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Arsenic Speciation in Food

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A B S T R A C T

Arsenic Speciation in Food

by

Simon Branch

A high performance liquid chromatography-inductively coupled plasma-mass spectrometry (HPLC-ICP-MS) method has been developed for the separation and quantification of $\mu g\ kg^{-1}$ levels of arsenobetaine, monomethylarsonic acid (MMAA), dimethylarsinic acid (DMAA), arsenite and arsenate. Using this coupling, arsenic species in fruit and vegetables grown on soils containing up to 1.4% w\w arsenic have been surveyed and DMAA, MMAA, arsenite and arsenate identified in the plants. Although extraction efficiencies were poor, typically 10%, total arsenic determinations demonstrated that arsenic uptake by the plants was low, with the highest arsenic level being 60-70 $mg\ kg^{-1}$ dry weight in unpeeled potato. Provided the plants are washed thoroughly they pose no dietary risk.

Using the same HPLC-ICP-MS coupling non-toxic arsenobetaine was identified as the major arsenic species in cod, dab, haddock, lemon sole, mackerel, plaice and whiting. Levels ranged between 1.0 $mg\ kg^{-1}$ dry weight in the mackerel, to 187 $mg\ kg^{-1}$ in the plaice. Mackerel also contained DMAA and possibly a lipid bound arsenic species. No degradation of arsenobetaine to more toxic species was observed when an enzymatic digestion procedure, based on the action of trypsin, was applied to fish except in the case of one of the plaice specimens for which DMAA was characterised in the digest at the $mg\ kg^{-1}$ level.

Ten volunteers participated in a dietary trial in which they were given set conventional meals. The main source of arsenic was fish and the predominant species was arsenobetaine. All of the arsenic, as arsenobetaine, was excreted in the urine within 72 hours of consumption. Urinary levels of MMAA, DMAA and inorganic arsenic were all below 10 μg.

For total arsenic determination in the urine nitrogen introduction ICP-MS was used to overcome the polyatomic ion $^{40}Ar^{35}Cl^{+}$. This method gave good agreement between observed and certified values for a range of reference materials.

A C K N O W L E D G E M E N T S

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C O N T E N T S

"What is food to one man may be

fierce poison to others".

Lucretius

1. INTRODUCTION

1.1 Trace Metal Speciation

The study of trace metal speciation, i.e. the study and quantification of the different physico-chemical forms of an element, is a relatively new subject, principally having developed in the last fifty years. However the concept of chemical speciation goes back at least one hundred years. A number of individuals had died in their sleep and it was postulated that possibly moulds living in the damp conditions in the bedrooms were converting the arsenic pigments used in paints and wallpapers into toxic arsine gas. This theory was confirmed by the work of Gosio (1; 2) who isolated the evolved gas from a culture of potato pulp accompanied by the mould Scopulariopsis brevicaulis. The garlic smelling gas, known as Gosio's gas, was incorrectly identified as diethylarsine. It was Frederick Challenger (3; 4) who identified "Gosio's gas" as trimethylarsine and simultaneously laid the foundation of modern organometallic speciation study with his work on the methylation of arsenic by the yeast S. brevicaulis. He found methylarsines were formed by S. brevicaulis grown on sterile bread crumbs in the presence of sodium methanearsonate, dimethylarsinic acid and a number of other arsenic compounds. However it was another thirty years before the methylation of inorganic arsenic in mammals was demonstrated by Braman and Foreback (5).

Speciation should not be thought of as referring only to

methylated forms of an element. Although numerous definitions of "species" and "speciation" have been proposed (6), in this study the term species refers to all the different physico-chemical forms of an element and speciation implies the identification and quantification of the different forms. Thus arsenite, the common environmental form of the +3 oxidation state, and arsenate, the common environmental form of the +5 oxidation state, are different arsenic species.

The developing trend towards speciating an element rather than determining total concentration stems from an understanding that certain essential properties such as bioavailability, toxicity and distribution are dependant on the chemical form of the element. Examples of species dependant phenomena are given below.

1. Iron absorption from the human gastrointestinal tract is determined by the form of the iron, <u>i.e.</u> Fe^{2+}, Fe^{3+} or heme iron. Fe^{2+} is far more readily absorbed than Fe^{3+} (7).

2. Total zinc measurements of food do not sufficiently characterise the nutritional quality of the food as phytates, present in foods of plant origin, combine with zinc to form zinc/calcium phytates which are insoluble at the intestinal pH (8).

3. Both organotins and trialkyleads inhibit the enzyme ATP synthase. This effect increases as alkyl chain length increases (9).

4. There is a considerable variation in mercury absorption through the intestinal wall. Methylmercury is readily absorbed, mercury (II) chloride is partially absorbed and mercury metal is even less well absorbed (10). The toxicity of methylmercury and other methylated mercury compounds was demonstrated in the infamous Minimata Bay poisoning incident of 1956-68. Organomercury was discharged from a chemical plant and passed along the food chain seriously affecting aquatic and terrestrial lifeforms. Hundreds of people were affected after ingesting contaminated fish. The toxic agents were identified as dimethyl-, diethyl- and di-n-propylmercury (11).

Arsenic is of particular interest as it is an element that has a pronounced species dependant toxicity. The arsenic species of specific interest are shown in Table 1.1. Their toxicity can be summarised (12-14).

Arsines (Organic or Inorganic) > arsenite > arsenoxides > arsenate > monomethylarsonic acid > dimethylarsinic acid > arsonium compounds > arsenobetaine > metallic arsenic

Kaise et al. (13) clearly demonstrated the great range of toxic effects by administering arsenite and arsenobetaine to mice. A selection of the results are shown in Table 1.2. Mice are most commonly used in arsenic studies in preference

Table 1.1. Selected Arsenic Species

AsO_2^-	Arsenite
AsO_4^{3-}	Arsenate
$(CH_3)AsO(OH)_2$	Monomethylarsonic Acid (MMAA)
$(CH_3)_2\ AsO(OH)$	Dimethylarsinic Acid (DMAA)
$(CH_3)_3\ As$	Trimethylarsine
$(CH_3)_3\ As^+(CH_2)_2OH$	Arsenocholine
$(CH_3)_3\ As^+\ CH_2COO^-$	Arsenobetaine

Table 1.2 Relationship Between Dose and Mortality for Arsenobetaine and Arsenic Trioxide (13)

Arsenobetaine		Arsenic Trioxide	
Dose (g kg^{-1})	Number of Deaths/Animals	Dose (mg kg^{-1})	Number of Deaths/Animals
0.5	0/10	25.00	0/10
1.0	0/10	28.75	1/10
5.0	0/10	33.06	2/10
10.0	0/10	38.02	9/10
		43.73	10/10

to rats because rats metabolise arsenic differently to humans (15). The Water Research Centre has reviewed the toxicity and toxic effects of a range of arsenic compounds to invertebrates, marine and fresh water fish and aquatic flora (16). In general trivalent arsenic was more toxic than pentavalent arsenic and both were more toxic than organo-arsenic compounds. Plants showed a wide range of toxicities being between 0.1 and 100 mg As l^{-1}. They also noted that in the field some species of algae were able to survive at concentrations twenty times those which were lethal in the laboratory.

From the foregoing the importance of chemical speciation, especially for toxic elements such as arsenic, is apparent. In the following pages the interactions of arsenic species within the environment will be reviewed and the implications for human health via the food web discussed.

1.2 Arsenic in the Environment

Arsenic is ubiquitous in nature, occurring in air, water, soil and living organisms. Natural processes such as the weathering of arsenic containing ores and volcanic action are the primary sources of arsenic. Man increases the arsenic in the environment indirectly by industrial actions such as the smelting of gold, zinc, lead, copper, silver and nickel, and directly by the use of arsenic containing herbicides and wood preservatives. Arsenic concentrations in the environment are given in Table 1.3.

