2} s^{-1}, t_i-t_d-t_c=5-20-2 h

The chemical forms and the levels of the possible OBAs in natural waters are not known with any degree of certainty. Sturgeon and coworkers [46] defined OBAs as any organic arsenic species that can be extracted using a C_{18} column. They reported a value of less than 0.01 μg of OBAs mL^{-1} in the NRCC Riverine Water CRM SLRS-1 (Table 5.6). Their solid-phase extraction method was modified in this thesis. A higher resin bed of a C_{18} column and a lower volume of water were used here. Acetonitrile was used to elute the OBAs instead of methanol because of its stronger eluting strength [325]. An attempt was also made to analyze three successive fractions (7-mL each) of the acetonitrile eluate from the C_{18} column. The OBAs level was found to be <0.005 ng mL^{-1} (detection limit) in all fractions.

Sturgeon and coworkers [46] also defined a term called "non-hydride active species" as the arsenic species not reactive towards $NaBH_4$ in 0.5M HCl. These species include AsB which is known to be non-reactive towards $NaBH_4$. They attributed a significant fraction of about 22% of the total arsenic in SLRS-1 as the non-hydride active species. We found that the concentration of AsB in SLRS-4, which is a riverine water similar to SLRS-1, to be <0.038 ng mL^{-1} (Table 5.6).

Results reported by other researchers for similar riverine water CRMs, namely SLRS-1, SLRS-2 and SLRS-3, are also listed in the Table 5.6. Detailed information on arsenic speciation in these materials is not available. Some general observations, however, can be made. In all cases, As(V) was found to be the predominant species. Some authors reported levels of As(III), MMA and DMA. It appears that the sum of all measured arsenic species accounted for 64% to 95% of the total certified arsenic concentrations.

5.2.3 Sensitivities and Detection Limits for Various Arsenic Species

The sensitivities and detection limits for As(III) and As(V) in natural water samples by solvent extraction NAA have already been described in Section 5.1.1. The values for OBAs, As(III), As(V), MMA, DMA and AsB by the speciation procedure described in Section 5.1.2 are shown in Table 5.7.

The detection limit of 0.005 to 0.006 ng OBAs mL^{-1} obtained in this work is an improvement by a factor of 2 of that reported by Sturgeon and coworkers [46] using a

similar separation procedure but an ET-AAS detector. Several steps were taken to achieve this low detection limit. For example, the SPE method used in this work extracted only the trace hydrophobic species from the natural water samples and removed almost all ionic species including the interfering elements such as sodium and chlorine. The use of longer irradiation and counting times as well as higher efficiency detection system further improved the detection limit and the sensitivity. It can be improved again if a higher neutron flux is available and if a higher volume of water, such as1 L used by Sturgeon and coworkers [46] is employed.

Table 5.7. Sensitivities and Detection Limits for Speciation of Arsenic in SLRS-4 by SPE-NAA and by HPLC-NAA

Species	t_i-t_d-t_c, h	Detection system	Sensitivity, Counts ng^{-1}	L_D, ng mL^{-1}
OBAs	2-22-8	System #2	564	0.006
	7-65-5	System #2	387	0.006
	7-30-5	System #2	974	0.005
DMA, AsB	5-20-5	System #2	740	0.038
As(III), As(V), MMA	5-20-2	System #2	296	0.21

Detection limits of 0.038 ng mL^{-1} for AsB and DMA and 0.21 ng mL^{-1} for As(III), As(V) and MMA were obtained using slightly different NAA timing schemes (Table 5.7). The lower detection limits for AsB and DMA were mainly obtained by using 10 times more water sample.

A comparison of detection limits of various arsenic species by a number of commonly used methods are summarized in Table 5.8. In order to facilitate comparison, the IUPAC definition of limits of detection (c_L) was used, and the NAA detection limits obtained in this work were re-calculated as three times the standard deviation of the blank. The detection limit of 0.033 μg L^{-1} for As(III) as well as As(V) by solvent

extraction NAA is superior to that of 0.5-2.1 μg L^{-1} for As(III) and 0.5-2.6 μg L^{-1} for As(V) by HPLC-HG-AAS [245], HPLC-ICP-MS [228, 282], HPLC-ET-AAS [234] and CE-ICP-MS [275]. Again the detection limits for MMA, DMA and AsB obtained in this work by HPLC-NAA are superior, where applicable, to those reported by the above methods. It appears that HPLC-HG-ICP-MS [258] detection limits are lower than most other methods. Two of the three other NAA methods [132, 163] used a considerably higher neutron flux and reported lower detection limits than ours for As(III) and As(V). The combination of solvent extraction NAA, SPE-NAA and HPLC-NAA methods developed in this thesis could be conveniently used to determine a total of 6 species, namely As(III), As(V), MMA, DMA, AsB and OBAs, in natural water samples with low detection limits.

Table 5.8. Species Analyzed and C_L Obtained by Reported Methods (μg L^{-1})

Species determined	As(III)	As(V)	MMA	DMA	AsB	OBAs
This work	0.033	0.033	0.17	0.021	0.021	0.005
HPLC-HG-ICP-MS [258]	0.01	0.051	0.029	0.018		
HPLC-HG-AAS [245]	0.5	0.5	0.5	0.5		
HPLC-ICP-MS [228]	0.9	2.6	3.0	1.5	1.4	
HPLC-ICP-MS [282]	1	1				
HPLC-ET-AAS [234]	1.6	1.6	1.6	1.9	1.9	
CE-ICP-MS [275]	2.1	1.3	1.6	1.7		
Co-precipitation NAA [161]	0.02	0.02				
Co-precipitation NAA [163]	0.001	0.001				
Solvent extraction NAA [132]	0.01	0.01				

6. SPECIATION OF ARSENIC IN SEAFOODS

The concentrations of various arsenic species in three samples of seafood using the analytical methods developed in Chapter 4 and the speciation scheme given in Section 6.2 are presented in this chapter. The precision and accuracy of the methods were evaluated using one tuna fish CRM. The total arsenic levels in selected food samples and two CRMs were determined by INAA. Lipid-soluble arsenic and residual arsenic were also determined by INAA after the methanol-MIBK-water extraction of arsenic from the samples. Five water-soluble arsenic species, namely As(III), As(V), MMA, DMA and AsB, were determined by HPLC-NAA methods. Analyses were also performed of a fish extract spiked with standards of the five arsenic species. Finally, the method developed here is compared with other methods reported in the literature. Details of the work are described below.

6.1 Method for Extraction of Arsenic Species from Fish

6.1.1 Selection of Solvents

The extraction of arsenic compounds from fish samples is an important step towards the speciation analysis. Past studies mostly concentrated on the identification of water-soluble arsenic species, which of course, account for much of the arsenic in marine organisms. Methanol-water mixed solvents were employed by many researchers to extract arsenic species from marine organisms [59, 64, 66-69, 77, 84, 88, 91, 97, 307]. Methanol-water-chloroform mixtures were also used, which helped to clean up lipids from raw extracts of these organisms [56, 60, 61, 63, 77, 96, 114, 307]. A methanol-MIBK-water system has also been used in this thesis for the extraction of various arsenic species. It was found that methanol-MIBK-water system worked better than methanol-water-chloroform in conjunction with NAA for the quantitative determination of water-soluble and lipid-soluble arsenic species and of residual arsenic.

6.1.2 Effect of Number of Extractions on Extraction Yield

The effect of number of extractions on the extraction yield with the methanol-MIBK-water system was evaluated using the Tuna Fish Tissue CRM 627. About 1 g of this tissue was extracted first by 10 mL of methanol-MIBK mixture (1:1), followed by three more extractions with 5 mL of the same mixture. The extraction yield of arsenic was calculated from the arsenic concentration of the original sample and that of the residual sample after each extraction. The results are graphically presented in Fig. 6.1. It was found that about 83% of the arsenic was extracted in the first extraction step. The yield was very high, >96%, after four extractions.

The same extraction procedure was applied to other 6 fish samples. The yields were quantitative (Table 6.1) in all cases except the Dogfish Liver CRM DOLT-2 which had a yield of about 81%.

6.1.3 Effect of Lipid Content on Extraction Yield

The difference in mass between the original sample and that in the residue after four extractions was taken as the approximate lipid content of the sample. The lipid contents of the fish samples are shown in Table 6.1. The extraction yield of arsenic is plotted against the lipid content of the fish samples, and the graph is shown in Fig. 6.2. The data indicate that the higher the lipid concentration of the sample the lower the extraction yields. More samples with varying lipid concentrations need to be analyzed to evaluate if such a relationship does exist.

6.2 Recommended Scheme for Speciation of Arsenic in Fish Samples

The recommended procedure for the speciation of arsenic in fish samples is shown in Fig. 6.3. The separation method developed in this thesis consists of the following steps.

(i) The first step was the extraction of all possible arsenic compounds from fish samples with a 1:1 mixture of methanol-MIBK. Between 1 and 2 g of the freeze-dried fish sample was mixed with 10 mL of methanol and 10 mL of MIBK in an extraction tube. The tube was shaken in a mechanical shaker for 2 h. The mixture

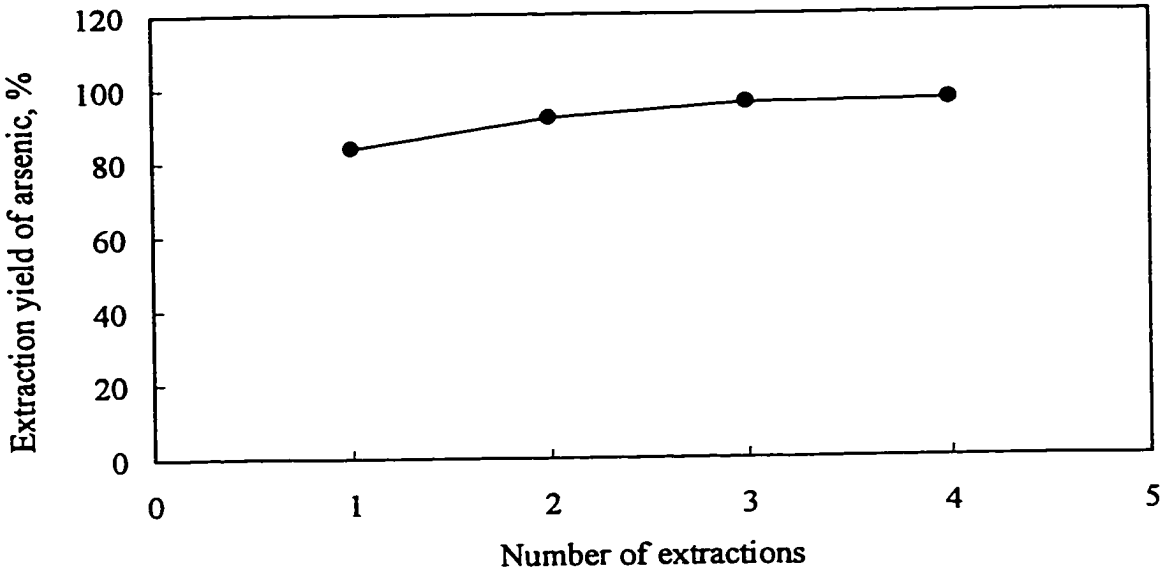

Fig. 6.1. Extraction Yield of Arsenic from Tuna Fish Tissue by Different Number of Extractions

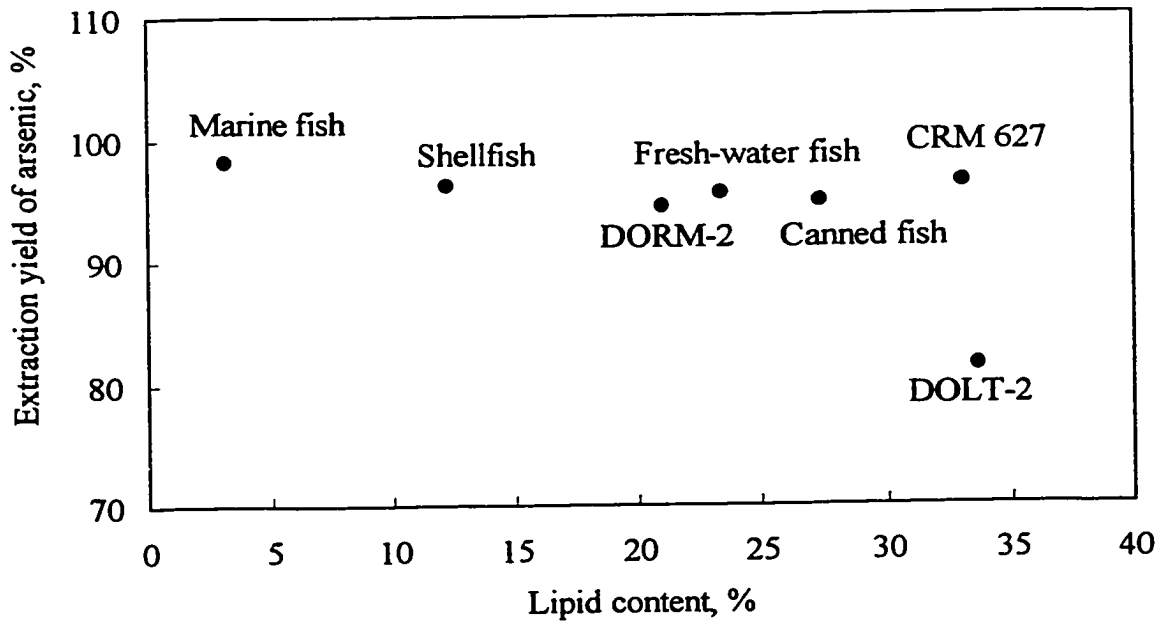

Fig. 6.2. Extraction Yields of Arsenic at Different Lipid Content

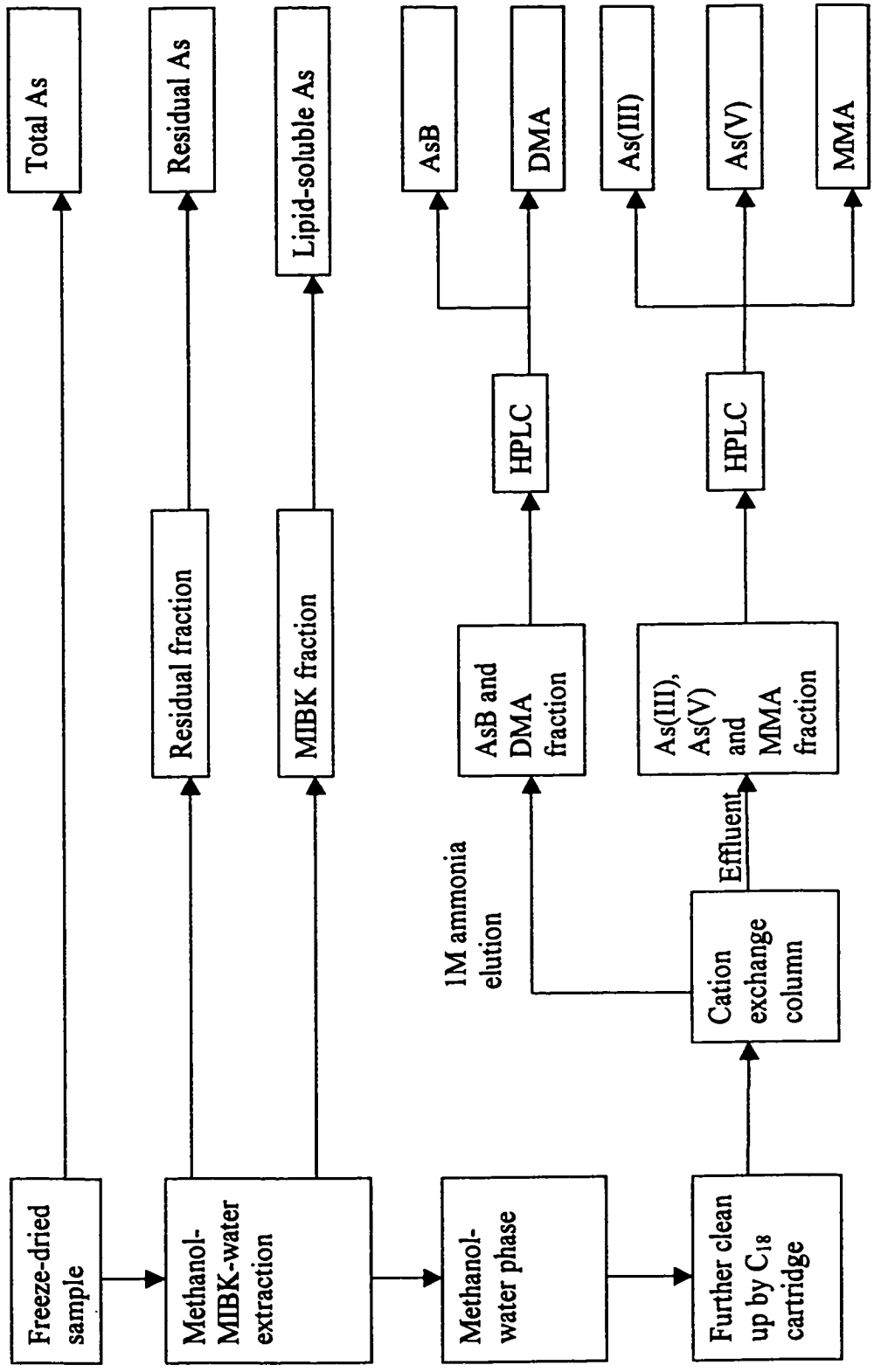

Fig. 6.3. Speciation Scheme for Arsenic in Fish Samples

Table 6.1. Extraction Yields of Arsenic from Different Samples

Sample	Sample mass used, g	Lipid, %	Extraction yield, %
Marine fish	1.0430	3.1	98.2
Fresh water fish	1.9958	23.4	95.6
Canned fish	2.1401	27.3	94.9
Shellfish	1.9240	12.2	96.2
Tuna Fish Tissue (CRM 627)	0.9630	33.0	96.4
Dogfish Muscle (DORM-2)	1.5596	21.2	94.5
Dogfish Muscle (DORM-2)	2.0250	21.0	92.3
Dogfish Liver (DOLT-2)	2.2280	33.6	81.4

was then centrifuged for 5 min, the liquid was decanted off, and poured into a separatory funnel.

(ii) The residual solid from step (i) was further extracted three more times using 10 mL of a 1:1 mixture of methanol and MIBK.

(iii) All extracts from step (ii) were combined with the extract from step (i) in a separatory funnel. About 25 mL of DDW were added to the combined extract, mixed thoroughly, and the phase separation was carried out using centrifugation.

(iv) The lower layer of the methanol-water phase from step (iii) was transferred to another separatory funnel and washed two times with 5 mL of MIBK. These resulting MIBK portions were combined with the upper layer of the MIBK phase from step (iii).

(v) The MIBK from step (iii) was washed twice with 5 mL of DDW, and the resulting aqueous solution was combined with the methanol-water phase from step (iv).

(vi) The volume of the MIBK phase from step (v), about 35 mL, was reduced to about 5-7 mL by evaporation on a rotary evaporator. It was quantitatively transferred to a 10-mL volumetric flask and made up to the mark with additional MIBK. About 1 mL of

this MIBK phase was pipetted into an irradiation vial and heat-sealed for the determination of lipid-soluble arsenic species by NAA.

(vii) The residue from step (iii) was dried in air in a fume hood and weighed again. The difference between mass before and after the extraction was taken as the approximate content of lipid in that sample. A portion of the dried residue was weighed into a small-size irradiation vial and analyzed for arsenic by INAA using an irradiation time of 30 min, a decay time of 24 h, and a counting time of 5-24 h using anti-coincidence gamma-ray spectrometry.

(viii) The methanol-water phase from step (v) was evaporated to dryness on a rotary evaporator, and then redissolved in 3 mL of DDW.

(ix) The solution from step (viii) was loaded onto a C_{18} cartridge. The cartridge was further flushed with an additional 5 mL of DDW. This effluent was transferred to a 10-mL volumetric flask with DDW.

(x) The solution from step (ix) was loaded on to a cation exchange column (Dowex 50W×8-200). An additional 10 mL of DDW were added to the column in several portions to completely flush the As(III), As(V) and MMA species from the column. The effluent and all washings, a total volume of about 20 mL, were collected.