Table 1.3 Arsenic Concentrations (mg kg^{-1}) in the Environment

Rocks	Range	Mean	Ref
Ultrabasic			
e.g. peridotite	0.034 - 15.8	1.5	17
	0.3 - 3.0	1.0	18
Basic			
e.g. basalt	0.18 - 113	2.3	17
	0.1 - 9.0	1.4	18
Intermediate			
e.g. latite	0.5 - 5.8	2.7	17
	0.5 - 5.8	2.2	18
Acid			
e.g. granite	0.18 - 15	1.3	17
	0.0 - 8.5	1.9	18
Sediments			
Ocean sediments	<0.4 - 445	33.7	17
River/harbour Sediments	1.1 - 37.5	-	19
Shales	0.3 - 500	14.5	17
Oil Shale	-	70.2	20
Limestone, dolomite	0.1 - 20.1	2.6	17
	0.1 - 23.5	3.5	18
Soils			
Tamar Valley, UK	0.02 - 880	45	21
Garden Soil, Cornwall	144 - 892	322	22
Memphis, USA	-	2.45	23
World, various sites	0.1 - 55	7.2	17
Water			
Stream, River and Lake waters	0.00025-22.4	0.0031	17
Estuary, Tamar, UK	0.0001-0.007	-	24
Estuary, Humber, UK	0.00094-0.024	-	25
The North Sea	0.00084-0.0016	0.00111	25
Californian Pacific Ocean	-	0.0015	26
Air			
United Kingdom	<0.5-12.3 ngm^{-3}	-	27
Japan	23 -692 pgm^{-3}	-	28

Arsenic is a constituent of over 200 minerals, or over 10 per cent of all known minerals, particularly those which contain a metal, e.g. silver, aluminium, nickel and iron, and sulphur. Typical examples are arsenopyrite (FeAsS), niccolite (NiAs), tennantite ($[Cu,Fe]_{12}$ $[As_4S_{13}]$), proustite (Ag_3AsS_3), orpiment (As_2S_3), realgar (As_4S_4), scorodite [(Fe, Al) $(AsO_4).2H_2O$] and arsenolite (As_2O_3). Of these arsenopyrites (or mispickel), orpiment and realgar are the commercially important ores. Almost all sulphides contain traces of arsenic, which is also commonly found in phosphates, sulphates and vanadates. Volcanic activity and natural weathering release arsenic to the pedosphere, usually as the arsenic (V) oxoanion, arsenate. Arsenic is generally located in the B horizon as a result of sorption to hydrous oxides of iron and aluminium which predominate in this horizon. Other metals e.g. Ni and Pb, react with soluble arsenates to form secondary arsenic minerals which themselves are subject to weathering, releasing arsenic to surface and groundwater.

Although numerous values for arsenic in soil have been reported in the literature (17; 21-23) these values will always be dependant on location, soil origin and anthropogenic inputs. Orchard soils often show the highest arsenic concentrations (29) as a consequence of spraying with arsenical insecticides or defoliants. In fact a considerable proportion of the industrial products of arsenic have a direct environmental fate. Industrial uses and production levels in several countries are given in

Tables 1.4-1.6. The use of arsenical herbicides and pesticides expanded in the second half of the 1970's after 2-4-5-t (2,4,5-trichlorophenoxy acetic acid) use was restricted. The principal examples are monosodium methanearsonate (MSMA), disodium methanearsonate (DSMA) and inorganic salts of lead and calcium, the production of which was severely curtailed in the 1970s. Several discussions of the herbicidal use of arsenic exist in the literature (34-36).

As previously stated arsenic in soil is tightly bound to the surface of soil particles with only a small proportion soluble and available for plant uptake. Depending on the soil pore water concentration and the plant species, the arsenic will either be phytotoxic or assimilated and translocated throughout the plant. The arsenic may then enter the food chain and move to higher trophic levels.

In addition to loss by plant uptake arsenic is lost from soil by bacteria-mediated volatilisation and leaching. McBride and Wolfe (37) demonstrated that the bacteria Methanobacterium could synthesise dimethylarsine. Under anaerobic conditions bacteria probably mediate the volatilisation of arsenic. Under aerobic conditions fungi are thought to be the active microorganisms. Numerous other reports of the formation of alkylarsines in the soil can be found in the literature (38; 39). However these mechanisms are a matter of some debate. Cheng and Focht (40) analysed soils and soil cultures containing Pseudomonas

Table 1.4 Estimates of Global Emissions of Arsenic (tonnes/year)

Natural Sources	Study 1(30)	Study 2(31)
Volcanoes	7000	17150
Low temperature Volatilisation	240	26200
Wind erosion	240	1980
Forest fires	160	125
Sea Spray	140	27
Total	7800	45480
Anthropogenic Sources		
Copper smelting	13000	12080
Lead smelting	-	1430
Zinc smelting	2200	780
Iron & steel production	4200	60[a]
Coal and coal use	550	6240
Wood fuel	600	425
Clearance for crops	-	1920
Agriculture burning	560	-
Burning pasture land	-	1000
Agricultural chemicals & wood preservatives	1920	3580
Glass manufacture	-	468
Cotton ginning	23	-
Waste Incineration	430	78
Other uses	217	2-3000[b]
Total	23700	28060

(a) Steel production only
(b) Estimated and not included in total

Table 1.5 Arsenic Emissions in Canada, 1972(32)

Source	Emissions Tons	Percent
Industry		
Primary Cu and Ni Production	661	16.2
Primary Pb Production	18	0.4
Primary Zn Production	359	8.8
Primary Fe and Steel Production	1041	25.6
Gold Processing	1934	47.5
Miscellaneous	15	0.4
Fuel Combustion/Stationary Sources		
Power Generation	25	0.6
Industrial & Commercial	13	0.3
Domestic	<1	<0.1
Transportation	<1	<0.1
Solid Waste Incineration	1	<0.1
Pesticide Application	6	0.2
Total	4073	100

Table 1.6 United States Markets for Arsenic (as As_2O_3), 1981(33)

Wood Preservatives	36%
Herbicides	31%
Cotton Dessicants	15%
Moly Flotation	8%
Glass Manufacture	5%
Miscellaneous	5%

Total Supply of As_2O_3 5000 tons (est.)

and Alcaligenes and found that the sole gaseous product of anaerobic incubation in the presence of arsenate or arsenite was arsine. Only when soil was incubated with dimethylarsinate was dimethylarsine detected, and even then it contributed only 25% of the gas produced. They concluded that reduction to arsine, not methylation to trimethylarsine, was the primary mechanism of arsenic loss from soils. They supported these conclusions further by a discussion on the shortcomings of the analytical protocols used by previous researchers. It is worth noting that Cheng and Focht did not include Methanobacterium in their cultures. More recently Mohan et al. (41) found traces of DMAA in soils incubated with MMAA but this evidence of methylation has to be treated with some scepticism as the DMAA concentration was too low to be quantified.

Arsenic is also lost from soil by leaching into ground and surface waters. This is most marked for sandy soils compared to clay soils as in the former the arsenic is more soluble hence more mobile. Clearly the different solubilities of the species will affect the degree of leaching. The chemistry and behaviour of arsenic in soil has recently been reviewed (42).

Soil leaching and anthropogenic inputs such as industrial discharges into estuaries are amongst the ways that arsenic passes into the rivers and oceans. Dissolved gases and particulate matter such as volcanic debris also increase the arsenic burden of natural waters. This arsenic is then

involved in a series of complex biochemical reactions between and within marine species. These reactions, and the position they occupy in the arsenic cycle, are described in detail in the following section. A simplified picture of the overall arsenic cycle is shown in Figure 1.1.

1.3 Arsenic Species in the Environment

The proposed generalised arsenic cycle shown in Figure 1.1 is relatively complex. In reality the picture is even more complicated, with a large range of interactions occurring within marine organisms alone (43). Table 1.7 lists some of the arsenic species identified in different parts of the environment. The Table is by no means exhaustive and is intended merely to demonstrate some of the known species, and their occurrence.