(xi) The solution from step (x) was evaporated to dryness and redissolved in 5 mL of DDW. About 200 μL of this solution was injected in the HPLC system. The As(III), As(V) and MMA were separated using a mobile phase of 75-mM $NH_4H_2PO_4$/ $(NH_4)_2HPO_4$ buffer at pH 5.5.

(xii) The cationic species, namely AsB and DMA, retained by the column from step (x) were eluted by 15 mL of 1M ammonia solution. The 15-mL eluate was then evaporated to dryness, and redissolved in 5 mL DDW. About 200 μL of this solution was injected in the HPLC system. The AsB and DMA species were separated using a mobile phase of 10-mM $NH_4H_2PO_4$/$(NH_4)_2HPO_4$ buffer at pH 6.5.

(xiii) The eluates from steps (xi) and (xii) were collected in 0.25 mL fractions in the small-size irradiation vials and dried under an IR lamp. A trimmed cap was placed inside the vial to keep a constant geometry for the irradiation and counting steps. All vials were then heat-sealed and irradiated for 2 to 5 h. The counting time varied between 2 and 12 h after a decay time of 20 h.

6.3 Determination of Arsenic by INAA

6.3.1 Determination of Total Arsenic

The determination of total arsenic in fish samples by INAA is fairly simple. It is a non-destructive method meaning that it does not require dissolution of the sample prior to measurement. Thus INAA is superior to other techniques that often require the digestion of the sample leading to either incomplete dissolution or possible contamination from reagents and sample handling. In non-INAA techniques, there can also be loss due to the formation of volatile components during digestion.

Several factors needed to be considered for the determination of arsenic by INAA. High sensitivities and low detection limits were required for samples with low levels of arsenic, such as the fresh water fish samples. Although the arsenic levels were found to vary in the fish samples and CRMs, they were relatively higher than those in natural waters. However, the sodium content of the marine fish samples were relatively high leading to high radioactivity after irradiations with neutrons. This high activity contributed to high background counts resulting in poorer detection limits. These poor limits as well as poor sensitivities were also obtained if longer decay times were allowed before counting to reduce the interference from sodium. Bromine is another element which is also present in seafood samples in fairly high concentrations. The 554.3-keV gamma-ray of ^{82}Br could interfere with the 559.1-keV gamma-ray of ^{76}As. The similar half-lives of these two nuclides make the procedure of adjusting the decay and counting times to reduce the interference rather ineffective.

Several NAA methods were developed and optimized in this thesis to obtain reliable measurements of arsenic in the fish samples. These include the selection of timing and other parameters for thermal INAA (TINAA) and epithermal INAA (EINAA) using conventional as well as anti-coincidence counting techniques. Adjustment to a relatively short irradiation time, a suitable length of decay and a long counting time could minimize the interference from sodium, as the product nuclide ^{24}Na has a half-life of 14.96 h compared to ^{76}As with a half-life of 26.3 h. An alternative method would be to use EINAA [320] since the analyte isotope ^{75}As has a large resonance integral (I) while the interfering isotope ^{23}Na does not (Table 3.1). It was soon found that another

potentially interfering isotope, namely ^{81}Br, also has a high resonance integral. Although the activity of sodium was suppressed, the activities of both ^{82}Br and ^{76}As were enhanced. The anti-coincidence counting technique can significantly suppress the bromine peak along with the suppression of the Compton background as earlier described by Zhang [304] from our laboratory. An anti-coincidence counting method was developed in this thesis.

The irradiation times for the determination of total arsenic in fish samples by TINAA and EINAA were selected as 10 and 30 min, respectively; decay and counting times as well as the counting technique were varied. The results from four fish samples and three CRMs are summarized in Tables 6.2 to 6.8. The methods were evaluated mainly on the basis of four parameters, namely signal-to-background ratio, As-to-Br counts ratio, sensitivity, and the detection limit L_D (calculated according to Equation 5.4).

Generally speaking, conventional counting technique gave the poorest signal-to-background ratios as well as detection limits for all samples. Although some of the differences in signal-to-background ratios were caused by the different efficiencies of the two detectors used, most of the differences in this ratio were mainly due to the suppression of the Compton background by the anti-coincidence counting technique. For example, a comparison of Expt. #2 and #4 (Table 6.2) for a marine fish sample and Expt. #1 and #2 (Table 6.7) for the NRCC CRM DORM-2 by TINAA show that the differences in the signal-to-background ratios between anti-coincidence and conventional counting techniques are 2.5 and 2.3, respectively under the nearly same irradiation, decay and counting times.

The suppression of the ^{82}Br counts by the anti-coincidence counting technique can be seen from the higher As-to-Br counts ratio obtained in all samples using both TINAA and EINAA methods. As expected, lower detection limits were obtained by anti-coincidence counting technique because of the suppression of the background counts. The lowest detection limits for total arsenic were obtained mostly by using EINAA in combination with the anti-coincidence counting technique.

Since arsenic was assayed through its long-live nuclide ^{76}As, the sensitivity expressed as number of counts per microgram of arsenic was found to be higher when longer irradiation and counting times and shorter decay times were used. The selection

Table 6.2. Evaluation of INAA Methods for Arsenic in a Marine Fish Sample

Sample	INAA Method	Exp. No.	Counting technique	Detector	t_i (min)-t_d (h)-t_c (h)	Signal-to-background ratio	As-to-Br counts ratio*	Sensitivity, counts μg^{-1} As	L_D, $\mu g\ g^{-1}$ food (dry)
Marine fish (D01)	TINAA	1	Conventional	Ge(Li)	10-58-2	0.17	1.07	927	1.90
		2	Conventional	Ge(Li)	10-76-5	0.30	0.97	1388	1.15
		3	Anti-coincidence	HPGe	10-75-2	0.83	1.13	1681	0.64
		4	Anti-coincidence	HPGe	10-74-5	0.76	1.15	4151	0.44
		5	Anti-coincidence	HPGe	10-98-1	1.31	1.13	465	1.01
		6	Anti-coincidence	HPGe	10-172-5	1.48	1.14	314	1.23
	EINAA	7	Anti-coincidence	HPGe	30-28-1	0.94	1.14	655	0.64
		8	Anti-coincidence	HPGe	30-52-3	1.48	1.10	1017	0.44
		9	Anti-coincidence	HPGe	30-140-24	0.86	1.10	618	0.74
Residue of D01	TINAA	10	Anti-coincidence	HPGe	30-85-8	0.052	0.54	14 288	0.14

* Normalized to t_i-t_d-t_c = 10 min - 48 h - 2 h.

Table 6.3. Evaluation of INAA Methods for Arsenic in a Fresh Water Fish Sample

Sample	INAA Method	Exp. No.	Counting technique	Detector	t_i (min)-t_d (h)-t_c (h)	Signal-to-background ratio	As-to-Br counts ratio*	Sensitivity, counts μg^{-1} As	L_D, $\mu g\ g^{-1}$ food (dry)
Fresh water fish (D02)	TINAA	1	Conventional	Ge(Li)	10-34-2	0.033	0.22	1745	0.81
		2	Anti-coincidence	HPGe	10-26-3	0.095	0.37	9046	0.58
		3	Anti-coincidence	HPGe	10-57-3	0.20	0.37	4000	0.29
		4	Anti-coincidence	HPGe	10-154-5	0.47	0.37	505	0.59
	EINAA	5	Conventional	HPGe	30-21-5	0.33	0.25	5233	0.18
		6	Anti-coincidence	HPGe	30-30-10	0.30	0.37	5535	0.14
Residue of D02	TINAA	7	Conventional	HPGe	20-53-4	0.020	0.17	15 916	0.10
		8	Anti-coincidence	HPGe	20-49-4	0.038	0.23	12 966	0.085

* Normalized to t_i-t_d-t_c = 10 min - 48 h - 2 h.

Table 6.4. Evaluation of INAA Methods for Arsenic in a Canned Fish Sample

Sample	INAA Method	Exp. No.	Counting technique	Detector	t_i (min)-t_d (h)-t_c (h)	Signal-to-background ratio	As-to-Br counts ratio*	Sensitivity, counts μg^{-1} As	L_D, $\mu g\ g^{-1}$ food (dry)
Canned fish (D03)	TINAA	1	Conventional	Ge(Li)	10-52-2	0.018	0.22	1086	1.65
		2	Anti-coincidence	HPGe	10-77-2	0.071	0.30	1595	0.74
		3	Conventional	Ge(Li)	10-108-5	0.047	0.15	598	1.24
		4	Anti-coincidence	HPGe	10-150-4	0.22	0.29	455	0.92
	EINAA	5	Conventional	HPGe	30-27-5	0.092	0.27	4468	0.33
		6	Anti-coincidence	HPGe	30-55-8	0.17	0.31	2351	0.28
Residue of D03	TINAA	7	Conventional	HPGe	20-83-5	0.014	0.067	8913	0.14
		8	Anti-coincidence	HPGe	20-105-22	0.040	0.11	13 050	0.085

* Normalized to t_i-t_d-t_c = 10 min - 48 h - 2 h.

Table 6.5. Evaluation of INAA Methods for Arsenic in a Shellfish Sample

Sample	INAA Method	Exp. No.	Counting technique	Detector	t_i (min)-t_d (h)-t_c (h)	Signal-to-background ratio	As-to-Br counts ratio*	Sensitivity, counts μg^{-1} As	L_D, $\mu g\ g^{-1}$ food (dry)
Shellfish (D04)	TINAA	1	Conventional	Ge(Li)	10-31-2	0.029	0.42	1888	0.82
		2	Conventional	Ge(Li)	10-81-2.4	0.10	0.44	604	0.95
		3	Anti-coincidence	HPGe	10-146-3	0.62	0.80	384	0.81
	EINAA	4	Anti-coincidence	HPGe	30-48-5	0.20	0.76	1836	0.44
		5	Anti-coincidence	HPGe	30-146-10	0.31	0.79	261	1.1
Residue of D04	TINAA	6	Conventional	HPGe	20-105-24	0.044	0.18	17 690	0.10
		7	Anti-coincidence	HPGe	20-85-5	0.034	0.19	13 180	0.18

* Normalized to t_i-t_d-t_c = 10 min - 48 h - 2 h.

Table 6.6. Evaluation of INAA Methods for Arsenic in the Tuna Fish Tissue CRM

Sample	INAA Method	Exp. No.	Counting technique	Detector	t_i (min)-t_d (h)-t_c (h)	Signal-to-background ratio	As-to-Br counts ratio*	Sensitivity, countsμg^{-1} As	L_D, $\mu g\ g^{-1}$ food (dry)
Tuna fish Tissue CRM 627	TINAA	1	Anti-coincidence	HPGe	10-67-4.1	0.38	0.41	4140	0.31
		2	Anti-coincidence	HPGe	10-125-24	0.72	0.39	4114	0.22
	EINAA	3	Conventional	Ge(Li)	30-50-5	0.18	0.29	761	0.55
		4	Conventional	HPGe	30-20-2	0.37	0.39	2234	0.36
		5	Anti-coincidence	HPGe	30-22-2	0.30	0.40	1514	0.35
		6	Anti-coincidence	HPGe	30-97-24	0.47	0.40	1918	0.24
		7	Anti-coincidence	HPGe	30-42-8	0.60	0.40	3311	0.16
		8	Anti-coincidence	HPGe	30-20-5	0.33	0.39	3838	0.19
Residue of CRM 627	EINAA	9	Anti-coincidence	HPGe	30-30-5	0.21	0.25	3064	0.15
	TINAA	10	Conventional	HPGe	30-50-5	0.07	0.19	14 870	0.068
		11	Anti-coincidence	HPGe	30-90-12	0.15	0.18	11 320	0.050

* Normalized to t_i-t_d-t_c = 10 min - 48 h - 2 h.

Table 6.7. Evaluation of INAA Methods for Arsenic in the Dogfish Muscle CRM

Sample	INAA Method	Exp. No.	Counting technique	Detector	t_i (min)-t_d (h)-t_c (h)	Signal-to-background ratio	As-to-Br counts ratio*	Sensitivity, counts μg^{-1} As	L_D, $\mu g\ g^{-1}$ food (dry)
Dogfish Muscle (DORM-2)	TINAA	1	Conventional	Ge(Li)	10-90-5	0.64	1.03	960	1.2
		2	Anti-coincidence	HPGe	10-81-5	1.44	1.24	3452	0.42
		3	Anti-coincidence	HPGe	10-125-5	1.51	1.20	1083	0.74
	EINAA	4	Conventional	Ge(Li)	30-56-5	0.07	0.98	650	0.67
		5	Anti-coincidence	HPGe	30-20-1	1.15	1.18	808	0.38
Residue of DORM-2	TINAA	6	Conventional	Ge(Li)	20-77-3	0.12	0.49	1660	0.37
		7	Anti-coincidence	HPGe	20-74-3	0.26	0.60	5100	0.23

* Normalized to t_i-t_d-t_c = 10 min - 48 h - 2 h.

Table 6.8. Evaluation of INAA Methods for Arsenic in the Dogfish Liver CRM

Sample	NAA method	Exp. No.	Counting technique	Detector	t_i (min)-t_d (h)-t_c (h)	Signal-to-background ratio	As-to-Br counts ratio*	Sensitivity, counts μg^{-1} As	L_D, $\mu g\ g^{-1}$ food (dry)
Dogfish Liver (DOLT-2)	TINAA	1	Conventional	Ge(Li)	10-68-5	0.27	1.07	1713	0.97
		2	Anti-coincidence	HPGe	10-93-4	0.93	1.37	2040	0.51
	EINAA	3	Conventional	Ge(Li)	30-115-5	0.28	0.82	137	2.49
		4	Anti-coincidence	HPGe	30-43-5	0.92	1.32	2094	0.27
Residue of DOLT-2	TINAA	5	Conventional	HPGe	20-74-3	0.35	1.15	7105	0.20
		6	Anti-coincidence	HPGe	20-78-3	0.27	1.09	4700	0.27

* Normalized to t_i-t_d-t_c = 10 min - 48 h - 2 h.

of the irradiation, decay and counting times in the actual experiments was a compromise among the factors such as the arsenic level in the sample, levels of sodium and bromine, the radioactivity after irradiation, and experimental time.

The suppression of the Compton background by EINAA in conjunction with anti-coincidence counting can be easily seen from the spectra given in Figs. 6.4 through 6.9. The high background counts from the marine fish sample with a high sodium content analyzed by TINAA using the conventional counting technique (Fig. 6.4) were significantly suppressed using the anti-coincidence counting system (Fig. 6.5). The latter spectrum also indicates a better resolution of the 554.3 and 559.1 keV peaks of ^{82}Br and ^{76}As, respectively. Four gamma-ray spectra obtained from a fresh water fish sample, which has the lowest arsenic level among all the fish samples and CRMs analyzed in this work, are shown in Fig. 6.6 to 6.9. Again, the high background and a poor resolution of the arsenic and bromine peaks in the TINAA-conventional counting method made an accurate determination of the arsenic level very difficult. However with the suppression of the background and of the 554.3-keV photopeak of ^{82}Br by anti-coincidence counting technique, the 559.1-keV photopeak of ^{76}As could be counted with higher precision. A longer decay period also appeared to improve the peak shape. A comparison of the gamma-ray spectra by TINAA (Fig. 6.7) and by EINAA (Fig. 6.9) shows that EINAA-anti-coincidence method gave a higher signal-to-background ratio and a better resolution. Because of the suppression of the ^{24}Na activity, the sample irradiated with epithermal neutrons could be counted for a longer period after a relatively shorter decay period without high background counts.

6.3.2 Determination of Residual Arsenic after Methanol-MIBK-Water Extraction

The arsenic content in the solid residue of the fish samples after the extraction of most of the arsenic species by methanol-MIBK-water was expected to be very low as discussed in Section 6.1. The INAA method for this type of analysis needs to have even higher sensitivity and lower detection limit than the INAA method for determination of total arsenic in unextracted fish samples. The normal practice for enhancing sensitivity is to use higher neutron flux, longer irradiation or counting times. Since the sodium content of the residue was low compared to that of the unextracted sample, it was

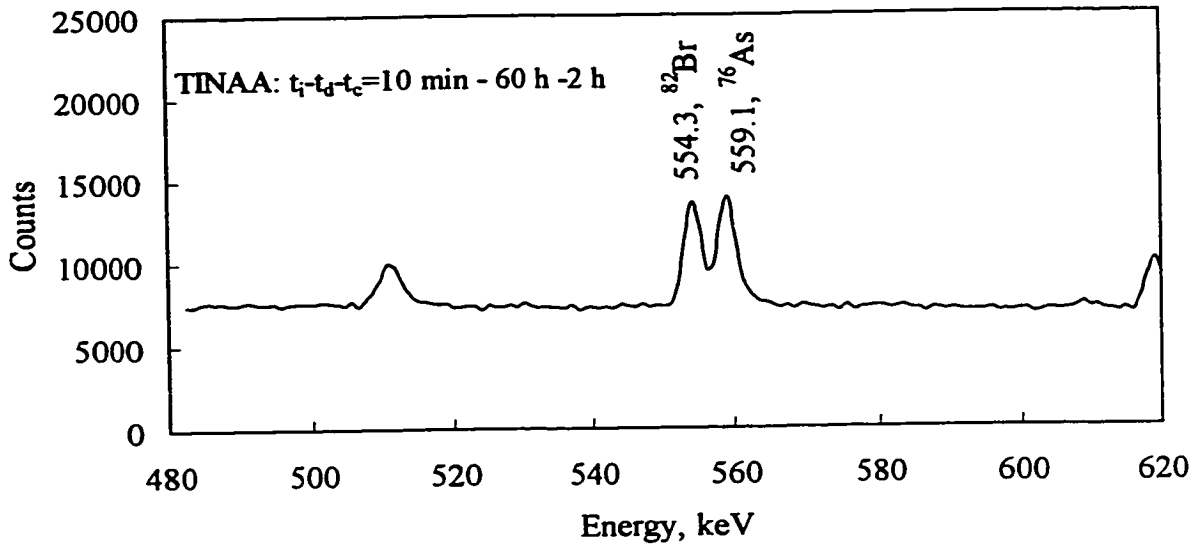

Fig. 6.4. Partial Gamma-ray Spectrum from a Marine Fish Sample by TINAA with Conventional Counting Technique

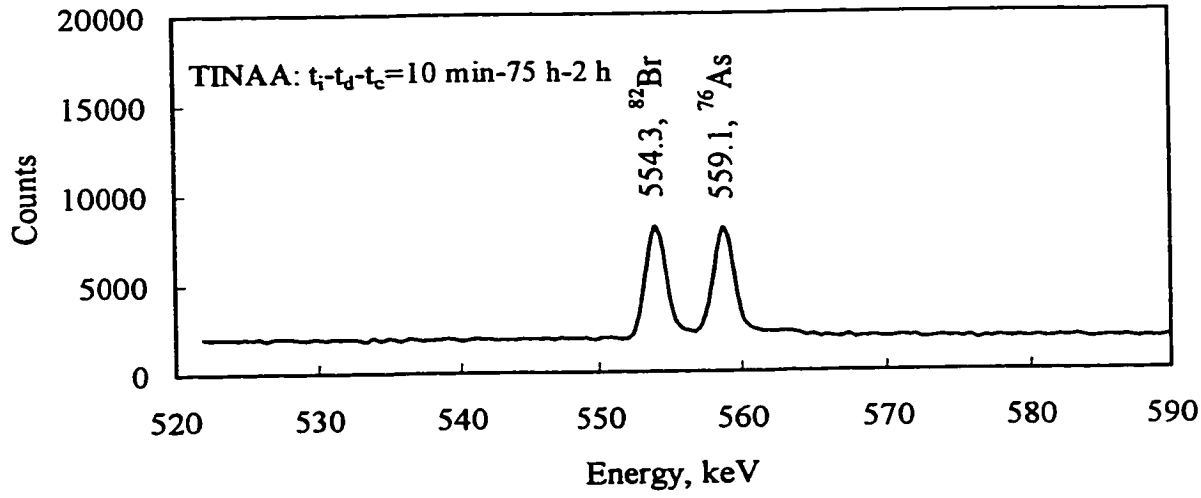

Fig. 6.5. Partial Gamma-ray Spectrum from a Marine Fish Sample by TINAA with Anti-coincidence Counting Technique