A number of chemical and biological mechanisms mediate the interconversions between species. These can be summarised:

(1) Redox reactions such as those between arsenate and arsenite which can be biologically or abiotically mediated,

(2) Reductive biomethylation of arsenic to form methylarsines, as proposed by Challenger (3; 4),

(3) The biosynthesis of organoarsenic compounds and their degradation.

FIGURE 1.1 : POSSIBLE ENVIRONMENTAL ARSENIC CYCLE

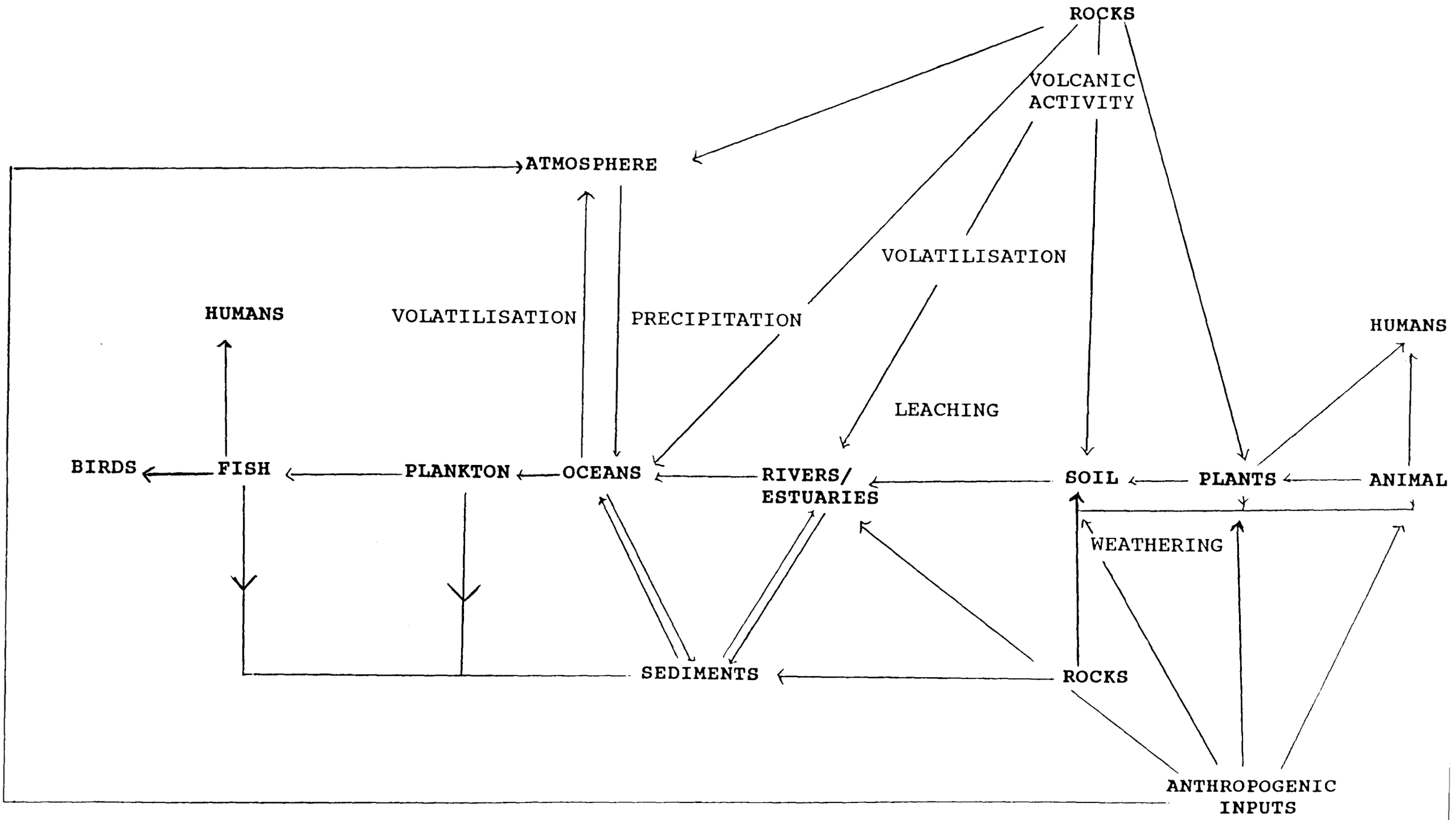


Table 1.7 Arsenic Species in the Environment

ROCKS	SPECIES	REF
Endogenous	As, As_2S_2, As_2S_3, FeAsS, Ag_2AsS_3, NiAs, NiAsS, CoAsS, $FeAs_2$	17
Supergenous	As_2O_3, $Zn_2AsO_4(OH)$, $Cu_2(AsO_4)OH$	17
SOIL		
Pore Water	Arsenite (1-5), MMAA (1-22), Arsenate (3-235)	44
WATER		
Estuaries, Southampton	Arsenite, MMAA, DMAA	45
Estuaries, Tamar	Inorganic As(4.69 ± 0.14), MMAA (0.69 ± 0.19), DMAA (0.43 ± 0.1)	25
Sediment, Tamar	MMAA (0.6 maximum), DMAA (2.0 max)	24
North Sea	Inorganic As (1.11 ± 0.18), MMAA (0.11 ± 0.04), DMAA 0.26 ± 0.15)	25
Sediment, Canada	Inorganic, mono- and trimethylarsines, MMAA and DMAA	46
AIR		
Over As treated Soil	Mono-, di- and trimethylarsine	47
Over Japanese Sea	Methylarsine (485), Dimethylarsine (45) and trimethylarsine (162)	28

Values for the species concentration are given in parenthesis. Units are $\mu g l^{-1}$ except for atmospheric results where concentration is in pgm^{-3}.

This third mechanism will be discussed in detail in the following section.

Further complexity is added to the arsenic cycle by man's perturbations. In addition to the anthropogenic inputs already discussed, complex organoarsenic compounds such as roxarsone are commonly used as growth promoters in poultry and swine (48; 49). Traces of these compounds will thus enter man and the environment. Several efforts have been made to draw together the transformations of arsenic species into a coherent series of pathways (50; 51). No truly satisfactory model pathway currently exists, partly because of a lack of knowledge of what is occurring at the cellular level, although this knowledge is constantly being updated. The marine environment has been the focus of most research and the following section is intended to summarise progress.

1.3.1 Arsenic and Arsenic Species in the Marine Environment

Much of the impetus for the research effort directed at arsenic species in the marine environment stems from the long held awareness of the high concentration of arsenic in fish (52; 53) a class of organisms that represent a significant proportion of the human diet in many areas of the world. In 1966 a review (54) of commercially available seafoods reported an arsenic range of 1.5-8.9 mg kg^{-1} in fish. These findings generated some concern that arsenic may accumulate in higher trophic levels. Penrose (12) surveyed the then available data and concluded this was not

the case. Penrose's review is significant in that he cited several research needs which included:

(1) studies on determining the biochemistry of arsenic in marine organism;
(2) arsenic should not be treated as "total arsenic" when assessing hazards, but as a range of compounds;
(3) the source of arsenic in marine animals needed to be identified.

Over the following sixteen years much research effort has been focused towards Penrose's goals.

Since the work of Chapman (53) it has been recognised that arsenic in marine organisms has distinct chemical and biological properties. Lunde (55) used molecular gel filtration to determine that arsenic in a range of fish and shellfish occurred predominantly as an organoarsenic compound. He concluded that the water and lipid soluble species isolated were possible derivatives of a simple organo arsenic molecule present in all marine organisms. In 1974 Penrose (12) observed that the arsenic of macroalgae was not accumulated, but excreted in a soluble organic form. In 1977 Irgolic et al. (56) grew Tetraselmis chuii (a marine alga) and Daphnia magna in media rich in inorganic arsenic. The organisms were found to grow and to incorporate arsenic from media containing up to a 1000 mg l^{-1} of arsenic. Extraction and isolation of the arsenic containing compound allowed the hypothesis that arsenic was present in lipids

as a replacement for nitrogen in choline groups. The group indicated that they had synthesised arsenobetaine and arsenocholine to help characterise the nature of the arsenic containing compounds. Also in 1977 Edmonds *et al*. (57) isolated arsenobetaine from the tail muscle of the Western Rock Lobster (*Panulirus longipes Cygnus George*). Identification was performed using nuclear magnetic resonance, thin layer chromatography and single crystal x-ray structure determination. Since this discovery arsenobetaine has been identified as the predominant arsenic species in a wide range of marine organisms (57-72) at all trophic levels.