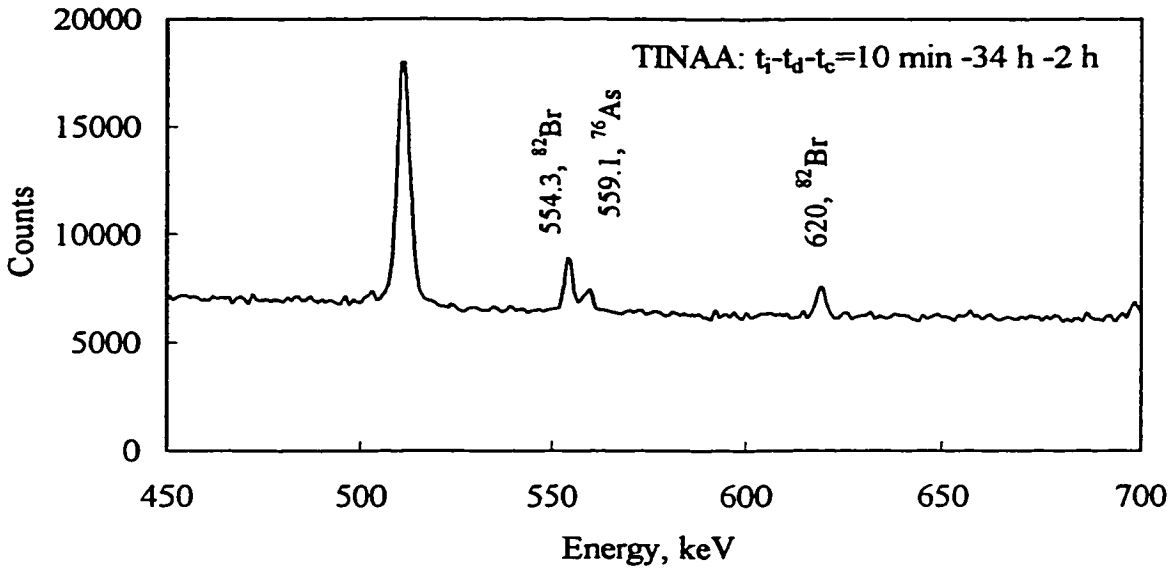

Fig. 6.6. Partial Gamma-ray Spectrum from a Fresh Water Fish Sample by TINAA with Conventional Counting Technique

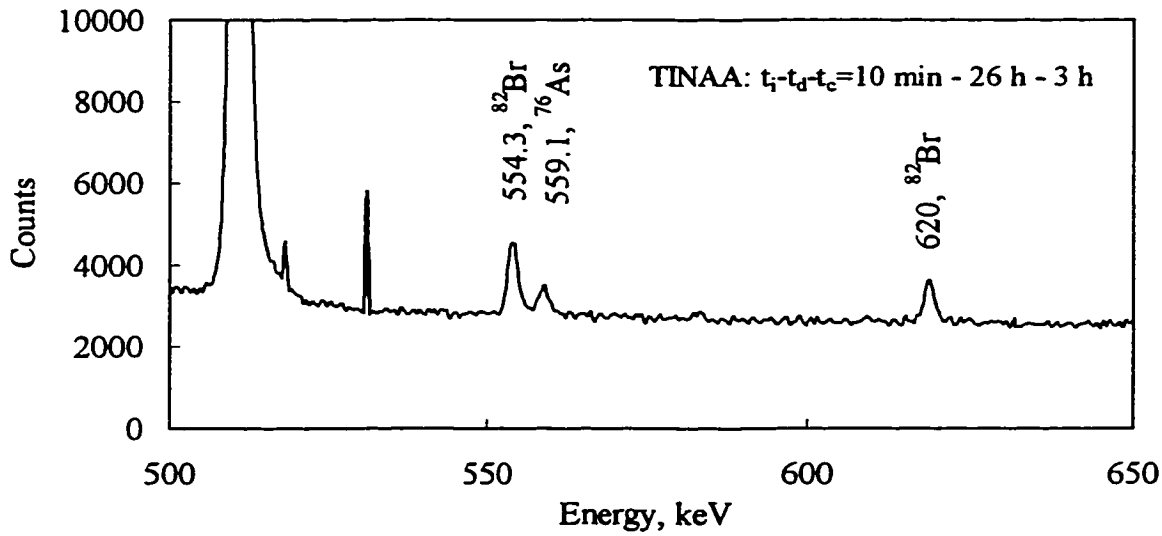

Fig. 6.7. Partial Gamma-ray Spectrum from a Fresh Water Fish Sample by TINAA with Anti-coincidence Counting Technique

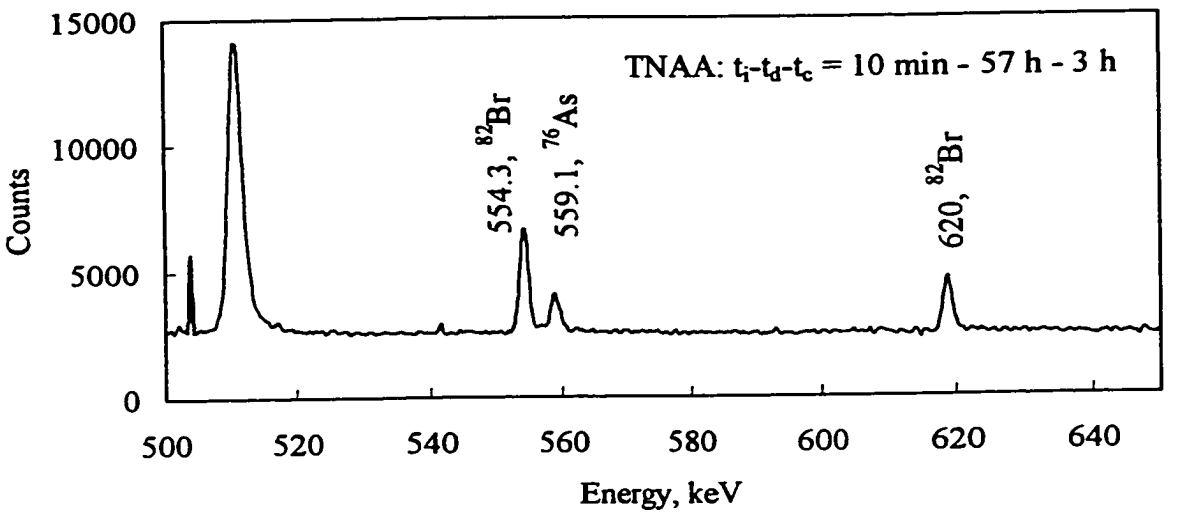

Fig. 6.8. Partial Gamma-ray Spectrum of a Fresh Water Fish Sample by TINAA with Anti-coincidence Counting Technique

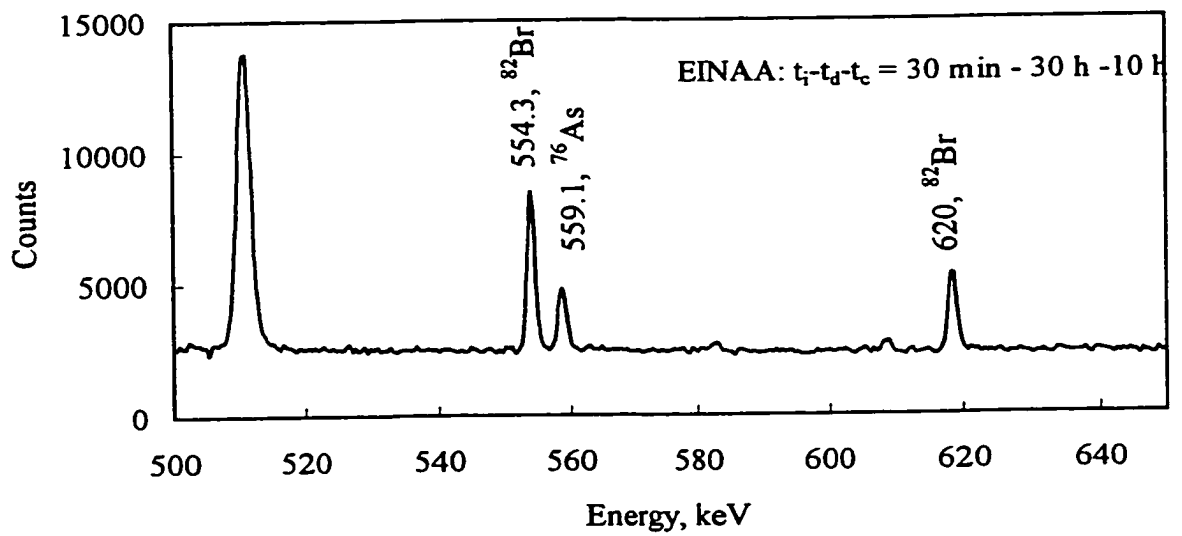

Fig. 6.9. Partial Gamma-Ray Spectrum of a Fresh Water Fish Sample by EINAA with Anti-coincidence Counting Technique

possible to use a longer irradiation time as well as TINAA with a higher neutron flux than that in EINAA. An irradiation time of 20 to 30 min at a neutron flux of 5×10^{11} cm^{-2} s^{-1} was finally selected for the analysis of residual arsenic.

The sensitivities and detection limits obtained using different decay times, counting times, and counting techniques for arsenic in the residue samples and those of the selected CRMs are listed in Tables 6.2 through 6.8. It is clear from these tables that both the sensitivity and detection limit were significantly improved using a longer irradiation time and the anti-coincidence counting technique. For example, a detection limit of 0.085 μg of residual arsenic g^{-1} of sample was obtained by a TINAA-anti-coincidence counting method (t_i-t_d-t_c=0.34-49-4 h) for a fresh-water fish sample (Table 6.3) containing about 0.10 μg g^{-1} of residual arsenic. Detection limits of 0.050, 0.085, 0.10, 0.14, 0.20 and 0.23 μg g^{-1} of residual arsenic were obtained for a Tuna Fish Tissue CRM (Table 6.6), a canned fish (Table 6.4), a shellfish (Table 6.5), a marine fish (Table 6.2), a Dogfish Liver CRM (Table 6.8) and a Dogfish Muscle CRM (Table 6.7) sample, respectively.

6.3.3 Determination of Lipid-soluble Arsenic

The MIBK phase of the methanol-MIBK-water extraction steps contained most of the lipid-soluble arsenic which was determined by INAA in four fish samples and three CRMs. Much less interference was encountered in this analysis because the sodium did not enter the MIBK organic phase. On the other hand, the lipid-soluble arsenic levels were expected to be lower than the total arsenic in the original food samples. A 20 to 30-min irradiation by thermal neutrons at a flux of 5×10^{11} cm^{-2} s^{-1} was found to be adequate for the determination of lipid-soluble arsenic. The sensitivities and detection limits obtained using different NAA time schemes are listed in Table 6.9. The concentrations of the lipid-soluble arsenic in these samples are also listed in Table 6.9. It is evident that the detection limits are about an order of magnitude lower than the measured arsenic levels.

Table 6.9. Evaluation of the INAA Methods for the Lipid-Soluble Arsenic in Fish Samples and CRMs

Sample	t_i(min.)-t_d(h)-t_c(h)	Sensitivity, counts μg^{-1} As	L_D, $\mu g\ g^{-1}$	Lipid-soluble As, $\mu g\ g^{-1}$
Marine fish	30-7-3	44 560	0.015	0.18 ± 0.02
	30-26-3	27 020	0.016	
Fresh water fish	20-10-1	9 410	0.026	0.16 ± 0.02
	20-8-2	19 580	0.014	
	20-25-2	12 520	0.018	
Canned fish	20-5-1	10 740	0.028	0.54 ± 0.06
	20-25-2	12 520	0.013	
Shellfish	20-30-2	10 970	0.029	0.19 ± 0.05
	20-49-3	9 850	0.025	
	20-35-4	18 750	0.028	
CRM 627	30-26-5	43 880	0.008	0.52 ± 0.01
	30-20-5	51 390	0.007	
DORM-2	30-3-1	16 940	0.037	0.57 ± 0.04
	30-20-1	18 020	0.031	
DOLT-2	30-3-1	16 940	0.023	3.61 ± 0.07
	30-20-1	18 020	0.024	

6.3.4 Quality Assurance for Arsenic by INAA

Both internal and external quality assessments for arsenic in fish samples were performed. Internal quality assessment was used to monitor the reproducibility of the measurement by INAA. A total of 13 arsenic standards of concentrations varying from 1 to 10 $\mu g\ mL^{-1}$ were prepared. At least one of these standards was analyzed with each

batch of samples using the same INAA method. An internal quality assessment control chart was prepared and is shown in Fig. 6.10. The upper and lower warning limits are given by $\pm 2\sigma$, and results more than $\pm 3\sigma$ from the mean are beyond the control limit and considered to be influenced by determinate errors [326]. It was found that all the 13 measurements were within the range of $\pm 3\sigma$, 12 of them were within $\pm 2\sigma$, and 9 of them were within $\pm 1\sigma$.

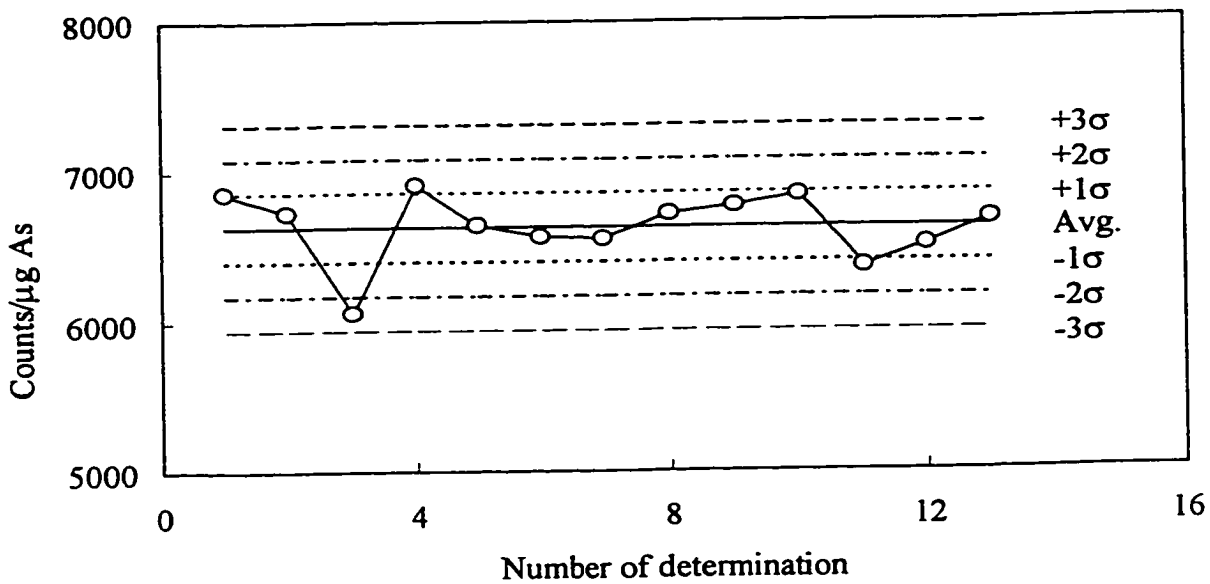

Fig. 6.10. Internal Quanlity Control Chart of the Determination of Arsenic by NAA

In order to evaluate the accuracy of total arsenic determination by INAA, three CRMs of marine organism origin were analyzed by EINAA in conjunction with anti-coincidence counting technique. The CRMs were: Dogfish Muscle (DORM-2), Dogfish Liver (DOLT-2), and Tuna Fish Tissue CRM 627. The results are shown in Table 6.10 and also in Fig 6.11. The measured values were found to be in good agreement with the certified values. The numbers in the brackets following the CRM numbers (Fig. 6.11) indicate the number of measurements performed. The good agreement between the certified value and the average of measured values can also be seen by another chart given in Fig. 6.12 where the individual arsenic concentrations of the CRM 627 are plotted against the certified value with their uncertainties.

Table 6.10. Results of Total Arsenic in CRM

CRM	CRM 627	DORM-2	DOLT-2
Measured value, $\mu g\ g^{-1}$	4.7 ± 0.5	19.0 ± 0.8	15 ± 1.1
Certified value, $\mu g\ g^{-1}$	4.8 ± 0.5	18.0 ± 1.1	16.6 ± 1.0
Number of measurements	7	4	3

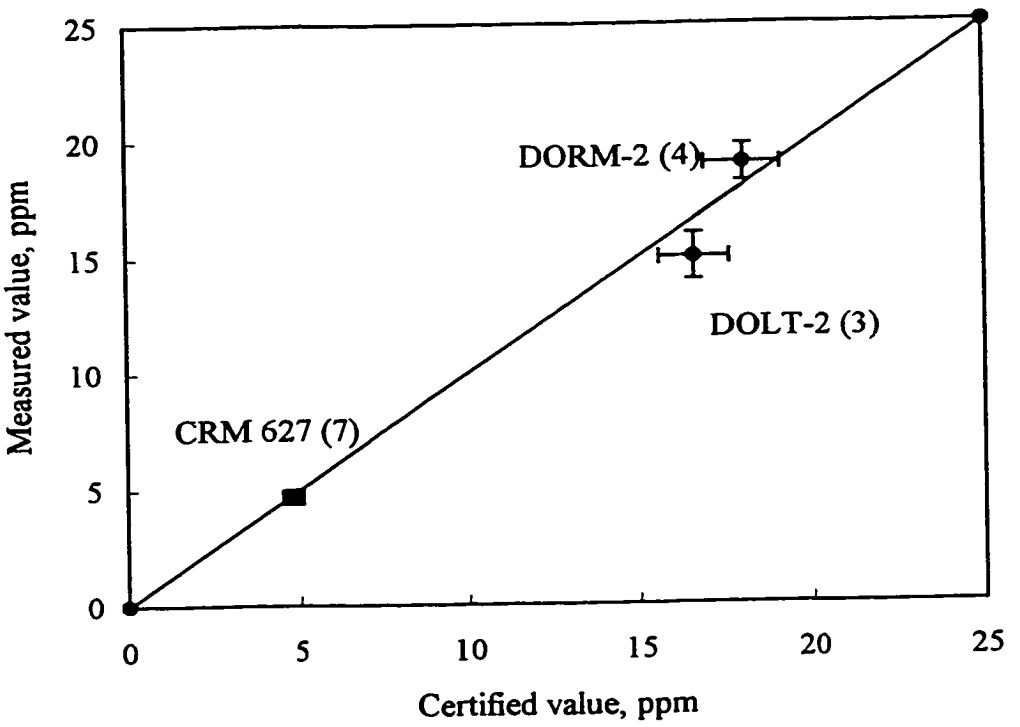

Fig. 6.11. External Quality Assessment Chart for Arsenic

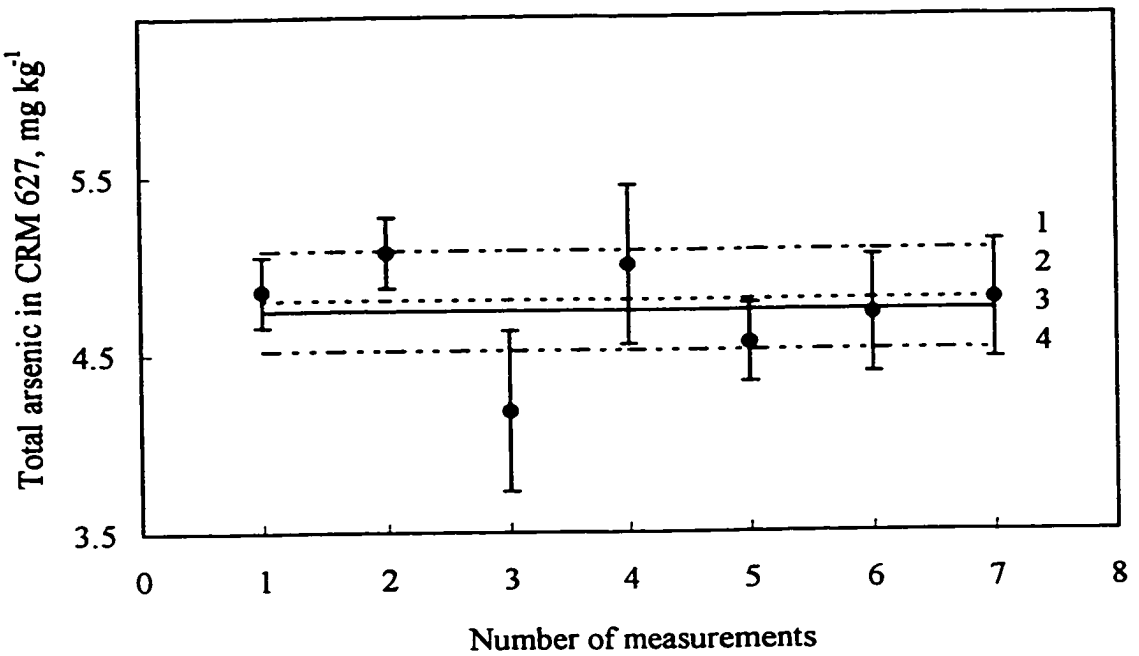

Fig. 6.12. External Quality Control Chart for Arsenic by INAA
Line1: certified value+uncertainty; line2: average measured value; line3: certified value; line4: certified value-uncertainty.

6.4 Speciation of Arsenic in Selected Fish Samples

6.4.1 Total Arsenic

Prior to the determination of individual arsenic species, four Canadian fish samples supplied by Health Canada were analyzed for the total arsenic content using the EINAA-anti-coincidence counting method developed in this work. The results are reported on a dry mass basis in Table 6.11. The moisture content of these samples is also listed in the table. Among the four fish, the marine fish sample had the highest arsenic content of 23±2 μg g^{-1} followed by 6±1 μg g^{-1} in the shellfish sample. The fresh water fish had a fairly low arsenic level of 2.6±0.3 μg g^{-1}. The arsenic concentration in the canned fish sample of unknown origin was also low and found to be 2.5±0.4 μg g^{-1}. The total arsenic levels measured in these four fish samples are within the range of values for many fish species reported by Francesconi and Edmonds [50-52], Cullen and Reimer [104] and Shiomi [49]. Larsen *et al.* [56] found total arsenic levels of 3.2 to 44.1 μg g^{-1} in tuna, plaice, oyster, mussel, crab, and shrimp samples. Velez *et al.* [120] reported a

range of 0.1 to 9.0 μg g^{-1} (wet weight) in fish, lamellibranchs, cephalopods, and crustaceans (fresh, frozen, canned or salted) where the moisture content varied between 62 and 84%.

Table 6.11. Total Arsenic Concentrations in Selected Fish Samples

Sample	Marine fish	Fresh-water fish	Canned fish	Shellfish
As, μg g^{-1} (dry weight)	23 ± 2	2.6 ± 0.3	2.5 ± 0.4	6.3 ± 1.1
Moisture, %	57.42	76.92	67.43	71.49
No. of measurements	6	5	5	3

6.4.2 Lipid-Soluble Arsenic

The concentrations of the lipid-soluble arsenic in 7 fish samples are given in Table 6.9. The DOLT-2 sample had the highest level of lipid-soluble arsenic which was about 3.6 μg g^{-1}, followed by the DORM-2, CRM 627 and the canned fish samples with their essentially same lipid soluble arsenic concentrations ranging from 0.5 to 0.54 μg g^{-1}. The levels of lipid-soluble arsenic in the marine fish, fresh water fish and shellfish samples were found to be low and also essentially same, from 0.16 and 0.19 μg g^{-1}. These results of the lipid-soluble arsenic in fish samples agree with the reported values in the literature. Shiomi [49] reported a range of 5.5 to 41.3 μg g^{-1} of total arsenic and of 0.22 to 7.6 μg g^{-1} of lipid-soluble arsenic in three fish, one sea squirt, one crustacean, and one shellfish samples.

It was found that two of the fish samples, namely the marine fish and the shellfish samples, had lower concentrations of lipid (Table 6.1) as well as lipid-soluble arsenic (Table 6.9). On the other hand, the Tuna Fish Tissue (CRM 627), Dogfish Muscle (DORM-2), Dogfish Liver (DOLT-2), and the canned fish sample had high lipid concentrations as well as high lipid-soluble arsenic. A correlation analysis between the lipid content as the mass percentage of fish (dry mass basis) and the lipid-soluble arsenic content as the mass percentage of the total arsenic was carried out. The graph is shown in

Fig. 6.13; a correlation coefficient of 0.76 was obtained using a t-test. Although more data points are needed to establish a more definite relationship between the concentrations of lipid and the lipid-soluble arsenic, the results obtained in this work clearly indicate that the higher the lipid content the higher is the lipid-soluble arsenic in the fish.

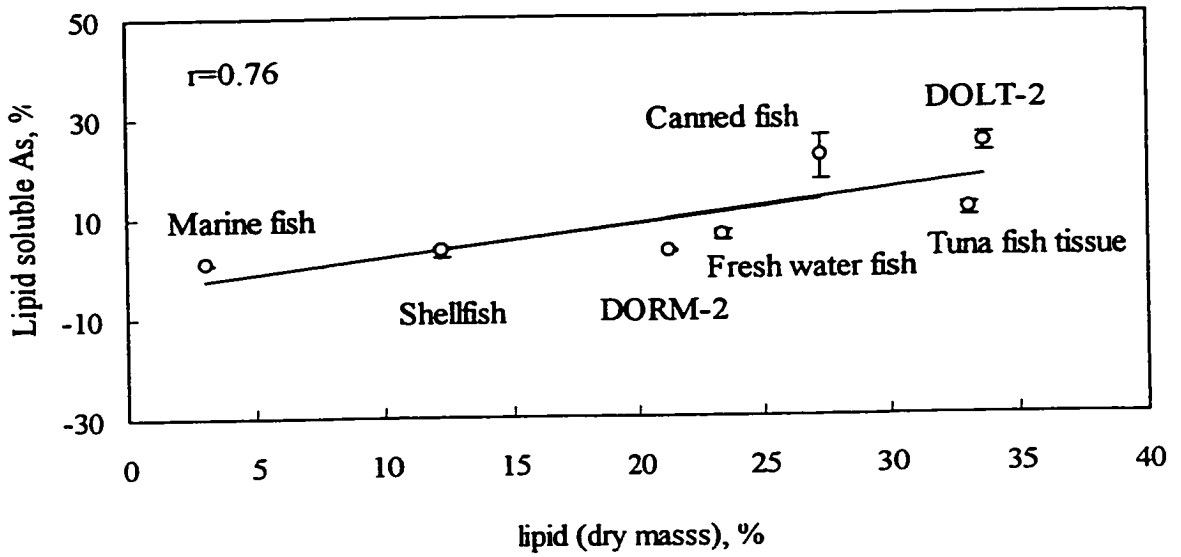

Fig. 6. 13. Correlation between Lipid Content (dry mass basis) and the Concentration of Lipid-soluble Arsenic Species

6.4.3 AsB, DMA, As(III), MMA, and As(V)

Three of the above four fish samples, namely marine fish, shellfish and canned fish, were then analyzed for various arsenic species according to the speciation scheme (Fig. 6.3) developed and described in Section 6.2 above. The levels of total arsenic (Table 6.11), residual arsenic, and lipid-soluble arsenic (Table 6.9) of the three fish samples have already been given. The HPLC-NAA chromatograms presented in Figs. 6.14, 6.15 and 6.16 show that AsB peaks are well separated from the DMA peaks, as expected. The separations were good even when the ratio of AsB-to-DMA was very high (*e.g.*, about 360 for the marine fish). All three fish samples had high levels of AsB compared to DMA. The HPLC-NAA chromatograms for As(III), MMA and As(V) of the fish samples are shown in Figs. 6.17, 6.18, and 6.19. All the three arsenic species were detected in the shellfish and canned fish samples. The level of As(V) in the marine

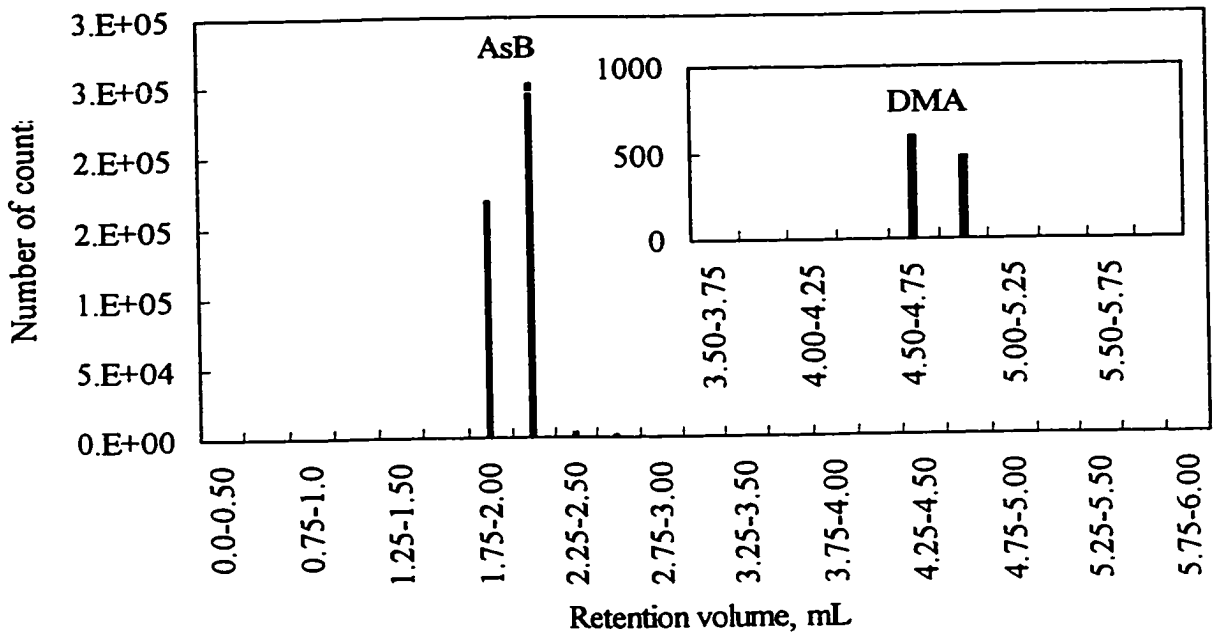

Fig. 6.14. Chromatogram of AsB and DMA in a Marine Fish Sample.
NAA: Flux=5X10^{11} n cm^{-2} s^{-1}; t_i-t_d-t_c=5-20-2 h.

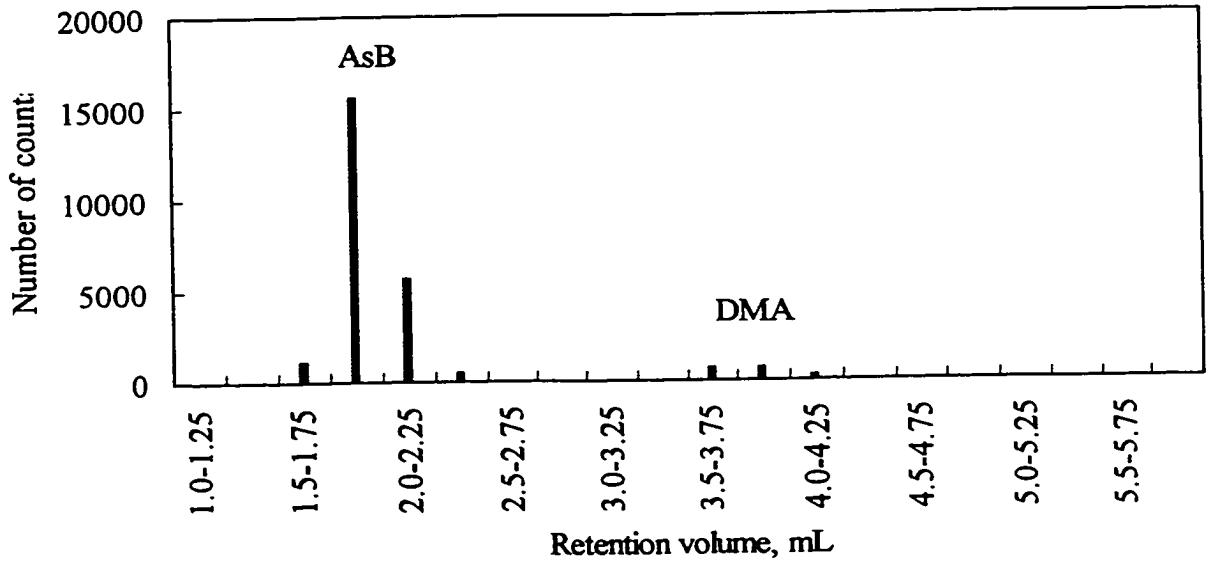

Fig. 6.15. HPLC-NAA Chromatogram of AsB and DMA in a Shellfish Sample
NAA: flux=5X10^{11}n cm^{-2}s^{-1}; t_i-t_d-t_c=5-20-2 h

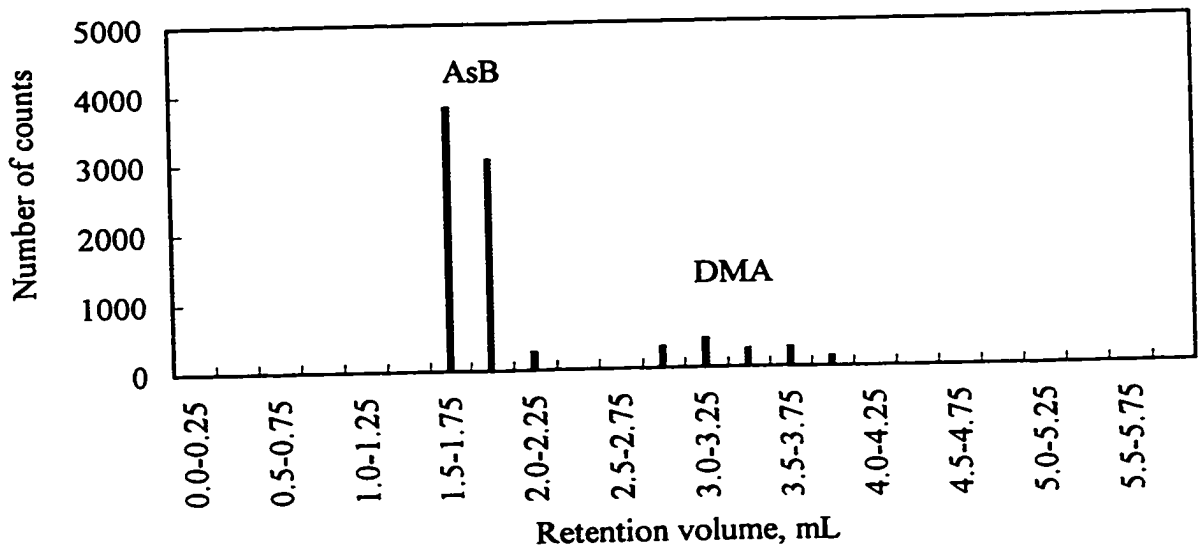

Fig. 6.16. HPLC-NAA Chromatogram of AsB and DMA in a Canned Fish Sample.
NAA: Flux=$5X10^{11}$n $cm^{-2}s^{-1}$; t_i-t_d-t_c=5-20-2 h

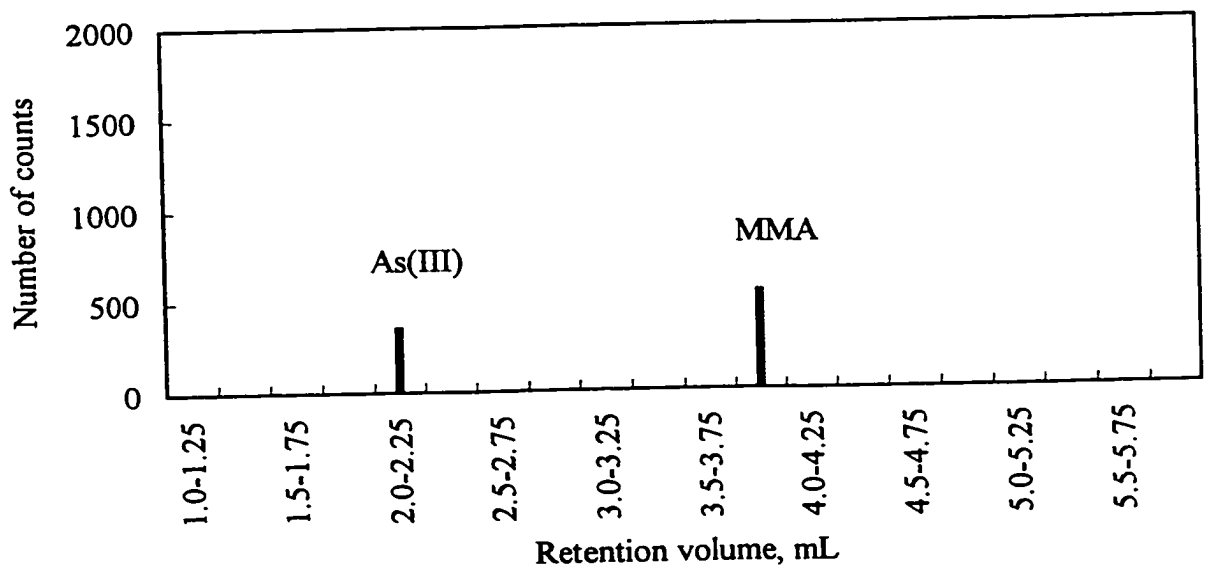

Fig. 6.17. HPLC-NAA Chromatogram of As(III), MMA and As(V) in a Marine Fish Sample.
NAA: flux=$5X10^{11}$n cm^{-2} s^{-1}; t_i-t_d-t_c=5-20-2 h

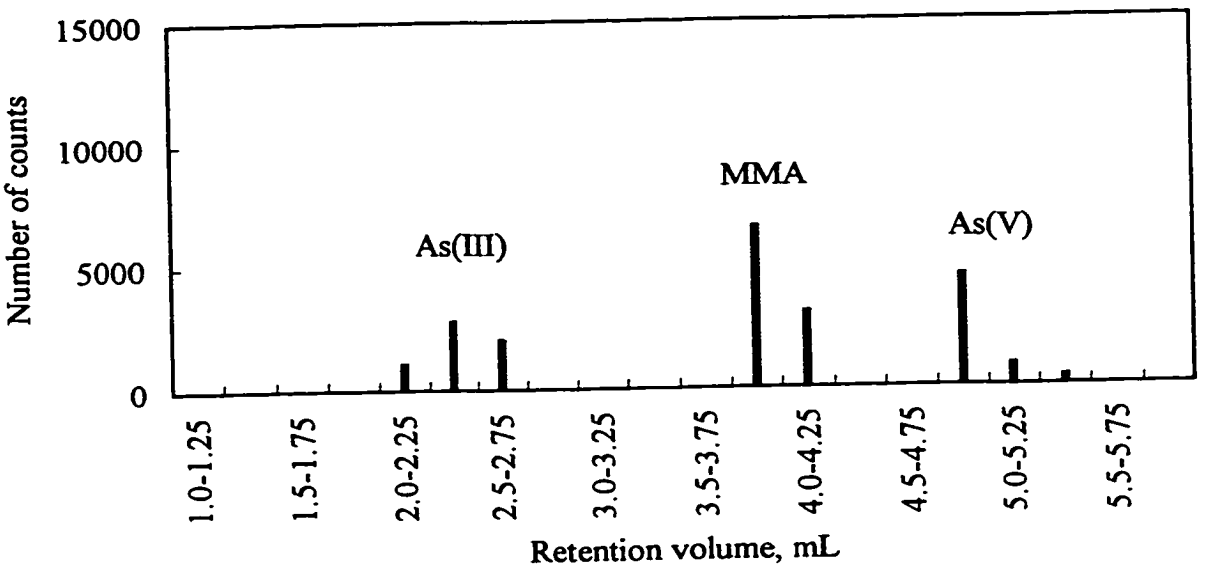

Fig. 6.18. HPLC-NAA Chromatogram of As(III), MMA and As(V) in a Shellfish Sample
NAA: Flux=$5X10^{11}$n cm^{-2} s^{-1}; t_i-t_d-t_c=5-20-3 h

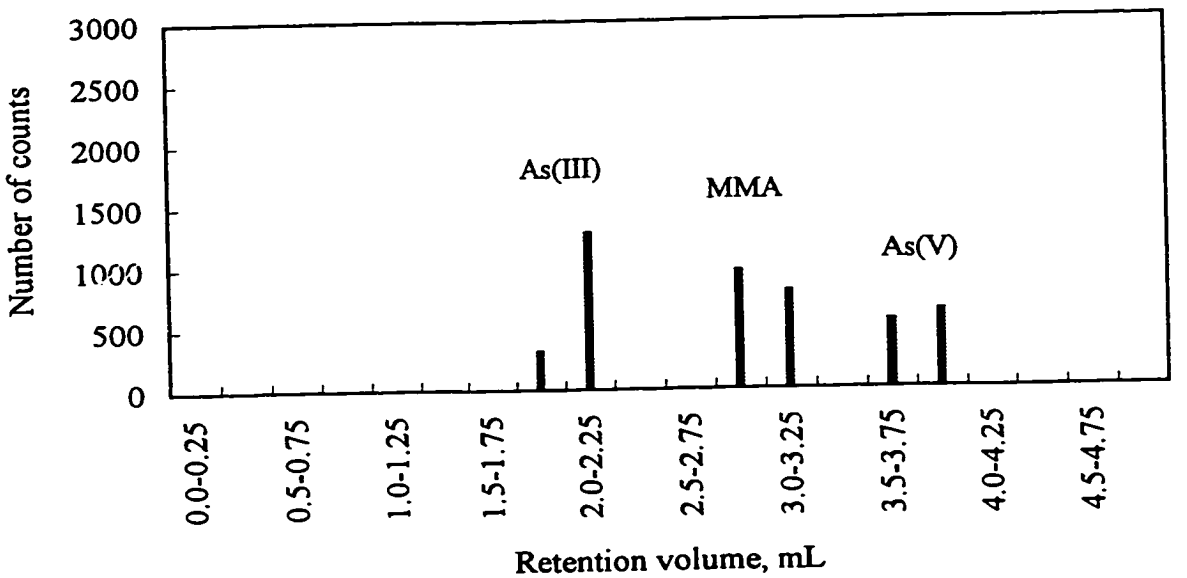

Fig. 19. HPLC-NAA Chromatogram of As(III), As(V) and MMA in a Canned Fish Sample
NAA: Flux=$5X10^{11}$ n cm^{-2} s^{-1}; t_i-t_d-t_c= 5-20-2 h

fish sample, however, was found to be below the detection limit of the NAA method used here. A summary of the above results is shown in Table 6.12.