Table 1.8 lists some examples of the presence of arsenobetaine in marine organisms. Luten *et al*. (61) determined arsenobetaine quantitatively as methylated arsenic species following alkaline digestion and generation of the corresponding hydride using sodium borohydride. Only in a few instances (63; 70) was a serious effort made to quantify arsenobetaine.

A number of other arsenic species have been isolated from marine organisms (Table 1.9). In reference (74) the arsenocholine and acetylarsenocholine concentrations lay between 0.04 and 0.5 nmol g^{-1}. The arsenocholines constituted a greater proportion of the total arsenic burden of the bay organisms than the marine organisms.

Table 1.8 **Arsenobetaine in Marine Organisms (Concentration in mg As Kg^{-1} Dry Weight)**

Organism	Species	Concentration	Ref
Sea Squirt	Styela clava	N.G	58
Bay Mussel	Mytilus edulis	N.G	58
Cockle	Meretrix lusoria	N.G	59
Sea Cucumber	Stichopus sp.	N.G	59
Clams	Various sp.	N.G	60
Shrimp	Nephrops norvegicus	(9.2-10.8)	61
Shrimp	Sergestas incens	N.G	62
Pacific Shrimp	N.G	15.8	63
Canadian Shrimp	N.G	0.58	63
Alaskan Snow Crab	Chionoecetes bairdii Rathbun	N.G	64
Alaskan King Crab	Paralithodes camtsc-hatica Tilesius	N.G	64
Crab	Cancer cancer	(9.2-10.8)	61
Red Crab	Chionoecetes opilio	N.G	66
Atlantic Lobster	N.G	4.5	63
Canadian Lobster	N.G	3.6	63
American Lobster	Humarus american	(6.5-26)	65
Western Rock Lobster	Panulires longipes Cygnus George	N.G	57
Octopus	Paroctopus dofleini	N.G	67
School Whiting	Sillago bassensis	7.7	68
Atlantic Cod	N.G	4.1	63
Pacific Cod	N.G	6.2	63
Plaice	Pleuronectes platessa	(3.0-166)	69
Sole	Solea solea	(4.8-9.0)	61
Pacific Sole	N.G	4.6	63
Atlantic Sole	N.G	11.3	63
Lemon Sole	Microstomus kitt	(2.0-11)	61
Dab	Limanda limanda	(19.0-21.2)	61
Flounder	Platichthys flesus	(0.5-5.6)	61
Spiny Dogfish (as a reference material)	Squalus acathias	15.7	70
Shark	Prionace glaucus	N.G	71
Blue Pointer Shark	Isurus oxyrhincus	N.G	72
Whitetip Shark	Carcarhinus longimanus	N.G	72

N.G = Not Given

Table 1.9 Organoarsenic Compounds in Marine Organisms

Compound	Organism	Ref
Arsenocholine	Shrimp	63; 73-75
Arsenocholine	Charonia sauliae (a marine gastropod)	76
Arsenocholine[a] and acetylarsenocholine	Myoxocephalus quadricornis (fourhorn sculpin)	74
Arsenocholine[a] and acetylarsenocholine	Lota lota (burbot)	74
Arsenocholine[a] and acetylarsenocholine	Esox lucius (pike)	74
Arsenocholine[a] and acetylarsenocholine	Perca fluviatilus (perch)	74
Arsenocholine[b] and acetylarsenocholine	Clupea harengus (Baltic Herring)	74
Arsenocholine[b] and acetylarsenocholine	Pleuronectus platessa (plaice)	74
Arsenocholine[b] and acetylarsenocholine	Homarus vulgaris (lobster)	74
Arsenocholine[b]	Cancer pagurus (crab)	74
Arsenocholine[b] and acetylarsenocholine	Glyptocephalus cyanoglossus (witch)	74
Arsenic containing ribofuranosides	Ecklonia radiata (Brown Kelp)	77
Arsenic containing ribofuranosides	Tridacna maxima (Giant Clam)	78
Arsenic containing ribofuranosides	Laminaria japonica (Edible Brown Seaweed)	79
Arsenic containing ribofuranosides and DMAA	Codium fragile (Green Seaweed)	80

(a) From polluted and unpolluted brackish water bays

(b) From marine water.

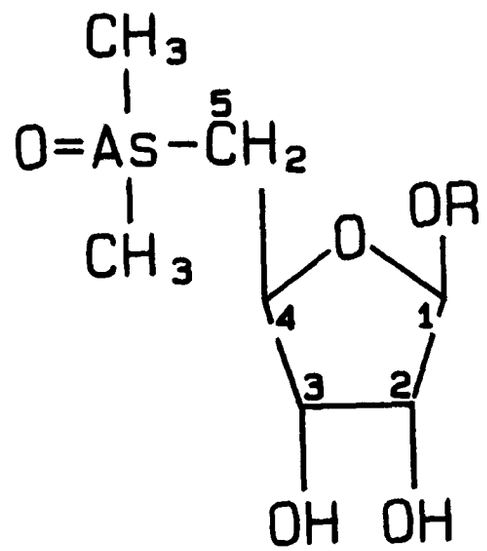
The arsenoribofuranosides constituted between 60 and 81% of the total arsenic in the organisms listed in Table 1.9. All of the sugars isolated to date have a 5-deoxy-5-dimethylarsinoylribofuranoside moiety in common and differ only in the nature of the side chain at the C_1 position (see Fig. 1.2). The arsenosugars may be the precursors of the arsenolipids which have been isolated in some algae (81; 82).

Another important intermediate in arsenobetaine synthesis, dimethyloxarsylethanol was identified by Edmonds et al. (83) in E. radiata following anaerobic decomposition.

Concurrent with the determination of arsenic species in marine organisms sensitive analytical techniques were being developed which allowed the determination of arsenic species in water and their uptake by marine organisms (26; 84-88). The most recent evidence indicates that the concentration and range of arsenic species, in water may have been underestimated due to the reliance on the hydride generation method, a method which does not detect the more complex arsenic species (88).

Several attempts have been made in the past decade to assess some of the interconversions of arsenic which result in the formation of arsenobetaine within the marine ecosystem (81;

Figure 1.2 Arsenic Containing Sugars Isolated to Date



R = $-CH_2CH(OH)CH_2OH$

R = $-CH_2CH(OH)CH_2SO_3H$

R = $-CH_2CH(OH)CH_2OSO_3H$

R = $-CH_2CH(NH_2)CH_2SO_3H$

R = $-CH_2CH(OH)CH_2O\overset{\overset{O}{\|}}{\underset{\underset{OH}{|}}{P}}OCH_2CH(OH)CH_2OH$

89; 90). The route to arsenobetaine as proposed by Phillips and Depledge (89) is the least satisfactory as it employs arsenoethanolamine as its starting point, a compound which has yet to be isolated (43).

Recently Edmonds and Francesconi (43) surveyed the available evidence for the origin of arsenobetaine in marine animals and concluded that it involved transformation of arsenic, as arsenate, at the early stages of the food chain. Evidence that contradicts the de novo synthesis of arsenobetaine from arsenate, the principal species in water (26), includes the findings that oral administration of sodium arsenate to estuarine catfish (Cnidoglanis macrocephalus) and school whiting (Sillago bassensis) led to an accumulation of trimethylarsine oxide rather than arsenobetaine, the levels of which were unchanged (91). However it should be said that the analytical protocol was not entirely satisfactory e.g. only one catfish was used as control.