Table 6.12. Concentrations of Arsenic Species in Some Fish Samples (dry weight)

Fish	Marine fish		Shellfish		Canned fish	
	Concentration	% of total	Concentration	% of total	Concentration	% of total
AsB, $\mu mol\ kg^{-1}$	286 ± 2	93.1	58 ± 3	69	12.8 ± 0.6	38.4
DMA, $\mu mol\ kg^{-1}$	0.8 ± 0.4	0.26	3.5 ± 1.3	4.2	2.7 ± 0.6	8.1
MMA, $\mu mol\ kg^{-1}$	0.3 ± 0.1	0.1	7.8 ± 0.5	9.3	3.2 ± 0.4	9.6
As(III), $\mu mol\ kg^{-1}$	0.2 ± 0.1	0.065	4.9 ± 0.6	5.8	3.0 ± 0.4	8.9
As(V), $\mu mol\ kg^{-1}$	<0.13	<0.04	4.4 ± 0.4	5.2	2.1 ± 0.4	6.3
Lipid-soluble As, $mg\ kg^{-1}$	0.18 ± 0.02	0.78	0.19 ± 0.05	3.0	0.54 ± 0.06	21.6
Sum, $mg\ kg^{-1}$	21.7 ± 2.0	94	6.1 ± 0.3	96	2.3 ± 0.2	92
Total As, $mg\ kg^{-1}$	23 ± 2		6.3 ± 1.1		2.5 ± 0.4	

The AsB species accounted for about 93% of the total arsenic in the marine fish sample, which is the highest percentage among the three fish samples and the Tuna Fish Tissue CRM 627 (Table 6.14). On the other hand, the percentages of the other minor arsenic species, namely DMA, MMA, As(III) and As(V), were found to be the lowest in this marine fish sample. In the shellfish sample, AsB was 69% of the total arsenic. The

canned fish sample had the lowest AsB concentration of 12.8 μmol kg^{-1} accounting for only 38% of the total arsenic. The concentration order of other four arsenic species, namely As(III), As(V), MMA and DMA, were: canned fish > shellfish > Tuna Fish Tissue CRM > marine fish.

The lower AsB level found in the canned fish sample compared to the other two samples agrees with the results reported by Velez *et al.* [120]. Their results on several seafood products, such as sardine, cockles, squid and small squid, showed that there was a lower percentage of AsB in the canned samples than in the fresh samples. They also reported a lower AsB percentage in frozen or preserved seafood products compared to the fresh ones.

The levels of the two inorganic arsenic species, namely As(III) and As(V), measured in the three fish samples are comparable with those reported in the literature. Oygard *et al.* [327] found the inorganic arsenic to range from 0.06% in cod to 7.5% in blue mussel of the total arsenic. Munoz *et al.* [328] reported values of 0.29% to 6.5% for several fish, mollusk and crustaceans. They also found [329] a higher percentage of inorganic arsenic in the canned seafood samples than in the fresh seafood samples, which agrees with the results obtained in our study.

6.4.4 Spiking Experiments

To examine the recovery of the analytical procedure used for the fish samples and to further identify the peaks of each of the arsenic species in the chromatogram, five arsenic species of interest, namely As(III), As(V), AsB, MMA, and DMA, were spiked into the aqueous extract of the canned fish sample before and after the clean-up of the C_{18} cartridge. The speciation scheme in Fig. 6.3 was followed. The recoveries of these species are given in Table 6.13. All species were recovered quantitatively both before and after the clean up of the C_{18} column with the exception of As(III). The recovery of As(III) was also quantitative when it was spiked after the clean up, however only about 8% of it was recovered if it was spiked before the clean-up.

The peak positions of AsB and DMA Fig. 6.20), and of As(III), MMA and As(V) (Figs. 6.21 and 6.22) in the HPLC-NAA chromatograms were found to be same as in the samples (Figs. 6.16 and 6.19) within the resolution of this technique.

Table 6.13. Recoveries of Arsenic Species in the Spiked Canned Fish Extract

Species	As(III)	As(V)	MMA	DMA	AsB
Recovery before the C_{18} clean up, %	8.0	100.8	98.7	98.5	103.9
Recovery after the C_{18} clean up, %	97.7	104.1	96.2	101.2	98.3

6.4.5 Accuracy of Measurements

The speciation method developed here was also applied to the Tuna Fish Tissue CRM to evaluate the accuracy of the method. The results obtained for 6 arsenic species as well as for the total arsenic are given in Table 6.14 together with the available certified values for AsB, DMA and total arsenic [77, 306-307]. It is evident that the measured concentrations of total arsenic, AsB and DMA agree well with the certified values. No certified or information values for MMA, As(V), As(III), and lipid-soluble arsenic were provided by the CRM issuing agency.

The percentages of the individual species based on the total arsenic are also listed in Table 6.14. About 83.3% of the total arsenic in this sample could be assigned to AsB and DMA on the basis of the certified values, which is comparable to 81.0% found in this work. The concentration of the lipid-soluble arsenic was found to account for 11% of the total arsenic making it the second most abundant species in this sample. Since more species were detected in this work, the percentage of the total arsenic that could be assigned to various species was as high as 92.2%. The HPLC-NAA chromatograms of this sample are shown in Fig. 6.23 and 6.24.

6.4.6. Detection Limits

The detection limits expressed as μg arsenic per gram of fish obtained using the recommended speciation procedure described in Section 6.2 are shown in Table 6.15. It is clear that the detection limits for the arsenic species in fish samples are of the

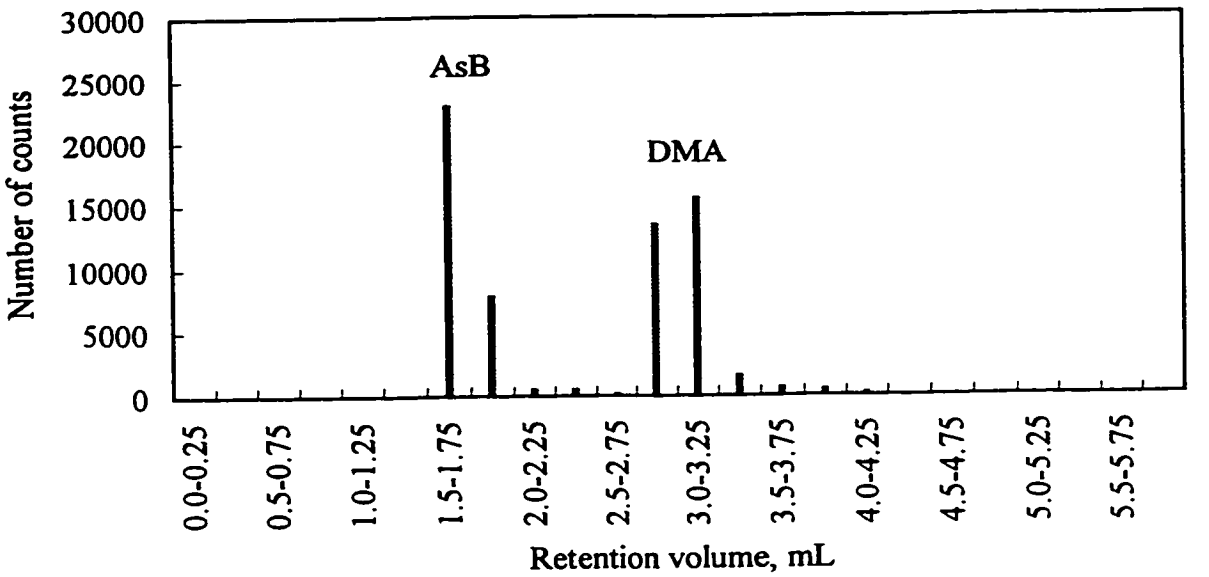

Fig. 6.20. HPLC-NAA Chromatogram of AsB and DMA in a Canned Fish Extract Spiked with Standards
NAA: Flux=$5X10^{11}$ n cm^{-2} s^{-1}; t_i-t_d-t_c=2-20-0.5 h

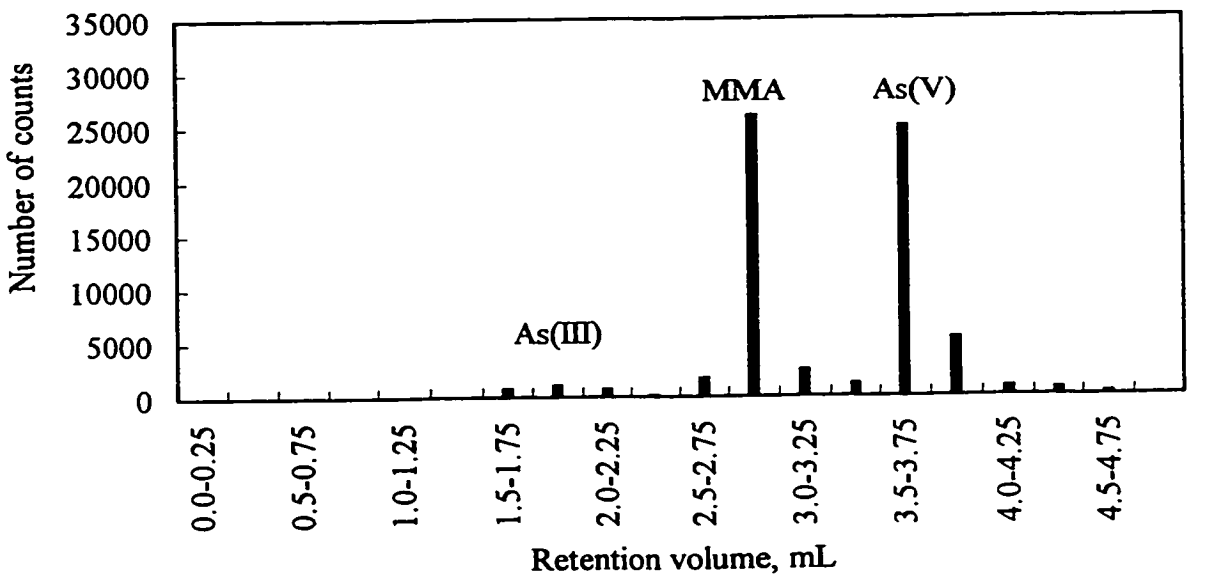

Fig. 6.21. HPLC-NAA Chromatogram of As(III), MMA and As(V) in a Canned Fish Extract Spiked with Standards before the Clean up
NAA: Flux=$5X10^{11}$n $cm^{-2}s^{-1}$; t_i-t_d-t_c= 2-20-0.25 h.

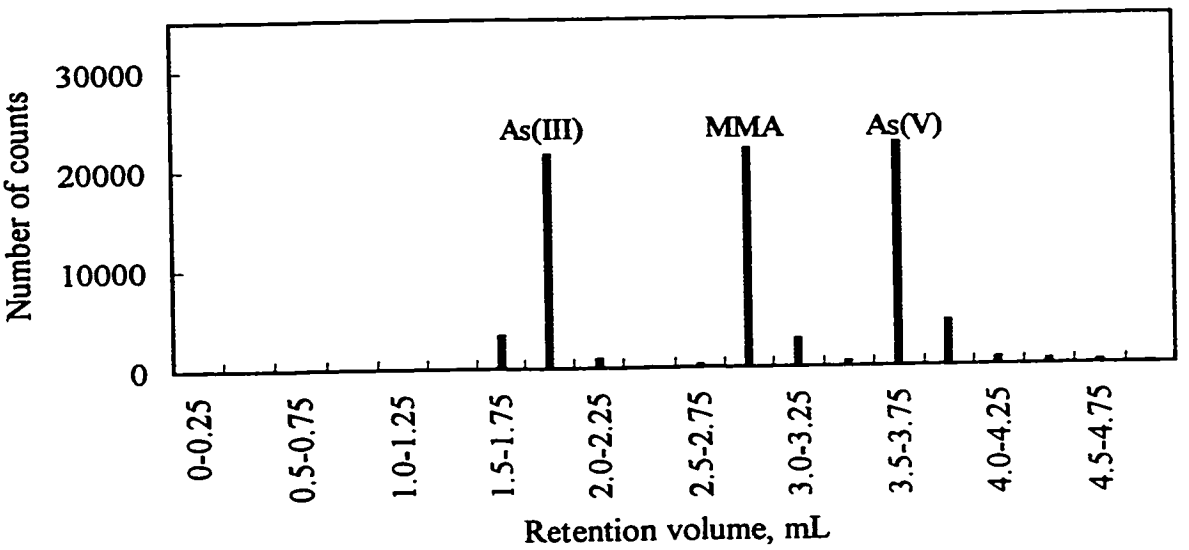

Fig. 6.22. HPLC-NAA Chromatogram of As(III), MMA and As(V) in a Canned Fish Extract Spiked with Standard after Clean up
NAA: Flux=$5X10^{11}$n $cm^{-2}s^{-1}$; t_i-t_d-t_c=2-20-0.5 h

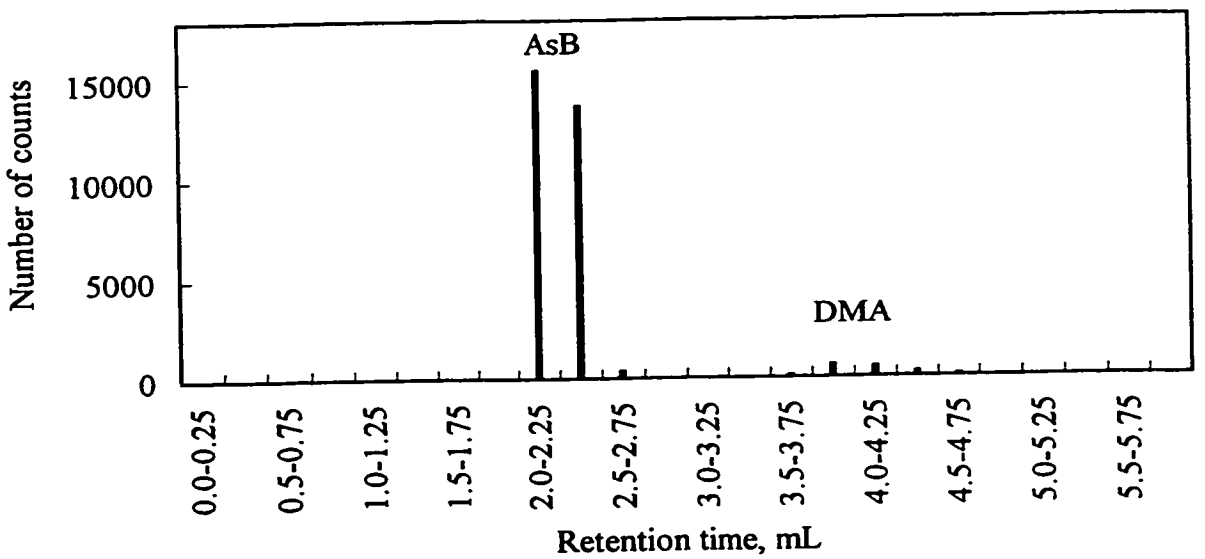

Fig. 6.23. HPLC-NAA Chromatogram of AsB and DMA in the Tuna Fish Tissue CRM
NAA: flux=$5X10^{11}$ n $cm^{-2}s^{-1}$; t_i-t_d-t_c=5-20-1 h.

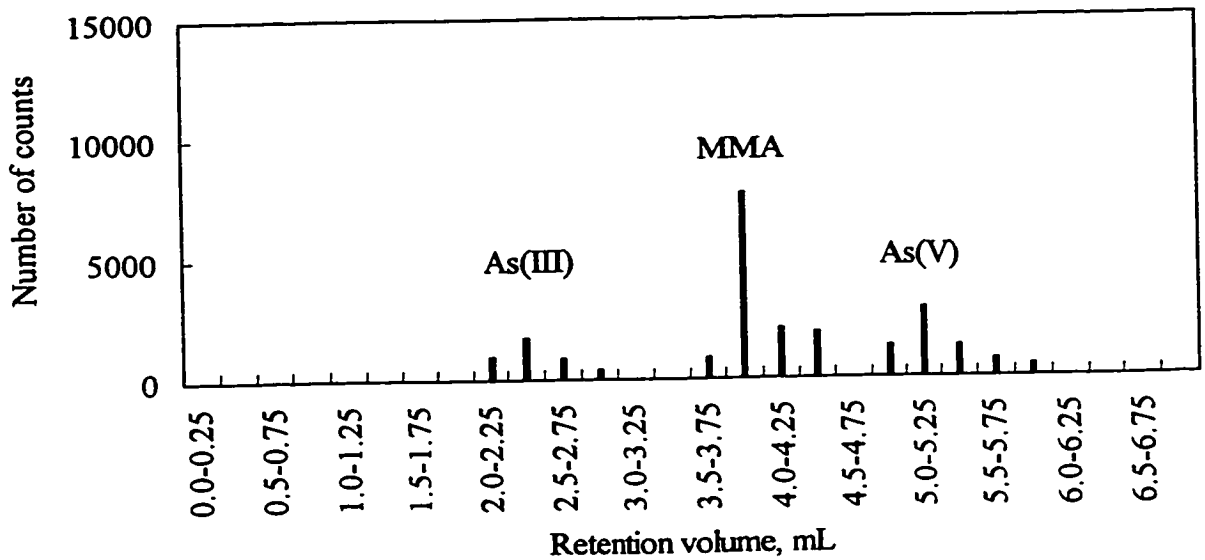

Fig. 6.24. HPLC Chromatogram of As(III), MMA and As(V) in the Tuna Fish Tissue CRM

NAA: Flux=$5X10^{11}$ n $cm^{-2}s^{-1}$; t_i-t_d-t_c=5-20-3 h

Table 6.14. Results of Arsenic Speciation in the Tuna Fish Tissue CRM

Species	This method		Certified value	
	Conc.	% of total	Conc.	% of total
AsB, μmol kg^{-1}	48.5 ± 1.2	77.3	52 ± 3	81.2
DMA, μmol kg^{-1}	2.3 ± 0.1	3.7	2.0 ± 0.3	3.1
MMA, μmol kg^{-1}	1.5 ± 0.1	2.4		
As(III), μmol kg^{-1}	0.5 ± 0.1	0.8		
As(V), μmol kg^{-1}	0.7 ± 0.1	1.1		
Lipid-soluble As, mg kg^{-1}	0.52 ± 0.01	11.0		
Sum, mg kg^{-1}	4.52 ± 0.09	96.3		
Total As, mg kg^{-1}	4.7 ± 0.3		4.8 ± 0.3	

Table 6.15. Detection Limits for Arsenic Species in Fish or Marine Organism Samples by Different Methods

Method	Detection limit, μg g^{-1}				
	AsB	DMA	As(III)	As(V)	MMA
HPLC-NAA*	0.020	0.020	0.019	0.019	0.019
HPLC-ICP-MS [56]	0.36	0.027	0.050	0.050	0.047
HPLC-ICP-MS [62]	0.050		0.050		
HPLC-ICP-MS [65]	0.040	0.040	0.048	0.13	0.072
HPLC-UV-HG-AFS [69]	0.040	0.040	0.040	0.040	0.040
HPLC-UV-HG-ICP-AES [59]	0.28	0.30		0.30	0.31
HPLC-MD-HG-AAS [58]	0.063	0.132		0.148	0.082

* This work

order of 0.020 μg g^{-1} and were low enough not only for the major species AsB but also for the other four minor species, namely MMA, DMA, As(III), and As(V). A few detection limits reported in the literature and obtained by other methods for the speciation of arsenic in fish or marine organisms are also shown in Table 6.15 for the purpose of comparison. It is evident that the detection limits for various arsenic species obtained by our HPLC-NAA method are lower by at least a factor of two (except DMA by HPLC-ICP-MS) than those by HPLC-ICP-MS [56, 62, 65], HPLC-UV-HG-AFS [69], HPLC-UV-HG-ICP-AES [59] and HPLC-MD-HG-AAS [58] methods.

7. CONCLUSIONS AND RECOMMENDATIONS

Several chemical separation methods in conjunction with neutron activation analysis (NAA) were developed for speciation of arsenic as well as antimony and selenium species. These included (i) a cation exchange chromatographic method for group separation of AsB and DMA from As(III), As(V) and MMA; (ii) a solvent extraction-NAA method for As(III), As(V), Sb(III), Sb(V), and Se(IV); (iii) a solid-phase extraction-NAA method for As(III) and Sb(III); and (iv) a HPLC-NAA method for As(III), As(V), MMA, DMA and AsB. Organically bound arsenic (OBAs), As(III), As(V), MMA, DMA and AsB in a Riverine Water CRM (NRCC SLRS-4) were determined using a speciation scheme based on the above methods. Inorganic species, namely As(III), As(V), Sb(III), Sb(V), and Se(IV) in several natural water samples were also measured. A speciation scheme for arsenic in fish samples was also developed. The concentrations of total arsenic, lipid-soluble arsenic, residual arsenic, As(III), As(V), MMA, DMA, and AsB were determined in three fish samples and in a Tuna Fish Tissue CRM using NAA. The separation methods in conjunction with NAA were found to have high selectivity, excellent sensitivity, low detection limits, and high accuracy.

In the cation exchange chromatographic method, the distribution coefficients for various species of arsenic, antimony and selenium on the Dowex 50WX8-200 resin in presence of different aqueous solutions were measured. The elution of arsenic species from a column was then carried out. This chromatographic column was found to retain the cationic arsenic species, namely DMA and AsB, from up to 250 mL of water samples and to let the anionic species including As(III), As(V) and MMA species pass through. The AsB and DMA retained on the column were then eluted by 1M ammonia solution. The ammonia solution was found to quantitatively elute the DMA and AsB but leave the interfering cations, especially, Na^+ on the column. This column was used as a group separation step for the speciation of arsenic in natural water and fish samples.

The solvent extraction-NAA method was mainly developed for the speciation of As(III), As(V), Sb(III), Sb(V), and Se(IV). Both APDC and DBDC were found to give similar extraction performances. Several solvents were tested and compared based on the extraction recovery and other properties relevant to the solvent extraction.

Between pH 4 to 6, As(III), Sb(III) and Se(IV) could be quantitatively extracted by APDC/MIBK while As(V), Sb(V) and Se(VI) were left behind, which allowed the separation of the lower valence species from the higher ones. Back extraction of the As(III) and Sb(III) by 4M HNO_3 solution allowed further enrichment of these species to a factor of 5; also it was easier to dry a smaller volume of the sample under an IR lamp for irradiation with neutrons for a longer time. Both aqueous-to-organic volume ratios in the extraction and the back extraction steps were studied. An extraction aqueous-to-organic phase volume ratio (V_a/V_o) of as high as 100 for Se(IV), and 10 for As(III) and Sb(III) species were obtained. Similarly, a back-extraction volume ratio (V_a/V_o) of 0.2 for As(III) and Sb(III) was achieved. The PDC complexes of As(III), Sb(III) and Se(IV) in MIBK phase at pH 4-6 were found to be stable for several days.

It was observed that the extraction of some of the organic arsenic species, including MMA, DMA and AsB, by the above method was negligible, which indicated that they did not interfere with the determination of As(III). The As(V) and Sb(V) species were reduced first to their trivalent species, and then extracted using the same solvent extraction method. L-cysteine was found to quantitatively reduce As(V) and Se(V) to As(III) and Sb(III) at pH from 1 to 6, while potassium thiosulfate required a pH of 1 for the reduction. Both reagents reduced Se(VI) to Se(0) directly, which made the determination of Se(VI) species impossible. Since both As(V) and Sb(V) were reduced by L-cysteine at pH 4-6 which is also the pH range for the APDC/MIBK solvent extraction, L-cysteine was more convenient to use. MMA and DMA were found to be reduced and extracted to a significant extent by both L-cysteine and potassium thiosulphate, which indicated a potential interference by MMA and DMA to the determination of As(V). However, this interference from DMA was eliminated in the present work by applying the group separation step prior to solvent extraction. The potential interference from MMA was negligible because of its low levels in water compared to As(V). AsB was neither reduced nor extracted along with As(V).

The sensitivities for arsenic and antimony by solvent extraction-NAA were found to be as high as 385 and 540 counts ng^{-1}, respectively, using a neutron flux of 5×10^{11} cm^{-2} s^{-1} and an irradiation-decay-counting time of 2-50-12 h. The corresponding detection limits were 0.027 and 0.020 ng mL^{-1}. The accuracy of the method was

evaluated by analyzing the NRCC CRM Seawater NASS-1 and Riverine Water SLRS-4, and was found to be good. This method was then applied to several natural water samples including rainwater, lake water, seawater, tap water, and spring water.

The SPE method was also developed for separation of As(III), Sb(III) and Se(IV). A chelating agent impregnated resin, namely DBDC/XAD-4, was synthesized by adsorbing DBDC in methanol-water onto XAD-4 resin. The DBDC in its acid form, and in ion-paired form with CTA or TMAm gave a satisfactory coating of the resin. A lower percentage of methanol in the solvent gave a higher percentage of coating. The adsorption was found to be complete within 3 h, and it appears to follow Freundlich model. The DBDC/XAD-4 resin was found to quantitatively extract As(III) and Sb(III), while the extractions of As(V) and Sb(V) were found to be less then 4%. The selectivity for Se(IV) and Se(VI) was poor; about 38% of Se(VI) was co-extracted with nearly 100% of Se(IV). A SPE column packed with 1.2 g of $DBDC^-$/XAD-4 resin could simultaneously as well as quantitatively extract As(III) and Sb(III) from up to 100 mL of water samples. The detection limits for arsenic and antimony in water samples were 0.093 and 0.028 ng mL^{-1}, respectively, using a neutron flux of 5×10^{11} cm^{-2} s^{-1} and an irradiation-decay-counting time of 2-50-10 h.

A HPLC-NAA method was developed for the determination of the arsenic species including As(III), As(V), MMA, DMA, and AsB. A fraction collection technique was used to couple the HPLC with NAA. An anion exchange column and a phosphate buffer were chosen as the stationary and the mobile phases, respectively, which gave a good separation and provided a good matrix for NAA. The retention volumes of various arsenic species using different buffer concentrations and pH were studied. Prior to HPLC, a group separation step was used to remove AsB and DMA from As(III), As(V) and MMA. A buffer of 10 mM of $NH_4H_2PO_4/(NH_4)_2HPO_4$ at pH 6.5 separated the AsB, and DMA peaks well. The same buffer at 75 mM and pH 5.5 was found to be a good mobile phase to separate As(III), MMA and As(V). The eluent was collected in 0.25-mL portions in which all five arsenic species were well separated from one another. The sensitivity for arsenic was 740 000 counts μg^{-1} and the detection limit was 0.64 ng arsenic using a neutron flux of 5×10^{11} cm^{-2} s^{-1} and an irradiation-decay-counting time of

5-20-5 h. The potential use of the HPLC-NAA method for the simultaneous speciation of arsenic, antimony and selenium was investigated.

The As(III), As(V), MMA , DMA, AsB and OBAs species in the Riverine Water CRM were separated by a scheme consisting of C_{18} column SPE, cation exchange chromatography, HPLC, and solvent extraction methods. The C_{18}-bound silica gel column was used for the isolation of OBAs. The As(III) and As(V) species were also separated by the APDC/MIBK solvent extraction method. Arsenic in these species was determined by NAA. The detection limits by this scheme were: 0.005 ng mL^{-1} for OBAs, 0.04 ng mL^{-1} for both As(III) and As(V), 0.038 ng mL^{-1} for both AsB and DMA, and 0.21 ng mL^{-1} for MMA. The NRCC Riverine Water CRM SLRS-4 was found to contain 0.39±0.08 ng mL^{-1} As(V), 0.22±0.035 ng mL^{-1} As(III), and 0.06±0.02 ng mL^{-1} DMA; the other arsenic species were below their detection limits.

The arsenic levels in fish samples were quantitatively determined by INAA after they were freeze-dried. Both TINAA and EINAA in combination with anti-coincidence counting technique were investigated, and EINAA with anti-coincidence counting technique gave better results for total arsenic in fish samples. Both internal and external quality assessments indicated that the measurements were done with good precision and accuracy.

Speciation of arsenic in the fish samples was carried out by the separation methods developed here. The arsenic species were first extracted by a methanol-MIBK-water system. The extraction yield of arsenic was found to increase with the increasing number of extractions; more than 90% of the arsenic could be recovered after 4 extractions. The lipid-soluble arsenic in the MIBK phase and the residual arsenic in the fish samples after the extraction were also determined by INAA. Higher sensitivity and lower detection limits were obtained by increasing the irradiation time. The As(III), As(V), DMA, MMA and AsB species in the fish extract were separated by a cation exchange chromatographic method followed by the HPLC method. The detection limits for As(III), As(V), MMA, DMA, and AsB were around 0.020 μg g^{-1} of fish under the experimental conditions used.

Four fish samples collected by Health Canada as marine fish, shellfish, fresh water fish, and canned fish were found to contain 23±2, 6±1, 2.6±0.3 and 2.5±0.4 μg g^{-1}

arsenic, respectively. Three CRMs of fish origin were also analyzed; the concentrations of total arsenic obtained by the method developed here were in good agreement with the certified values. The levels of lipid-soluble arsenic in the four fish samples as well as in the three CRMs were determined. A range from 0.16 to 3.6 $\mu g\ g^{-1}$ lipid-soluble arsenic was found, and a correlation between the levels of lipid-soluble arsenic and the lipid content was indicated.

Four fish samples, namely marine fish, canned fish, shellfish, and the Tuna Fish Tissue CRM, were analyzed by these methods for various arsenic species. The AsB was found to be the predominant arsenic species in all samples as expected. Small amounts of DMA, MMA, As(III) and As(V) species were also detected. The concentration of AsB expressed as the percentage of total arsenic follows the order of marine fish > tuna fish > shellfish > canned fish. An opposite order of canned fish > shellfish > tuna fish > marine fish was observed for As(III), As(V), MMA and DMA. Lower percentage of AsB was found in the canned fish than in the other samples. The concentrations of total arsenic, AsB and DMA obtained for the Tuna Fish Tissue CRM were found to be in very good agreement with the certified values provided.

The NAA detection in combination with various chemical separation methods were found to be very useful. The solvent extraction separation-NAA and SPE-NAA methods were suitable for the simultaneous analysis of low levels of inorganic arsenic, antimony and selenium species in natural water samples. More work is needed to develop an appropriate reduction method for Se(VI) to Se(IV). The SPE method needs to be further studied to improve the selectivity and in conjunction with a reduction step for the inorganic arsenic, antimony and selenium at higher oxidation states. The HPLC-NAA method can be further improved by applying both anion and cation exchange columns to give a more complete separation of all arsenic species of interest, instead of a group separation by open-column cation exchange chromatography prior to the HPLC separation. The HPLC separation using a cation exchange column is also expected to include other possible cationic arsenic species, such as AsC and TMA, upon the availability of suitable standards.

REFERENCES

1. R. Lobinski, *Appl. Spectrosc.*, 51, 260A-278A (1997).

2. P. Apostoli, *Fresenius J. Anal. Chem.*, 363, 499-504 (1999).

3. S. J. Fairweather-Tait, *Fresenius J. Anal. Chem.*, 363, 536-540 (1999).

4. J. Szpunar and R. Lobinski, *Fresenius J. Anal. Chem.*, 363, 550-557 (1999).

5. S. Caroli, in *Element Speciation in Bioinorganic Chemistry* (editor: S. Caroli), 1-20, John Wiley & Sons, INC., New York (1996).

6. B. Michalke and P. Schramel, *Fresenius J. Anal. Chem.*, 363, 429-430 (1999).

7. B. Michalke, *Fresenius J. Anal. Chem.*, 363, 439-445 (1999).

8. R. Cornelis, C. Camara, L. Ebdon, L. Pitts, B. Welz, R. Morabito, O. Donard, H. Crews, E. H. Larsen and B. Neidh, *Fresenius J. Anal. Chem.*, 363, 435-438 (1999).

9. T. Berg and E. H. Larsen, *Fresenius J. Anal. Chem.*, 363, 431-434 (1999).

10. R. Harrison and S. Rapsomanikis (editors), *Environmental Analysis Using Chromatography Interfaced with Atomic Spectroscopy*, Ellis Horwood Limited, Chichester (1989).

11. I. S. Krull (editor), *Trace Metal Analysis and Speciation, Journal of Chromatography Library*, Vol. 47, Elsevier, Amsterdam (1991).

12. M. Burguera and J. L. Burguera, *Talanta*, 44, 1581-1604 (1997).

13. C. Sarzanini, *J. Chromatogr.* A, 850, 213-228 (1999).

14. B. Welz, *J. Anal. At. Spectrom.*, 13, 413-417 (1998).

15. T. Guerin, A. Astruc and M. Astruc, *Talanta*, 50, 1-24 (1999).

16. J. Szpunar-Lobinska, C. Witte, R. Lobinski and F. C. Adams, *Fresenius J. Anal. Chem.*, 351, 351-37 (1995).

17. K. Pyrzynska, *Analyst*, 121, 77R-83R (1996).

18. P. Smichowski, Y. Madrid and C. Camara, *Fresenius J. Anal. Chem.*, 360, 623-629 (1998).

19. X. Dauchy, M. Potin-Gautier, A. Astruc, and M. Astruc, *Fresenius J. Anal. Chem.*, 348, 792-805 (1994).

20. M. S. Alaejos and C. D. Romero, *Chem. Rev.*, 95, 227-257 (1995).

21. J. Versieck, in *Trace Element Analysis in Biological Specimens* (editor: R. F. M. Herber and M. Stoeppler), 147-164, Elsevier, Amsterdam (1994).

22. H. A. Das and R. W. Woittiez, in *Element Speciation in Bioinorganic Chemistry* (editor S. Caroli), 155-192, John Wiley & Sons, INC., New York (1996).

23. R. H. Filby, *Pure & Appl. Chem.*, 67, 1929-1941 (1995).

24. D. De Soete, R. Gijbels and J. Hoste, *Neutron Activation Analysis,* Wiley-Interscience, London (1972).

25. A. J. Blotcky, G. T. Hansen L. R. Opelanio-Buencamino and E. P. Rack, *Anal. Chem.*, 57, 1937-1941 (1985).

26. A. J. Blotcky and G. T. Hansen, N. Borkar, A. Ebrahim and E. P. Rack, *Anal. Chem.*, 59, 2063-2066 (1987).

27. C. Chai, X. Mao, Y. Wang, J. Sun, Q. Qian, X. Hou, P. Zhang, C. Chen, W. Feng, W. Ding, X. Li, C. Li and X. Dai, *Fresenius J. Anal. Chem.*, 363, 477-480 (1999).

28. M. S. Gorby, in *Arsenic in the Environment* (editor: J. O. Nriagu), 27, 1-16, John Wiley and Sons, INC., New York (1994).

29. R. W. Squibb and B. A. Fowler, in *Biological and Environmental Effects of Arsenic* (editor: B. A. Fowler), 233-269, Elsevier, Amsterdam, New York (1983).

30. H. Yamauchi and B. A. Fowler, in *Arsenic in the Environment* (editor: J. O. Nriagu), 27, 35-54, John Wiley and Sons, INC., New York (1994).

31. R. W. Boyle and I. R. Jonasson, *J. Geochem. Explor.*, 2, 251-296, (1973).

32. Huang Yan-Chu, in *Arsenic in the Environment* (editor: J. O. Nriagu), 26, 17-49, John Wiley & Sons INC. New York (1994).

33. K. J. Irgolic, H. Greschonig and A. G. Howard, in *Arsenic* in *Encyclopedia of Analytical Science* (editor A. Townshend), 1, 168-184, London; San Diego; Academic Press (1995).

34. X. Chris Le, *Can. Chem. News*, 18-20 (1999).

35. M. O. Andreae, J. T. Byrd and P. N. Froelich, *J. Environ. Sci. Technol.*, 17, 731 (1983).

36. L. C. D. Anderson and K. W. Bruland, *J. Environ Sci. Technol.*, 25, 420-427 (1991).

37. A. G. Howard, M. H. Arbab-Zavar and S. Apte, *Mar. Chem.*, 11, 493-498 (1982).

38. R. S. Braman, in *Biological and Environmental Effects of Arsenic* (editor: B. A. Fowler), 141-154, Elsevier, Amsterdam, New York (1983).

39. M. O. Andreae, *Anal. Chem.*, 49, 820-823 (1977).

40. S. J. Santosa, H. Mokudai, M. Takahashi and S. Tanaka, *Appl. Organomet. Chem.*, 10, 697-705 (1996).

41. M. O. Andreae, *Deep-Sea Research*, 25, 391-402 (1978).

42. D. L. Johnson and R. M. Burke, *Chemosphere*, 645-648 (1978).

43. S. D. W. Comber and A. G. Howard, *Anal. Proc.*, 26, 20-22 (1989).

44. P. Michel, B. Averty and V. Colandini, *Mikrochim. Acta*, 109, 35-38 (1992).

45. A. G. Howard and A. D. W. Comber, *Appl. Organomet. Chem.*, 3, 509-514 (1989).

46. R. E. Sturgeon, K. W. Michael Siu, S. N. Willie and S, S. Berman, *Analyst*, 114, 1393-1396 (1989).

47. H. Hasegawa, M. Matsui, S. Okamura, M. Hojo, N. Lewasaki and Y. Sohrin, *Appl. Organomet. Chem.*, 13, 113-119 (1999).