Edmonds and Francesconi (43) proposed that the principal steps in the formation of arsenobetaine were the uptake of arsenate by algae, possibly by the mechanisms used for nitrogen, and the subsequent microbially mediated methylation by S- adenosylmethionine by the mechanisms proposed by Challenger (92) leading to the formation of a variety of arsenoribosides. These arsenoribosides could then be transformed by bacteria in sediments to dimethyloxarsylethanol (83). Reduction, methylation and

oxidation of this intermediate, either by conversion within sediments or following release to the water column and ingestion by marine animals, will yield arsenobetaine. Recent research (46) supports this hypothesis as methylarsines and methylarsenic (V) compounds were identified in marine sediments. Once again the limiting factor in obtaining a clear picture of the species present was the choice of hydride generation as the detection method which as the author observed, precluded the identification of more complex arsenic species. A number of key intermediates still remain to be identified and the methylation step between dimethyloxarsylethanol and arsenobetaine needs to be demonstrated, before Edmonds' hypothesis can be accepted.

The ultimate fate of arsenobetaine appears to be microbial degradation within sediments (93; 94) leading to the formation of trimethylarsine oxide. Whether or not this then degrades to arsenate to cycle back into the start of the food chain remains to be elucidated.

1.3.2 Arsenic and Arsenic Species in Humans

Generally humans only consume levels of arsenic at the rate of 0.62 mg/week (95). Higher consumption takes place when contamination occurs, or when the diet contains high levels of seafood.

Compounds entering the gastro-intestinal tract undergo

attack from bacteria and enzymes. Those that are ingested enter the portal venous system and then pass through the liver before reaching the general circulation. During the 1970's and 1980's considerable evidence was gathered that indicated a number of changes occurred during these processes. It was Braman and Foreback (5) who first demonstrated MMAA, DMAA, arsenite and arsenate in human urine. Lakso and Peoples (96) further demonstrated that over 50% of inorganic arsenic ingested by cows and dogs was excreted as methanearsonates. The nature of the organo-arsenic species was not elucidated. Crecelius (97) investigated the transformations that occurred when humans were fed arsenite-rich wine, arsenate-rich water and crab meat which contained organo-arsenic species. He found that after ingesting the wine there was a peak in arsenate and arsenite in the urine that persisted for about twenty hours, but levels of MMAA and DMAA peaked and persisted over forty hours. Eighty percent of ingested arsenic was excreted within sixty-one hours. When the water was ingested levels of arsenate rose rapidly and returned to normal within ten hours, and DMAA rose gradually over a seventy hour period. Only 50% of the ingested arsenic was excreted over seventy hours. Following ingestion of crab meat none of the aforementioned arsenic species were identified in the urine until it was subjected to alkaline hydrolysis, when high concentrations of DMAA were observed. The indications were that the organo-arsenic compound (which was probably arsenobetaine) was excreted within forty-eight hours of

ingestion, without any degradation to reducible species occurring. It should be noted that Creclius' findings cannot be regarded as being typical of the population as a whole as they were performed on only one subject. Numerous papers have subsequently appeared adding to our understanding of arsenic metabolism in man. Buchet and Lauwerys (98) demonstrated that ingested methanearsonate was converted to DMAA prior to excretion, and that DMAA, ingested as sodium dimethylarsinate, was excreted unchanged. Hence arsenic species are apparently not subject to demethylation reactions in humans.

As the levels of arsenic in fish may represent dietary risk several studies have focussed on arsenic in urine following fish-based meals (98-100). These have generally demonstrated that the arsenic, probably as arsenobetaine, was excreted rapidly and unchanged.

Determining normal base-line levels of arsenic in urine is extremely difficult as factors such as occupational exposure, diet, drinking water, and wine consumption all affect the results. However several papers have been published which detail arsenic concentration in the urine of the general population (101; 102). Table 1.10 draws together some of the evidence of urinary arsenic species. Foa et al. (105) observed that urinary arsenic species correlated significantly with blood arsenic species and that blood arsenic concentration was proportional to exposure.

Table 1.10 Arsenic Species Reported in Human Urine (μg As l^{-1})

No. of Subjects	Arsenate	Arsenite	MMAA	DMAA	Total As	R
4	3.9 (0.8-7.9)	1.9 (0.1-5.1)	1.8 (0.6-2.5)	15.0 (8.9-25.2)	22.5 (10.4-30.0)	
14	ND	1.0	5.5 (1-29)	8.2 (1-31)	14.6 (3.0-53.0)	1
1	ND	ND	0.7 ± 0.7	4.4 ± 0.6	NG	104
1	1.9 ± 1.0	27.5 ± 11.0	0.8 ± 0.5	5.8 ± 1.1	NG	104
300	8.3 ± 9.8		NG	NG	30.8 ± 36.8	101
1	2	2	3	8	15	97

ND = Not Detected

NG = Not Given

In conclusion it appears that methylation is the principal mechanism in humans following exposure or ingestion of inorganic or simple organoarsenic compounds. The end product, and major urinary arsenic species is DMAA. There is some evidence that DMAA may be further methylated to trimethyarsine oxide (106).

Complex arsenic species such as arsenobetaine and tetramethylarsonium salts are rapidly excreted in the urine without further transformation (107).

The mechanism and site of methylation have not yet been fully elucidated. Vahter and Marafante (106) recently reviewed the available evidence and concluded the probable site was the liver and that the process was that postulated by Challenger (92), i.e., alternating reduction and oxidative methylation by addition of a carbonium ion to trivalent arsenic. It is thought thiols provide the electrons for the reduction steps and that the methyl donor is S-adenosylmethionine. The purpose of these reactions is probably to serve as a detoxification mechanism.

1.4 Arsenic and Arsenic Species in Food

Arsenic has long been known as a poison; thus it is inevitable that considerable effort has been expended in determining the levels in foodstuffs, many of which are

known to contain natural levels in excess of 1 mg kg^{-1}. As early as 1903 a Royal Commission published guidelines for arsenic (as arsenic trioxide) levels in solid food, declaring that levels "in excess of 1/100th grain or more in a pound of food" (1.4 mg kg^{-1}) would be penalised under the Sale of Food and Drugs Act. These regulations followed a poisoning incident arising from beer made with glucose which itself was contaminated with arsenical pyrites. At least 70 people died as a result (95).

Nowadays numerous government agencies and international organisations lay down guidelines for arsenic consumption from food and concentrations acceptable in drinking water. The United Kingdom Ministry of Agriculture, Fisheries and Food in 1959 set a limit for arsenic in food of 1 mg kg^{-1} and in beverages of 0.1 mg kg^{-1}. Exceptions were granted for seafood if the arsenic could be shown to be naturally occurring (95). The European Community has published a Directive (108) which lists the maximum allowable arsenic concentration in human drinking water as 50 μg l^{-1}. The World Health Organisation (109) cites a maximum daily arsenic intake of no more than 50 μg As kg^{-1} body weight as being acceptable. Arsenic in food from the United Kingdom is monitored via the Total Diet Study (95), in which analysis is made of composite samples of all the staple foods and a significant proportion of the minor food components. The study covers everything from cereals and meats to curry powder. The weighted average intake in the

UK is 89 μg/day, or 0.62 mg/week. Similar surveys are carried out worldwide, such as the Market Basket Survey in the USA.

None of the regulations make definite provision for the variation in toxicity of arsenic species. Many studies have therefore been directed at determining what compounds are present in foods, particularly those that are derived from marine produce as these, as already noted, often contain arsenic in excess of 1 mg kg^{-1}. A wide range of edible algae (77; 79), marine crustacea (57; 61; 63; 65) and fish (61; 63; 68; 69) have been surveyed and the predominant form of arsenic identified as non-toxic arsenosugars or arsenobetaine. To date very little evidence has been accumulated as to what species comprise the rest of these animals arsenic burden, or what affect, if any, the human digestive system has on speciation.