48. A. C. Chapman, *Analyst*, 51, 548-563 (1926).

49. K. Shiomi, in *Arsenic in the Environment* (editor: J. O. Nriagu), 27, 261-282, John Wiley & Sons INC. New York (1994).

50. K. A. Francesconi and J. S. Edmonds, *Oceanogr. Mar. Biol. Annnu. Rev.*, 31, 111-151 (1993).

51. K. A. Francesconi and J. S. Edmonds, *Adv. Inorg. Chem.*, 44, 147-189 (1997).

52. K. A. Francesconi and J. S. Edmonds, in *Arsenic in the Environment* (editor: J. O. Nriagu), 26, 221-262 (1994).

53. A. Shinagawa, K. Shiomi, H. Yamanaka and T. Kikuchi, *Nippon Suisan Gakkiaishi*, 49, 75-78 (1983).

54. T. Kaise, H. Yamauchi, T. Hirayama and S. Fukui, *Appl. Organomet. Chem.*, 2, 339-347 (1988).

55. J. S. Edmonds and K. A. Francesconi, *Nature (London)*, 265, 436 (1977).

56. E. H. Larsen, G. Pritzl and S. H. Hansen, *J. Anal. At. Spectrom.*, 8, 1075-1084 (1993).

57. G. M. Momplalsir, T. Lei and W. D. Marshall, *Anal. Chem.*, 66, 3533-3539 (1994).

58. K. J. Lamble, S. J. Hill, *Anal. Chim. Acta*, 334, 261-270 (1996).

59. J. Alberti, R. Rubio and G. Rauret, *Fresenius J. Anal. Chem.*, 351, 415-419 (1995).

60. J. Alberti, R. Rubio and G. Rauret, *Fresenius J. Anal. Chem.*, 351, 420-425 (1995).

61. D. Beauchemin, M. E. Bednas, S. S. Berman, J. W. McLaren, K. W. Siu, and R. E. Sturgeon, *Anal. Chem.*, 60, 2209-2212 (1988).

62. D. Beauchemin, K. M. W. Siu, J. M. Mclaren, S. S. Berman, *J. Anal. At Spectrom.*, 4, 285-289 (1989).

63. S. Branch, L. Ebdon and P. O'Neill, *J. Anal. At. Spectrom.*, 9, 33-37 (1994).

64. W. Goesser, D. Kudhnelt, C. Schlagenhanfen, Z. Slejkovec and K. J. Irgolic, *J. Anal. At. Spectrom.*, 13, 183-187 (1998).

65. S. Londesborough, J. Mattusch and R. Wennrich, *Fresenius J. Anal. Chem.*, 363, 577-581 (1999).

66. K. L. Ackley, C. Bhymer, K. L. Sutton and J. A. Caruso, *J. Anal. At. Spectrom.*, 14, 845-850 (1999).

67. J. W. Mckiernan, J. T. Creed, C. A. Brockhoff, J. A. Caruseo and R. M. Lorenzana, *J. Anal. At. Spectrom.*, 14, 607-613 (1999).

68. A. El Moll, R. Heimburger, F. Lagrade. M. J. F. Leroy and E. Maier, *Fresenius J. Anal. Chem.*, 354, 550-556 (1996).

69. Z. Slejkovec, J. T. van Elteren and A. R. Byrne, *Talanta*, 49, 619-627 (1999).

70. S. Kurosawa, K. Yasuda, M. Taguchi, S. Yamazaki, S. Toda, M. Morita, T. Uehiro, and K. Fuwa, *Agric. Biol. Chem.*, 44, 1993-1994 (1980).

71. J. R. Cannon, J. S. Edmonds, K. A. Francesconi, C. L. Raston, J. B. Sanders, B. W. Skelton, and A. H. White, *Aust. J. Chem.*, 34, 787-798 (1981).

72. J. S. Edmonds and K. A. Francesconi, *Mar. Pollut. Bull.*, 12, 92-96 (1981).

73. J. B. Luten, G. Biekwel-Booy, J. van de Greef and M. C. Noever de Brauw, *Chemsphere*, 12, 131-141 (1983).

74. K. Hanaoka and S. Tagawa, *Nippon Suisan Gakkaishi*, 51, 681-685 (1985).

75. K. Hanaoka and S. Tagawa, *Nippon Suisan Gakkaishi*, 51, 1203 (1985).

76. K. Hanaoka, H. Yamamoto, K. Kawashima, S. Tagawa and T. Kaise, *Appl. Organomet. Chem.*, 2, 371-376 (1988).

77. E. A. Maier, C. Demesmay, M. Olle, A. Lamotte, F. Lagarde, R. Heimburger, M. J. F. Leroy and Z. Asfari, *The certification of the contents of arsenobetaine in solution (CRM626) and of total arsenic, arsenobetaine dimethylarsinic acid in tuna fish tissue (CRM 627)*, European Commission (1997).

78. J. S. Edmonds and K. A. Francesconi, *Chemosphere*, 10, 1041-1044 (1981).

79. H. Norin and A. Christakopoulos, *Chemosphere*, 11, 287-298 (1982).

80. H. Norin, R. Ryhage, A. Christakopoulos and M. Sandstrom, *Chemosphere*, 12, 299-315 (1983).

81. K. A. Francesconi, P. Micks, R. A. Stockton and K. J. Irgolic, *Chemosphere*, 14, 1443-1453 (1985).

82. S. Matsuto, K. A. Stockton and K. J. Irgolic, *Sci. Total Environ.*, 48, 133-140 (1986).

83. T. Dagnac, A. Padro, R. Rubio and G. Rauret, *Anal. Chim. Acta*, 364, 19-30 (1998).

84. T. Dagnac, A. Padro, R. Rubio and G. Rauret, *Talanta*, 48, 763-776 (1999).

85. K. Shiomi, M. Orii, H. Yamanaka and T. Kikuchi, *Nippon Suisan Gakkaishi*, 53, 103-108 (1987).

86. K. Shiomi, Y. Kakehashi, H. Yamanaka and T. Kikuchi, *Appl. Organomet. Chem.*, 1, 177-183 (1987).

87. K. Shiomi, Y. Sakamoto, H. Yamanaka and T. Kikuchi, *Nippon Suisan Gakkaishi*, 54, 539 (1988).

88. W. Goessler, W. Maher, K. J. Irgolic, D. Kuehnelt, C. Schlagenhaufen and T. Kasie, *Fresenius J. Anal. Chem.*, 359, 434-437 (1997).

89. K. A. Francesconi, J. S. Edmonds and B. G. Hatcher, *Comp. Biochem. Physiol.*, 90C, 313-316 (1988).

90. W. R. Cullen and M. Dodd, *Appl. Organomet. Chem.*, 3, 79-88 (1989).

91. Y. Shibata and M. Morita, *Anal. Chem.*, 61, 2116-2118 (1989).

92. J. F. Lawrence, P. Michalik, G. Tam and H. B. S. Conacher, *J. Agric. Food Chem.*, 34, 315-319 (1986).

93. H. Norin, A. Christakopoulos, M. Sandstrom and R. Ryhage, *Chemosphere*, 14, 313-323 (1985).

94. E. H. Larsen and S. Sturup, *J. Anal. At. Spectrom.*, 9, 1099-1105 (1994).

95. P. Thomas and K. Sniatecki, *Fresenius J. Anal. Chem.*, 351, 410-414 (1995).

96. K. Hanaoka, W. Goessler, T. Kaise, H, Ohno, Y. Nakatani, S. Ueno, D. Kuehnnelt, C. Schlagenhaufen and K. J. Irgolic, *Appl. Organomet. Chem.*, 13, 95-99 (1999).

97. S. X .C. Le, W. R. Cullen and K. J. Reimer, *Environ. Sci. Technol.*, 28, 1598-1604 (1994).

98. X. C. Le, M. Ma and N. A. Wong, *Anal. Chem.*, 68, 4501-4506 (1996).

99. J. S. Edmonds and K. A. Francesconi, *Nature (London)*, 289, 602-604 (1981).

100. W. A. Maher and S. M. Clarke, *Marine Pollut. Bull.*, 15, 111-112 (1984).

101. Y. Shibata, M. Morita, J. S. Edmonds, *Agric. Biol. Chem.*, 51, 391-398 (1987).

102. Y. Shibata and M. Morita, *Agric. Biol. Chem.*, 52, 1087-1089, (1988).

103. V. W. M. Lai, W. R. Cullen and S. Ray, *Mar. Chem.*, 66, 81-89 (1999).

104. W. R. Cullen and K. J. Reimer, *Chem. Rev.*, 89, 713-764, (1989).

105. R. W. Dabeka, A. D. McKenzie, G. M. A. Lacroix, C. Cleroux, S. Bowe, R. A. Graham, H. B. S. Conacher and P. Verder, *J. AOAC Int.*, 76, 14-25 (1993).

106. National Food Agency of Denmark, *Food Monitoring in Denmark 1983-1987*, Publication Nr. Soborg, Denmark (1990).

107. Ministry of Agriculture, *Fisheries and Food, Food Surveilance Paper Nr. 52.* London (1997).

108. S. Shinichiro, A. Hayase, M. Murakami, I. Hatai, K. Higashigawa, C. S. Moon, Z-W. Zhang, T. Watanabe, H. Igushi and M. Ikeda, *Food Addit. Contamin.*, 13, 775-786 (1996).

109. M. L. Cerversa and R. Montoro, *Fresenius J. Anal. Chem.*, 348, 331-340 (1994).

110. P. Fecher and G. Ruhnke, *At. Spectrosc.*, 19, 204-206 (1999).

111. A. Lasztity, A. Krushevska, M. Kotrebai, R. M. Barnes and D. Amarasiriwardena, *J. Anal. At. Spectrom.*, 10, 505-510 (1995).

112. P. N. Fedorov, G. N. Ryabchuk and A. V. Zverev, *Spectrochimica Acta Part B*, 52, 1517-1523 (1997).

113. J. C. Lopez, C. Reija and R. Montoro, *J. Anal. At. Spectrom.*, 9, 651-656 (1994).

114. U. Ballin, R. Kruse and H. A. Russel, *Fresenius J. Anal. Chem.*, 350, 54-61 (1994).

115. D. Velez and R. Montoro, *J. Food Protect.*, 61, 1240-1245, (1998).

116. J. T. van Elteren and Z. Slejkovec, *J. Chromatogr. A*, 789, 339-348 (1998).

117. J. P. Buchet, D. Lison, M. Ruggeri, V. Foa and G. Elia, *Arch. of Toxicol.*, 70, 773-778 (1996).

118. D. L. MacIntosh, P. L. Williams, D. J. Hunter, L. A. Sampson, S. C. Morris, W. C. Willet and E. B. Rimm, *Cancer Epidemiology, Biomarkers & Prevention*, 6, 1043-1050 (1997).

119. R. A. Schoof, L. J. Yost, J. Eickhoff, E. A. Crecelius, D. W. Cragin, D. M. Meacher and D. B. Menzel, *Food and Chemical Toxicology*, 37, 839-846 (1999).

120. D. Velez, N. Ybanez and R. Montoro, *J. Agric. Food Chem.*, 43, 1289-1294 (1995).

121. A. Yashi, C. Tsutsumi and A. Teda, *Agric. Biol. Chem.*, 42, 2139 (1978).

122. J. Chappell, B. Chiswell and H. Olszowy, *Talanta*, 42, 323-329 (1995).

123. M. Izawa, T. Korenaga and T. Takahashi, *Bunseki Kagaku*, 39, 193-195 (1990).

124. U. Rohr and L. Michael, *Fresenius J. Anal. Chem.*, 342, 370-375 (1992).

125. Y. Kanda and N. Suzuki, *Radiochem. Radioanal. Lett.*, 39, 221 (1979).

126. N. Suzuki, F. Jitoh, H. Imura and Y. Kanda, *Anal. Chim. Acta*, 193, 239 (1987).

127. F. Jitoh, H. Imura and N. Suzuki, *Anal. Chim. Acta*, 228, 85-91 (1990).

128. K. Venkaji, P. P. Naidu and T. J. P. Rao, *Talanta*, 41, 1281-1290 (1994).

129. S. Tesfalidet and K. Irgum, *Fresenius J. Anal. Chem.*, 338, 741-744 (1990).

130. L. Li, P. Yan, W. Gao and Y. Li, *Fresenius J. Anal. Chem.*, 363, 317-319 (1999).

131. S. Gohda, *Bull. Chem. Soc. Jpn.*, 48, 1213-1216 (1975).

132. W. M. Mok and C. M. Wai, *Anal. Chem.*, 59, 233-236 (1987).

133. A. M. Yusof, Z. B. Ikhsan and A. K. H. Wood, *J. Radioanal. Nucl. Chem. Articles*, 179, 277-283 (1994).

134. C. H. Chung, E. Iwamoto, M. Yamamoto and Y. Yamamoto, *Spectrochim. Acta.*, 39B, 459-466 (1984).

135. P. Bernejo-Barreara, M. C. Barciela-Alonso, M. Ferron-Novais and A. Bermejo-Barrera, *J. Anal. At. Spectrom.*, 10, 247-252 (1995).

136. M. M. Smith, M. A. White and H. K. Wilson, *J. Anal. At. Spectrom.*, 10, 349-352 (1995).

137. T. Kamada and Y. Yamamoto, *Talanta*, 24, 330-333 (1977).

138. M. Beatriz de la Calle-Guntinas, Y. Madrid and C. Camara, *Anal. Chim. Acta*, 247, 7-11 (1991).

139. N. L. Chutke, M. M. N. Ambulkar, R. G. Weginwar and A. N. Garg, *J. Radioanal. Nucl. Chem. Articles*, 185, 145-156 (1994).

140. W. M. Mok and C. M. Wai, *Talanta*, 35, 183-186 (1988).

141. E. Taskaev, I. Penev and L. Kinova, *J. Radioanal. Nucl. Chem.*, 120, 85-88 (1988).

142. G. Zhuang, Y. Wang, M. Zhi, W. Zhou, J. Yin, M. Tan and Y. Cheng, *J. Radioanal. Nucl. Chem. Articles*, 129, 459-464 (1989).

143. L. Tandon, B. F. Ni, X. X. Ding, W. D. Ehmann, E. J. Kasarskis and W. R. Markesbery, *J. Radioanal. Nucl. Chem. Articles*, 179, 331-339 (1994).

144. C. J. Peacock and S. C. Singh, *Analyst*, 106, 931-938 (1981).

145. A. Celkova, J. Kubova and V. Stresko, *Fresenius J. Anal. Chem.*, 355, 150-153 (1996).

146. X. Yin, E. Hoffmann and C. Ludke, *Fresenius J. Anal. Chem.*, 355, 324-326 (1996).

147. A. M. Garcia, M. C. R. Rodriguez, J. E. S. Uria and A. S. Medel, *Fresenius J. Anal. Chem.*, 353, 128-132 (1995).

148. F. Challenger, *Chem. Rev.* 36, 315 (1945).

149. W. R. Cullen, C. L. Froese, A. Liu, B. C. McBride, D. J. Patmore and M. J. Reimer, *J. Organomet. Chem.*, 139, 61 (1977).

150. H. Hasegawa, Y. Sohrin, M. Matsul, M. Hojo and M. Kawashima, *Anal. Chem.*, 66, 3247-3252 (1994).

151. M. Kanke, *Anal. Chim. Acta*, 247, 13-18 (1991).

152. C. R. Lan, Y. C. Sun, J. H. Chao, C. Chung, M. H. Yang, N. Lavi and Z. B. Alfassi, *Radiochim. Acta*, 50, 225-229 (1990).

153. S. J. Yeh, C. L. Tseng, and J. C. Wei, *J. Radioanal. Nucl. Chem.*, 151, 131-138 (1991).

154. S. J. Yeh, J. C. Wei, C. N. Ke, C. Y. Yang, *J. Radioanal. Nucl. Chem., Articles,* 179, 221-229 (1994).

155. N. V. Suc, M. M. Hung and N. V. Hung, *J. Radioanal. Nucl. Chem., Lett.*, 213, 65-70 (1996).

156. S. Nakashima, *Fresenius J. Anal. Chem.*, 341, 570-571 (1991).

157. J. T. van Elteren, G. J. M. Gruter and H. A. Das, *Int. J. Environ. Anal. Chem.*, 43, 41-54 (1990).

158. A. G. Howard, M. Volkan and D. Y. Ataman, *Analyst*, 112, 159-162 (1987).

159. E. Russeva, I. Havezov, and A. Detcheva, *Fresenius J. Anal. Chem.*, 347, 320-323 (1993).

160. S. Arpadjan, L. Vuchkova and E. Kostadinova, *Analyst*, 122, 243-246 (1997).

161. J. T. van Elteren and H. A. Das, *Anal. Chim. Acta*, 222, 159-167 (1989).

162. A. M. Yusof, Z. Hanafiah and A. K. H. Wood, *Sci. Total Environ.*, 214, 247-252 (1998).

163. Y. C. Sun and J. Y. Yang, *Anal. Chim. Acta*, 395, 293-300 (1999).

164. R. Ritsema, *Mikrochim. Acta.*, 109, 61-65 (1992).

165. D. Pozebon, V. L. Dressler, J. A. Gomes Neto, A. J. Curtius, *Talata*, 45, 1167-1175 (1998).

166. C. J. Hsieh, C. H. Yen and M. S. Kuo, *Anal. Sci.*, 15, 669-673 (1999).

167. S. Latva, S. Peraniemi and M. Ahlgren, *Analyst*, 124, 1 105-1108 (1999).

168. X. Yan, R. Kerrich and M. J. Hendry, *Anal. Chem.*, 70, 4736-4742 (1999).

169. S. Garbos, E. Bulska, A. Hulanicki, N. I. Shcherbinina and E. M. Sedykh, *Anal. Chim. Acta*, 342, 167-174 (1997).

170. K. Pyrzynska, P. Drzewicz and M. Trojanowicz, *Anal. Chim. Acta*, 363, 141-146 (1998).

171. J. L. Gomez-Ariza, J. A. Pozas, I. Giraldez and E. Morales, *Analyst*, 124, 75-78 (1999).

172. M. C. Valencia, E. A. Nicolas, L. F. Capitan-Vallvey, *Talanta*, 49, 915-921 (1999).

173. R. S. Braman and C. C. Foreback, *Science*, 182, 1247-1249 (1973).

174. A. G. Howard, *J. Anal. At. Spectrom.*, 11, 75-84 (1996).

175. A. U. Shaikh and D. E. Tallman, *Anal. Chim. Acta*, 98, 251-259 (1978).

176. R. J. A. van Cleuvenbergen, W. E. van Mol and F. C. Adams, *J. Anal. At. Spectrom.*, 3, 169-176 (1988).

177. J. C. Ng, D. Johnson, P. Imray, B. Chiswell and M. R. Moore, *Analyst*, 123, 929-933 (1998).

178. A. G. Howard and M. H. Arbab-Zavar, *Analyst*, 106, 213-220 (1981).

179. P. J. Craig, S. N. Forster, R. O. Jenkins and D. Miller, *Analyst*, 124, 1243-1248 (1999).

180. S. C. Pinillos, J. S. Asensio and J. G. Bernal, *Anal. Chim. Acta*, 300, 321-327 (1995).

181. J. Sanz-Asensio, M. T. Martinez-Soria, M. Plaza-Medina and M. P. Clavijo, *Anal. Chim. Acta*, 381, 331-339 (1999).

182. X. Le, W. R. Cullen and K. J. Reimer, *Anal. Chim. Acta*, 285, 277-285 (1994).

183. H. Chen, I. D. Brindle, and X. C. Le, *Anal. Chem.*, 64, 667 (1992).

184. A. G. Howard and C. Salou, *Anal. At. Spectrom.*, 13, 683-686 (1998).

185. U. Pyell, A. Dworschal, E. Nitschke and B. Neidhart, *Fresenius J. Anal. Chem.*, 363, 495-498 (1999).

186. W. W. Ding, and R. E. Sturgeon, *J. Anal. At. Spectrom.*, 11, 225-230 (1996).

187. M. G. Haywood and J. P. Riley, *Anal. Chim. Acta*, 85, 219-230 (1976).

188. E. A. Crecelius, *Anal. Chem.*, 50, 826-827 (1978).

189. J. T. van Elteren, H. A. Das, C. L. Deligny, J. Agterdenbos and D. Bax, *J. Radioanal. Nucl. Chem. Articles*, 179, 211-219 (1994).

190. R. C. Fry, M. B. Denton, D. L. Windsor and S. J. Northway, *Appl. Spectrosc.*, 33, 399-403 (1979).

191. S. T. G. Anderson and R. V. D. Robert, *J. Anal. At. Spectrom.*, 9, 1107-1110 (1994).

192. A. M. Featherstone, E. C. V. Butler, B. V. O'Grady and P. Michel, *J. Anal. At. Spectrom.*, 13, 1355-1360 (1999).

193. S. J. Santosa, S. Wada, and S. Tanaka, *Appl. Organomet. Chem.*, 8, 273 (1994).

194. Y. Odanaka, N. Tsuchlya, O. Matano and S. Goto, *Anal. Chem.*, 55, 929-932 (1983).

195. M. Dodd, S. A. Pergantis, W. R. Cullen, H. Li, G. K. Eigendorf and K. J. Reimer, *Analyst*, 121, 223-228 (1996).

196. Z. Mester, G. Vianyi, R. Morabito, P. Fodor, *J. Chromatogr. A*, 832, 183-190 (1999).

197. L. Ebdon, S. Hill, A. P. Walton and R. W. Ward, *Analyst*, 113, 1159-1165 (1988).

198. E. Orvini and R. Delfanti, *Radiochem. Radioanal. Lett.*, 37, 199-206 (1979).

199. Y. Talmi and D. T. Bostick, *Anal. Chem.*, 47, 2145-2150 (1975).

200. C. Feldman, *Anal. Chem.*, 51, 664-669 (1979).

201. G. Bomback, A. Pierra and W. Klemm, *Fresenius J. Anal. Chem.*, 350, 49-52 (1994).

202. P. H. Masscheleyn, R. D. Delaune and W. H. Patrick, *J. Enviro. Qual.*, 20, 96-100 (1991).

203. K. J. Reimer and J. A. J. Thompson, *Biogeochemistry*, 6, 211-237 (1988).

204. J. L. Burguera, M. Burguera, C. Rivas and P. Carrero, *Talanta*, 45, 531-542 (1998).

205. T. R. Rude and H. Puchelt, *Fresenius J. Anal. Chem.*, 350, 44-48 (1994).

206. M. A. Lopez-Gonzalves, M. M. Gomez, C. Camara and M. A. Palacios, *Mikrochim. Acta*, 120, 301-308 (1995).

207. R. K. Anderson, M. Thompson and E. Cullbard, *Analyst*, 111, 1153-1158 (1986).

208. R. K. Anderson, M. Thompson and E. Cullbard, *Analyst*, 111, 1143-1152 (1986).

209. J. Aggett and R. Kadwani, *Analyst*, 108, 1495-1499 (1983).

210. W. H. Ficklin, *Talanta*, 37, 831-834 (1990).

211. E. A. Dietz, Jr., and E. Perez, *Anal. Chem.*, 48, 1088-1092 (1976).

212. R. K. Elton and W. E. Geiger, Jr., *Anal. Chem.*, 50, 712-717 (1978).

213. W. A. Maher, *Anal. Chem. Acta*, 126, 157-165 (1981).

214. J. A. Persson and K. Irgum, *Anal. Chim. Acta*, 138, 111-119 (1982).

215. O. J. Blas, S. V. Gonzalez, R. S. Rodriguez, and J. H. Mendez, *J. AOAC int.*, 77, 441-445 (1994).

216. F. T. Henry and T. M. Thorpe, *Anal. Chem.*, 52, 80-83 (1979).

217. G. E. Pacey and J. A. Ford, *Talanta*, 28, 935-938 (1981).

218. A. A. Grabinski, *Anal. Chem.*, 53, 966-968 (1981).

219. Z. Slejkovec, A. R. Byrne and M. Dermelj, *J. Radioanal. Nucl. Chem. Articles*, 173, 357-364 (1993).

220. Z. Slejkovec, A. R. Byrne, B. Smodis and M. Rossbach, *Fresenius J. Anal. Chem.*, 354, 592-595 (1996).

221. E. G. Soto, E. Rodriguez, P. L. Mahia, S. Lorenzo, and P. Rodriguez, *Anal. Lett.*, 28, 2699-2718 (1995).

222. E. G. Soto, E. A. Rodriguex, D. P. Rodriguez and E. F. Fernandez, *Anal. Lett.*, 29, 2701-2712 (1996).

223. E. G. Soto, M. C. V. Lojo, D. A. Rodriguez, J. N. Dourado, D. P. Rodriguez and E. F. Fernandez, *Fresenius J. Anal. Chem.*, 355, 713-715 (1996).

224. X .C. Le, W. R. Cullen and K. J. Reimer, *Talanta*, 41, 495-502 (1994).

225. H. Ding, J. Wang, J. G. Dorsey and J. A. Caruso, *J. Chromatogr. A*, 694, 425-431 (1995).

226. S. L. Cleland, L. K. Olson and J. A. Caruso, *J. Anal. At. Spectrom.*, 9, 975-979 (1994).

227. J. Gailer, K. A. Francesconi, J. S. Edmonds and K. J. Irgolic, *Appl. Organomet. Chem.*, 9, 341-355 (1995).

228. P. Thomas and K. Sniatecki, *J. Anal. At. Spectrom.*, 10, 615-618 (1995).

229. S. H. Hansen, E. H. Larsen, G. Pritzl and C. Cornett, *J. Anal. At. Spectrom.*, 7, 629-634 (1992).

230. M. A. Lopez, M. M. Gomez, M. A. Palacios and C. Camara, *Fresenius J. Anal. Chem.*, 346, 643-647 (1993).

231. D. L. Tsalew, M. Sperling and B. Welz, *Analyst*, 123, 1703-1710 (1998).

232. R. Sur, J. Begerow and L. Dunemann, *Fresenius J. Anal. Chem.*, 363, 526-530 (1999).

233. A. J. L. Murer, A. Abildtrup, O. M. Poulsen, and J. M. Christensen, *Analyst*, 117, 677-680 (1992).

234. Han Heng-bin, Liu Yan-bing, Mou Shi-fen and Ni Zhe-ming, *J. Anal. At. Spectrom.*, 8, 1085-1090 (1993).

235. R. Rubio, A. Padro, J. Alberti and G. Rauret, *Anal. Chim. Acta*, 283, 160-166 (1993).

236. P. Boucher, M. Accomionotti and J. J. Vallon, *J. Chromatogr. Sci.*, 34, 226-229 (1996).

237. M. A. Lopez-Gonzalvez, M. M. Gomez, C. Camara and M. A. Palacios, *J. Anal. At. Spectrom.*, 9, 291-295 (1994).

238. C. Demesmay, M. Olle and M. Porthault, *Fresenius J. Anal. Chem.*, 348, 205-210 (1994).

239. J. Mattusch and R. Wennrich, *Anal. Chem.*, 70, 3649-3633 (1998).

240. F. E. Brinckman, W. R. Blair, K. L. Jewett and W. P. Iverson, *J. Chromatog. Sci.*, 15, 493 (1977).

241. R. A. Stockton and K. J. Irgolic, *Int. J. Environ. Anal. Chem.*, 6, 313-319 (1979).

242. S. J. Haswell, P. O'Neil and K. C. C. Bancroft, *Talanta*, 32, 69-72 (1985).

243. B. S. Chana and N. J. Smith, *Anal. Chim. Acta*, 197, 177-186 (1987).

244. R. Rubio, A. Padro and G. Rauret, *Fresenius J. Anal. Chem.*, 351, 331-333 (1995).

245. J. Stummeyer, B. Harazim and T. Wippermann, *Fresenius J. Anal. Chem*, 354, 344-351 (1996).

246. E. Hakala and L. Pyy, *J. Anal. At. Spectrom*, 7, 191-196 (1992).

247. M. G. Cobo-Fernandez, M. A. Palacios, D. Chakraborti, P. Quevauviller and C. Camara, *Fresenius J. Anal. Chem.*, 351, 438-44 (1995).

248. M. A. Palacios, M. Gomez, C. Camara and M. A. Lopez, *Anal. Chim. Acta*, 340, 209-220 (1997).

249. X. Zhang, R. Cornelis, J. De Kimpe and L. Mees, *Anal. Chim. Acta*, 319, 177-185 (1996).

250. R. Cornelis, X. Zhang, L. Mees, J. M. Christensen, K. Byrialsen and C. Dyrschel, *Analyst*, 123, 2883-2886 (1999).

251. M. Vilano, A. Padro, R. Rubio and G. Rauret, *J. Chromatogr. A.*, 819, 211-220 (1998).

252. W. D. Spall, J. G. Lynn, J. L. Anderson, J. G. Valdez and L. R. Gurley, *Anal. Chem.*, 58, 1340-1344 (1986).

253. K. E. Lafreniere, V. A. Fassel and D. E. Eckels, *Anal. Chem.*, 59, 879-887 (1987).

254. N. Furuta and T. Shinofuji, *Fresenius J. Anal. Chem.*, 355, 457-460 (1996).

255. D. Schlegel, J. Mattusch and K. Dittrich, *J. Chromatogr. A*, 683, 261-267 (1994).

256. G. Rauret, R. Rubio and A. Padro, *Fresenius J. Anal. Chem.*, 340, 157-160 (1991).

257. F. Vanhaecke and L. Moens, *Fresenius J. Anal. Ch*em., 364, 440-451 (1999).

258. C. Hwang and S. Jiang, *Anal. Chim. Acta*, 289, 205-213 (1994).

259. D. Beauchemin, *J. Anal. At. Spectrom.*, 13, 1-5 (1998).

260. A. Woller, Z. Mester and P. Fodor, *J. Anal. At. Spectrom.*, 10, 609-613 (1995).

261. Z. Mester and P. Fodor, *Anal. Chim. Acta*, 386, 89-97 (1999).

262. X. C. Le, M. S. Ma, *Anal. Chem.*, 70, 1926-1933 (1998).

263. Z. Slejkovec, J. T. van Elteren and A. R. Byrne, *Anal. Chim. Acta.*, 358, 51-60 (1998).

264. P. Morin, M. B. Amran, S. Favier, R. Heimburger and M. Leroy, *Fresenius J. Anal. Chem.*, 339, 504-509 (1991).

265. J. F. Jen, Y. J. Yang and C. H. Cheng, *J. Chromatogr. A*, 791, 357-360 (1998).

266. J. M. Costa-Fernandez, F. Lunzer, R. Pereiro-Garcia and A. Sanz-Medel and N. Bordel-Garcia, *J. Anal. At. Spectrom.*, 10, 1019-1025 (1995).

267. D. Schlegel J. Mattusch and R. Wennrich, *Fresenius J. Anal. Chem.*, 354, 535-539 (1996).

268. C. Casiot, M. C. B. Alonso, J. Boisson, O. F. X. Donard and M. Potin-Gautier, *Analyst*, 123, 2887-2893 (1999).

269. K. Li, S. F. Y. Li, *Analyst*, 120, 361-366 (1995).

270. E. P. Gil, P. Ostapczuk and H. Emons, *Anal. Chim. Acta*, 389, 9-19 (1999).

271. H. Greschonig, M. G. Schmid and G. Gubitz, *Fresenius J. Anal. Chem.*, 362, 218-223 (1998).

272. M. L. Magnuson, J. T. Creed and C. A. Brockhoff, *Analyst*, 122, 1057-1061 (1997).

273. B. Michalke, P. Schramel and P. Schramel, *J. Chromatogr. A*, 834, 341-348 (1999).

274. B. Michalke, O. Schramel and A. Kettrup, *Fresenius J. Anal. Chem.*, 363, 456-459 (1999).

275. M. van Holderbeke, Y. N. Zhao, F. Vanhaecke, L. Moens, R. Dams and P. Sandra, *J. Anal. At. Spectrom.*, 14, 229-234 (1999).

276. J. J. Thompson and R. S. Houk, *Anal. Chem.*, 58, 2541-2548 (1986).

277. D. Heitkemper, J. Creed, J. Caruso and F. L. Fricke, *J. Anal. At. Spectrom.*, 4, 279-284 (1989).

278. B. S. Sheppard, W. L. Shen, J. A. Caruso, D. T. Heitkemper and F. E. Fricke, *J. Anal. At. Spectrom.*, 5, 431-435 (1990).

279. B. S. Sheppard, J. A. Caruso, D. T. Heitkemper and K. A. Wolnik, *Analyst*, 117, 971-875 (1992).

280. S. C. K. Shum, R. Neclderson and R. S. Houk, *Analyst*, 117, 577-582 (1992).

281. P. M. Yehl and J. F. Tyson, *Anal. Comm.*, 34, 49-51 (1997).

282. A. Woller, H. Garraud, J. Boisson, A. M. Dorthe, P. Fodor and O. F. X. Donard, *J. Anal. At. Spectrom.*, 13, 141-149 (1998).

283. H. Helgesen and E. H. Larsen, *Analyst*, 123, 791-796 (1998).

284. J. Lintschinger, P. Schramel, A. Hatalak-Rauscher, I. Wendler and B. Michalke, *Fresenius J. Anal. Chem.*, 362, 313-318 (1998).

285. P. Kavanagh, M. E. Farago, I. Thornton, W. Goessler, D. Kuehnelt, C. Schlagenhaufen and K. J. Irgolic, *Analyst*, 123, 27-29 (1998).

286. T. Lindemann, A. Prange, W. Dannecker and B. Neidhart, *Fresenius J. Anal. Chem.*, 364, 462-466 (1999).

287. G. E. M. Hall, J. C. Pelchat and G. Gauthier, *J. Anal. At. Spectrom.*, 14, 205-213 (1999).

288. W. R. Cullen and M. Dodd, *Appl. Organomet. Chem.*, 3, 401-409 (1989).

289. R. Rubio, A. Padro, J. Alberti and G. Rauret, *Mikrochim. Acta*, 109, 39-46 (1992).

290. T. Taniguchi, H. Tao, M. Tominaga, A. Miyazaki, *J. Anal. At. Spectrom.*, 14, 651-655 (1999).

291. T. Prohaska, M. Pfeffer, M. Tulipan, G. Stingeder, A, Mentler and W. W. Wenzel, *Fresenius J. Anal. Chem.*, 364, 467-470 (1999).

292. J. Zima and C. M. G. van den Berg, *Anal. Chim. Acta.*, 289, 291-298 (1994)

293. H. Li and R. B. Smart, *Anal. Chim. Acta.*, 325, 25-32 (1996)

294. I. Eguiarte, R. M. Alonso and R. M. Jimenez, *Analyst*, 121, 1835-1838 (1996)

295. G. Henze, W. Wagner and S. Sender, *Fresenius J. Anal. Chem.*, 358, 741-744 (1997).

296. Y. Sun, J. Mierzwa and M. Yang, *Talanta*, 44, 1379-1387 (1997)

297. M. Kopanica and L. Novotny, *Anal. Chim. Acta.*, 368, 211-219 (1998)

298. L. Jurica, A. Manova, J. Dzurov, E. Beinrohr, *Fresenius J. Anal. Chem.*, 366, 260-266 (2000).

299. H. C. Beard, *Radiochemistry of Arsenic*: Nuclear Science Series. National Research Council (U.S.), (1965).

300. Z. Slejkovec, J. T. van Elteren, A. R. Byrne and J. J. M. de Goeij, *Anal Chim. Acta*, 380, 63-71 (1999).

301. D. E. Ryan, D. C. Stuart and A. Chattopadhyay, *Anal. Chim. Acta*, 100, 87-93 (1978).

302. J. Holzbecher, A. Chatt and D. E. Ryan, *Can. J. Spectrosc.*, 30, 67-72 (1985).

303. D. E. Ryan, A. Chatt and J. Holzbecher, *Anal. Chim. Acta*, 200, 89-100 (1987).

304. W. Zhang, *Studies on Anticoincidence Gamma-ray Spectrometry in Neutron Activation Analysis*, Ph.D. Thesis, Dalhousie University, Halifax, 187-198 (1997).

305. D. R. Helmer, R. J. Gehrke and J. R. Davidson, *Gamma-ray Spectrum Catalogue: Ge(Li) and Si(Li) Gamma-ray Spectra*, Idaho National Engineering and Environmental Laboratory (1999).

306. F. Lagarde, M. B. Amran M. J. F. Leroy, C. Demesmay, M. Olle, A. Lamotte, H. Muntau, P. Michel, P. Thomas, S. Caroli, E. Larsen, P. Bonner, G. Rauret, M. Foulkes, A. Howard, B. Griepink and E. A. Maier, *Fresenius J. Anal. Chem.*, 363, 5-11 (1999).

307. F. Lagarde, M. B. Amran M. J. F. Leroy, C. Demesmay, M. Olle, A. Lamotte, H. Muntau, P. Michel, P. Thomas, S. Caroli, E. Larsen, P. Bonner, G. Rauret, M. Foulkes, A. Howard, B. Griepink and E. A. Maier, *Fresenius J. Anal. Chem.*, 363, 18-22 (1999).

308. A. J. Quick and R. Adams, *J. Am. Chem. Soc.*, 44, 805-809 (1922).

309. C. K. Banks, J. F. Morgan, R. L. Clark, E. B. Hatlelid, F. H. Kahler, H. W. Paxton and E. J. Cragoe, *J. Am. Chem. Soc.*, 69, 927-930 (1947).

310. S. Budavari, M. J. O'Neil, A. Smith, P. E. Heckelman and J. F. Kinneary, *The Merck Index, 12th edition*, Merck & Co., Inc., Whitehouse, NJ (1996).

311. Y. Shi, E. E. Sullivan, J. Holzbecher and A. Chatt, *Biological Trace Element Research*, 71-72, 377-386 (1999).

312. J. A. Dean, *Analytical Chemistry Handbook*, McGraw-Hill Inc. New York, 2-20 (1995).

313. D. R. Lide (Editor-in-Chief), *CRC Handbook of Chemistry and Physics, 79th edition*, 8-120, 15-14 to 15-18, CRC Press. (1998-99).

314. J. L. Lundgren and A. A. Schilt, *Anal. Chem.*, 49, 974-980 (1977).

315. R. J. Philips and J. S. Fritz, *Anal. Chem.*, 50, 1504-1508 (1978).

316. K. Isshiki, F. Tsuji, and T. Kuwamoto, *Anal. Chem.*, 59, 2491-2495 (1987).

317. K. Isshiki and E. Nakayama, *Anal. Chem.*, 59, 291-295 (1987).

318. G. Persaud and F. F. Cantwell, *Anal. Chem.*, 64, 89-105 (1992).

319. D. A. Skoog and J. J. Leary, *Principles of Instrumental Analysis, 4th addition*, 5-9, Saunders College Publishing (1992).

320. W. D. Ehmann and D. E. Vance, *Radiochemistry and Nuclear Methods of Analysis*, John Wiley & Sons, New York Chichester, Brisbane, Toronto Singapore (1991).

321. G. L. Long and J. D. Winefordner, *Anal. Chem.*, 55, 712A-724A (1983).

322. H. Kaiser, *Anal. Chem.*, 42, 53A-58A (1970).

323. L. A. Currie, *Anal. Chem.*, 40, 586-593 (1968).

324. C. Vandecasteele and C. B. Block, *Modern Methods for Trace Element Determination*, 68-74, John Wiley and Sons, Chichester (1993).

325. R. M. Smith, *Gas and Liquid Chromatography in Analytical Chemistry*, 273-286 John Wiley and Sons, Chichester (West Sussex); New York (1988).

326. J. K. Taylor, *Quality Assurance of Chemical Measurements*, 129-146, Lewis Publishers, Inc., Chelsea, Michigan (1987).

327. J. K. Oygard, A. Lundebye and K. Julshamn, *J. AOAC Int.*, 82, 1217-1223 (1999).

328. O. Munoz, D. Velez and R. Montoro, *Analyst*, 124, 601-607 (1999).

329. O. Munoz, D. Velez, M. L. Cevere and R. Montoro, *J. Anal. At. Spectrom.*, 14, 1607-1613 (1999).