The increasing popularity of 'health' foods, vegetarianism and veganism has focussed attention on the diet supplements that devotees of such eating habits ingest to maintain adequate mineral intakes. These supplements are often manufactured from kelps which themselves contain high levels of arsenic and iodine. Norman et al. (110) analysed a number of such formulations and found that in every case, except one, reducible arsenic, which was considered a suitable indicator of dietary risk, was below 1 mg kg^{-1}. One product, sold in Australia, contained 50 mg kg^{-1}.

Normal dosing with this product would exceed recommended intakes.

As previously noted, (97), wine can contain relatively high levels of arsenic, possibly as a result of herbicide application to grapes and vineyard soils. Aguilar et al. (111) determined arsenate, arsenite, MMAA and DMAA in musts, rosè and red wines from Mentrida, Spain. In all cases the concentrations were very low; the highest recorded levels being arsenite - 4.0 $\mu g\ l^{-1}$, arsenate - 6.2 $\mu g\ l^{-1}$, MMAA - 0.4 $\mu g\ l^{-1}$ and DMAA - 5.3 $\mu g\ l^{-1}$. There was some evidence that fermentation during red wine production leads to an increase in arsenite and a decrease in arsenate and DMAA.

The increasing use of arsenic containing food additives for poultry, swine and sheep has generated a number of studies on whether this leads to elevated levels in the animal tissues (48; 49). Recently Dean (112) speciated the growth promoter roxarsone in chickens and found roxarsone concentrations in leg meat of three of the birds to be between 15 and 18 $\mu g\ kg^{-1}$ fresh weight. Total arsenic in both leg and breast of the chickens was in the range 39-355 $\mu g\ kg^{-1}$ fresh weight.

An increasingly large proportion of the human diet is occupied by plant derived foodstuffs. Numerous papers exist which detail arsenic concentrations in fruit and vegetables,

but a remarkably small proportion of the literature details the species present, especially considering the previously widespread application of arsenical pesticides.

Normal arsenic levels in plants rarely exceed 1 mg kg^{-1}, a consequence of the binding of arsenic to metal oxides in the soil. Arsenic absorption occurs via the leaves or the root system. The uptake of arsenic is partially determined by its form and element availability. Following absorption arsenic is rapidly translocated throughout the plant, either symplastically (cell cytoplasm-to-cytoplasm transport) or apoplastically (extracellular transport). The generalised route is root → xylem → leaves → phloem and leaves → phloem → roots, tops and xylem. The rate and mode of translocation are dependent upon the arsenic species. This is partly the reason that rapidly translocated methanearsonates are preferred as contact herbicides over inorganic herbicides, such as sodium arsenite. The evidence for these theories has been reviewed (113-115).

As previously indicated natural arsenic concentrations in terrestrial plants lie in the range 0.1-10 mg kg^{-1} (113; 114). Contamination can lead to much higher concentrations and grasses growing on spoil heaps at arsenic mines were found to contain over 1000 mg kg^{-1} of arsenic (116). That plant species do vary markedly in their arsenic tolerance has been firmly established (115).

A number of authors (115; 117) have highlighted the need for speciation techniques to be applied to plants, but the paucity of data in the literature remains.

Pyles and Woolson (118) surveyed crops grown on soil treated with 100 mg kg^{-1} of arsenic. They found total arsenic ranged from <0.01-3 mg kg^{-1}, the highest levels being recorded in potato peel. A methanol/water extraction was applied to the crops and arsenate or MMAA identified in lettuce, potato flesh, potato peel and Swiss chard. The available evidence indicated that the majority of the arsenic in the extract was present as a complex organic molecule. In tomatoes they found arsenic to predominate as a non-polar lipid material.

O'Neill and Bancroft (119) speciated arsenic in crops from the south-west of England and found arsenite, arsenate and traces of MMAA in spring onion, tomato, beetroot and parsnip, and additionally traces of DMAA in spring onion and tomato. The concentrations of the species were not reported, only the relative abundances.

1.5 Aims of the Study

Devon and Cornwall are historically linked with arsenic mining (120) and soils at the mines and in the surrounding areas are known to contain relatively high levels of arsenic (21). The mines have now fallen into disuse and the sites

are employed in some instances as market gardens.

The aim of this study was to develop suitable analytical procedures for the determination of total arsenic and arsenic species in a range of food crops grown on soils from former mines in which elevated levels of arsenic had been identified.

These analytical methods would also be applied to fish caught from the seas around the south-west of England. There is considerable data on total arsenic in fish caught around the United Kingdom (95) but data on the actual forms of arsenic is scarce. Using the results from the analyses of the various foods dietary risk posed by arsenic would be assessed.

A dietary trial was also proposed whereby volunteers would be asked to eat a number of meals, over a period of two days, known to contain naturally occurring arsenic. The volunteers would be asked to collect sub-samples of all urine passed during the trial and these samples would be analysed to determine if the human metabolism transforms arsenic species. Arsenic in the food would be compared to arsenic output in urine to identify whether or not tissue accumulation occurs.

Throughout the study a high priority would be given to quality control by use of certified reference materials

(CRM's), blind spiking trials etc.

The techniques and protocols will be described in the forthcoming pages.

2. COUPLED LIQUID CHROMATOGRAPHY-ATOMIC SPECTROMETRY

2.1 Introduction

An obvious approach to trace metal speciation in non-volatile forms is to couple the separatory powers of liquid chromatography, principally high performance liquid chromatography (HPLC), with the inherent selectivity of atomic spectrometry. The following sections describe the various components of such combinations. In addition instrumentation used for determining total arsenic in the study is described. Finally a review of research citing the use of coupled techniques for speciating arsenic is presented.

2.2 Instrumentation

2.2.1 High Performance Liquid Chromatography

High performance liquid chromatography (HPLC) involves the separation of mixtures of solutes in, typically, ng-mg quantities by passing the sample solution through a column, typically 5-30 cm in length and 0.4 cm in diameter, packed with a solid stationary phase. The solution is pushed through the column by a pressurised flow of a liquid mobile phase. Flow rates are normally 0.1-4.0 ml min^{-1}. The nature of the interaction between the stationary and mobile phase allows several separation mechanisms to be utilised:

(a) Adsorption Chromatography. Molecules bond to the surface of the solid stationary phase by dipole-dipole interactions. The strength of this interaction varies

depending on the substance and thus the retention time varies, effecting separation of the solutes. Polar, non-ionic compounds are separated by liquid-solid adsorption chromatography.

(b) Partition Chromatography. This is the most common separation mechanism. Both the stationary and mobile phase are liquids and separation is achieved by the different partition coefficients of the solutes in the sample. In normal phase partition, used for very polar organic solutes, the stationary phase is more polar than the mobile phase. In reversed phase, for non-polar or weakly polar compounds, the mobile phase is more polar than the stationary phase. The stationary phases are either coated or chemically bonded onto a support.

(c) Ion-exchange Chromatography. This is used for separating ionic compounds, and involves migration between acidic or basic functional groups, bonded to a resin or silica gel polymeric matrix, and a mobile phase containing charged species.

(d) Size Exclusion Chromatography. Also known as gel permeation chromatography, this mechanism features a porous stationary phase permeated by mobile phase molecules. Small solute molecules enter the pores and are retarded. Larger molecules are excluded.

Migration is thus size dependant. Size exclusion chromatography is used for polymers and oligomers.

Qualitative information in HPLC is obtained by comparing the retention times of compounds in the sample mixture with the retention times of injections or co-injections of pure standards. Quantitative information is found by connecting the column outlet to a range of detectors, examples of which are described in the following sections.

Numerous texts have been published which detail the development and fundamentals of HPLC (121; 122) and the various separation mechanisms.

2.2.2 Atomic Absorption Spectrometry

Atomic absorption spectrometry (AAS) is one of the best established, well documented and widely used of all modern analytical techniques (123; 124).

Atomic absorption involves the absorption, by free atoms of an element, of electromagnetic radiation (energy) of a wavelength specific to that element. The valence electrons of metals and metalloids can be excited from their ground state to higher energy levels by photons in the wavelength range 190-900 nm. In general ground state populations are greater than those in higher energy levels, hence absorption is greatest for resonance lines which give transitions from the ground state.

For individual elements, the energy levels occupied by the electrons are characteristic of that element. If light of a narrow enough fixed wavelength is passed through an atom cloud, only atoms of one particular element will absorb photons. Hence the selectivity of AAS.

An essential instrumental feature of AAS, the development of which by Walsh (125) lead to the commercial realisation of AAS, is the hollow cathode lamp. This overcame the practical difficulty of how to isolate narrow atomic lines, typically 0.002 nm, from the continuum. The lamp cathode is constructed from the element of interest and a low pressure discharge produced within the cathode. The filler gas, normally neon or argon, sputters atoms from the cathode. The emission spectrum so produced contains lines from the cathode element. As atomic absorption and emission lines have the same wavelength the narrowness of the absorption line is a major advantage - it precludes the overlap of the absorption line of one element on the emission line of another. The selectivity of AAS is due to this 'lock and key' effect.

In outline, the operation of the flame AAS instrument involves the aspiration of the sample solution into a flame, typically an air/acetylene flame, to produce an atomic vapour. Radiation from the lamp passes through an atom cloud and into a monochromator with a band pass of 0.2-5 nm

and the signals are amplified by a photomultiplier tube and passed to a digital display. Absorption is proportional to analyte concentration.

A number of alternatives to the flame as atom cell have been described, the two most popular being described below.

2.2.2.1 Graphite Furnace Atomic Absorption Spectrometry

There are a number of disadvantages using flames as atom sources such as dilution effects, the requirement for large sample volumes and the inability to analyse solid samples. One alternative is to use atomisation by thermal means following resistive heating - electrothermal atomisation. A number of furnace designs have been suggested by such as King, L'vov, Massman and West (123). Commercially available furnaces are based on the Massman design. The advantages of graphite furnace AAS (GFAAS) are:

(1) high sensitivity; limits of detection are in the ng-μg l^{-1} range. The higher sensitivity of the furnace compared to flame AAS is because it avoids the inefficient nebulisation step, and because the atoms are maintained within the furnace rather than being diluted and rapidly transported from the optical path by a flame;

(2) Only small samples (5-20 μl) are required; this is very useful in biological and clinical studies where only a few ml of samples such as blood and serum are available;

(3) Solids can be directly analysed.

The main disadvantages are:

(1) the need for drying and ashing stages means it takes several minutes for each analysis;

(2) unless an autosampler is used precision is rarely better than in the range 2-5%;

(3) the only way of increasing the dynamic range (of about 1 order of magnitude) is the use, if available, of an alternative resonance line;

(4) increased cost due to the increased complexity of the instrument compared to flame AAS.

Numerous texts discuss the fundamentals of GFAAS (124, 126).

2.2.2.2. Hydride Generation

Some elements, e.g. antimony, arsenic, bismuth, germanium, selenium, tellurium and tin, form gaseous covalent hydrides.

The favoured reducing agent is sodium borohydride, usually 1-4% m/v, because of the rapid formation of the hydrides. A major advantage of hydride generation is gaseous sample transport is possible, which leads to an improvement in detection limits for most of the above elements as it avoids the inefficient nebulisation processes.

Hydride generation can be in batch or continuous mode. The acidified sample reacts with the aqueous sodium borohydride, the gaseous sample hydride is then flushed by co-generated hydrogen plus argon or nitrogen from a gas liquid separator along a transfer line to the atom cell which may be of several designs (123):

(1) A flame, typically an argon-hydrogen diffusion flame. This is advantageous as the argon-hydrogen flame only absorbs 15% of the incident radiation, compared to the conventional air-acetylene flame which absorbs 62%.

(2) A narrow silica tube externally heated by an air-acetylene flame.

(3) A narrow silica tube externally heated by the passage of electrical current through a suitable resistance wire wrapped around the tube.

The arsenic +3 valence state gives a better response than

the +5 state therefore samples are often reduced with potassium iodide prior to analysis (123).

The use of hydrides in atomic absorption spectroscopy has been extensively reviewed (127).

2.2.3 Inductively Coupled Plasma-Mass Spectrometry

The coupling of an argon inductively coupled plasma (ICP) to a quadrupole mass spectrometer has recently been commercially realised, following the development work of Date and Gray (128) and others.

The main stages are:

(1) ion production in the ICP;

(2) ion extraction from the interface region;

(3) separation and measurement of the ionic species by the mass spectrometer.

The plasma is initiated by an electrical discharge from a small Tesla coil. This seeds the argon gas, flowing in a Fassel torch, with electrons which accelerate in the AC magnetic field produced by a radio frequency generator delivering 1.2-1.8 kW at 27.12 MHz from an induction coil. The electrons rapidly reach ionizing energies and collisions with the gas atoms produce an avalanche effect. The magnetic field causes the ions and electrons to flow in

closed circular horizontal paths. These eddy currents heat the neutral argon by collisional energy exchange and a fireball is produced. Pneumatic nebulisation is conventionally used to introduce aqueous samples and the aerosol so produced is punched into the bottom of the plasma producing an annulus. Beyond the induction coil the sample vapour enters the excitation zone of the plasma. The temperature here is in excess of 7000 K and provides enough energy to ionise significantly all the elements of the periodic table except He, F and Ne, whose first ionisation energy exceeds that of argon at 15.7 eV.

Ion extraction occurs at the interface which consists of two nickel cones, known as the sampler and skimmer cones, with apertures of 1.0 and 0.7 mm respectively, located on the axis of the plasma torch which itself is mounted horizontally. The pressure between the cones is maintained at 100 Pa by a rotary pump, and further reduced by a diffusion pump behind the skimmer, in the intermediate chamber to 0.1 Pa. Excited species are continuously extracted from the 1.5-3.0 mm core of the plasma. In the zone between the cones the rapid increase in mean free path freezes reactions between species, giving representative sampling. However, there is evidence that gas reactions occur here, giving polyatomic and oxide species, two of the major sources of interference in ICP-MS. The bulk of the gas is pumped away but about 10^{-4}% passes through the skimmer into the mass analyser.

The mass analyser is maintained by a further diffusion pump at 1-2 x 10^{-4} Pa. The ion lens directs the ion beam along the central axis of the quadrupole. Within this there is a two dimensional oscillating electrical field. At a given set of voltages only ions of a certain mass:charge ratio have stable trajectories and hit the electron multiplier (the detector). The whole mass spectrum can be analysed in 30 ms, although typically scan times of 100-1000 ms are used.

The great complexity of the instrumentation means that the inductively coupled plasma-mass spectrometer is a high cost instrument. This is its principal disadvantage. The advantages are high sensitivity, rapid multi-element analysis, the potential for isotope studies and a long linear working range, potentially eight orders of magnitude with both a pulse and analogue detector.

2.3 Coupled Techniques for Arsenic Speciation

To quantify a range of chemically different compounds it is obvious a powerful separation and a sensitive detection technique used in tandem are required. For trace element speciation the favoured approach is to couple the separatory powers of chromatography with the selectivity and sensitivity of atomic spectrometry as the means of detection. The linking of gas (129), liquid (130) and

chromatography in general (131) to atomic spectrometry have recently been reviewed. The coupled techniques that have been applied to arsenic speciation are outlined below.

2.3.1 Gas Chromatography-Atomic Absorption Spectrometry

In general most important arsenic species are not volatile enough to be chromatographed directly although exceptions exist e.g. Limentani and Uden (132) described a gas chromatography (GC)-microwave induced plasma (MIP) method to determine a range of arsoles and arsines. However, normally a derivitisation step is required, the most popular of which is to form the volatile gaseous hydride by acidifying aqueous solutions of the sample and then reacting them with sodium borohydride. This technique was first described by Andreae (84) who collected the arsines after volatilisation in a liquid nitrogen cryogenic trap. The arsines were then separated by either allowing the trap to warm slowly which gave a sequential separation based on the boiling points of the arsines or by switching the carrier stream of a GC to the nitrogen trap which was rapidly warmed by immersion in hot water. This technique has been used by a large number of authors (45; 98; 133; 134;). Initially detection was by conventional flame AAS but Ward (135) recognised that for true trace level analysis the residence time of atoms must be prolonged. This he achieved by using a flame heated ceramic tube suspended over a flame in various configurations. Van Cleuvenbergen et al. (136) took this idea a step further by using a quartz furnace as the atom

cell. Five arsenic compounds were speciated in environmental water samples.

The selective volatilisation of arsines from a cryotrap have also been used with d.c. plasma emission spectrometry (5) and a MIP (137) as the means of detection.

Another derivatisation approach for the analysis of DMAA and MMAA is to form the volatile methylthioglycolate derivatives (138). However Edbon et al. (139) in a comparative study of a number of coupled techniques noted that the sensitivity was not suitable for environmental analysis, the detection limit being of the order of 10 μg l^{-1}. Furthermore Haraguchi and Takatsu (140) noted that the derivatisation reaction was of too low an efficiency to be applicable to trace analysis and that the derivative showed some decomposition in the GC column.

2.3.2 High Performance Liquid Chromatography-Hydride Generation-Atomic Absorption Spectrometry

Hydride generation (HG) is used with HPLC/AAS systems to overcome the poor nebulisation and transport efficiency of AAS. Additionally the separation of volatile hydrides from the sample matrix reduces interferences in the flame. Typically the HPLC column eluate is acidified, reacted with sodium borohydride and after gas-liquid separation the hydrides are burnt in a flame heated silica tube or an

inverted 'Y' glass burner or are electrothermally atomised in a quartz cell (139).

Examples of papers that have been published detailing HPLC-HG-AAS are given in Table 2.1.

HPLC-HG can also be coupled to an ICP to overcome the poor transport and nebulisation efficiency of the ICP, typically 1-2%. Bushee et al. (145) described just such a coupling for determining arsenate, arsenite and DMAA in well water samples. They used a range of C_{18} columns in conjunction with tetrabutylammonium phosphate as the mobile phase. The gaseous products were passed directly to the nebuliser of the plasma. The limitation of this , and any other technique involving hydride generation is that not all arsenic species are reducible e.g. arsenobetaine. Recently Kaise et al. (146) described a HG-GC-Mass spectrometry method in which the samples were first subjected to alkaline hydrolysis (2M NaOH, 85°C, 3 hours) prior to introduction to the hydride generator. Quantitative conversion of arsenobetaine to trimethylarsine oxide was observed, although some organoarsenicals were still unchanged.

2.3.3 High Performance Liquid Chromatography-Inductively Coupled Plasma-Atomic Emission Spectrometry

The attraction of this coupling is that no complicated interfacing is required. The column outlet of the HPLC system is connected to the uptake capillary of the ICP,

TABLE 2.1 HPLC-HG-AAS for Arsenic Speciation

Chromatography	Sample	Comments	Ref
Column - Dionex anion precolumn, 15 x 3 mm and Dionex anion separator, 500 x 3 mm i.d. Mobile Phase - 2.4 mM $NaHCO_3$/1.9 mM Na_2CO_3/1 mM $Na_2B_4O_7$ switched to 5 mM $Na_2B_4O_7$. Both delivered at 2.6 $mlmin^{-1}$.	Arsenite, arsenate, MMAA, DMAA and p-aminophenyl arsonate, in air. No results for real samples presented.	Detection by quartz furnace at 800°C and EDL at 193.7 nm. Blank acid saturated with potassium persulphate. Re-equilibration required after 10-15 samples.	141
Column - 3μm Hypersil ODS, 250 x 5 mm i.d. Mobile phase - 1.8×10^{-5} M H_2SO_4 at 0.5ml min^{-1} for 5 minutes then 1.5 $mlmin^{-1}$.	Arsenite, arsenate, MMAA and DMAA standards. No real samples analysed.	Inverted 'Y' glass burner with H_2/Ar flame was used for detection. L.O.D. for each species was < 50 ppb.	135
Column - 40 μm Zipax anion exchange, 100 x 5 mm i.d., followed by 5 μm Benson anion exchange resin, 200 x 5 mm i.d. Mobile phase - 10^{-4}% H_2SO_4 at 4 $mlmin^{-1}$ used for loading, followed by 0.1 M $(NH_4)_2CO_3$ at 4 $mlmin^{-1}$.	Arsenite, arsenate, MMAA and DMAA in bottled waters, and soil waters.	Detection by silica tube heated by an air/acetylene flame. Wavelength was 193.7 nm.	142

Chromatography	Sample	Comments	Ref
Column - 50 μm silica saturation column, 250 x 4.6 mm i.d., followed by 10 μm Nucleosil SB, 250 x 4.6 mm i.d. Mobile phase - 0.05M phosphate buffer, pH 4.5 delivered at 1.1 ml min^{-1}.	Arsenite, arsenate and DMAA in the liver of male Wistar rats.	Same detection system as (141). L.O.D. between 25-35 ng.	143
For Seaweed Analysis: Column - Nucleosil 10SB anion exchange, 250 x 0.4 mm i.d. Mobile Phase - 50 mM phosphate buffer, pH 6.75, at a flow rate of 0.5 ml min^{-1}.	Arsenite, arsenate, MMAA and DMAA in seaweed extract and mouse liver.	Detection by a quartz absorption tube heated by an air/acetylene flame. Eluate acidified by HCl or 10% oxalic acid. Ligand and protein bound arsenic species were detected in the mouse liver.	144
For Liver Analysis: Column - TSK GEL G3000SW, 600 x 7.5 mm i.d. Mobile Phase - 50 mM Tris-HCl buffer, pH 7.2, at 1 ml min^{-1}.			
Column - 1.5 cm guard column of RP18 followed by 10 μm Ionospher anion exchange, 250 x 0.45 mm i.d. Mobile Phase - 0.03M phosphate buffer, pH 6.2, at 1.7 ml min^{-1}.	Arsenite, arsenate, MMAA and DMAA in human urine.	Quartz furnace at 900°C used for detection. Arsenic EDL used as source. A conditioning column was used to saturate the mobile phase with silica and thus prolong the lifetime of the analytical column.	103

which is of approximately the same flow rate as HPLC mobile phases. The principal drawback is that ICP-AES does not have the required sensitivity, particularly at the arsenic wavelengths. However a number of studies have been performed using this coupling. Some examples are given in Table 2.2.

There are a number of other reports in the literature, e.g. (64; 71) in which ICP-AES was used as the HPLC detector, but these papers tend to concentrate on sample preparation and results and give little or no details of the coupling. Irgolic and Stockton (153) and Irgolic and Brinckman (154) have reviewed the ICP as an element specific detector and compared performance with other techniques, particularly HPLC-GFAAS. Uden (155) has published a general overview of coupled chromatography-plasma emission.

2.3.4 High Performance Liquid Chromatography - Graphite Furnace Atomic Absorption Spectrometry

This technique has proved popular as it offers the required sensitivity for arsenic analysis, and because it responds to all arsenic species. A fairly complicated interface is usually required to introduce the sample into the cuvette. The major problem is that real time detection is impossible due to the furnace cycle and hence discontinuous chromatograms are given. Furthermore, only small volumes, typically 20-50 μl, can be introduced into the furnace and thus very slow flow rates need to be used, at most being 0.5

Table 2.2 HPLC-ICP-AES for the Determination of Arsenic Species

Chromatography	Sample	Comments	Ref
Column - Bondapak, 10 μm Nucleosil $N(CH_3)_3$ and 10 μm Nucleosil SO_3H separately packed into 300 x 4.0 mm columns. Mobile Phase - phosphate, borate, Tris-HCl, NaCl and tributyl ammonium phosphate at various flow rates.	Arsenite, arsenate, MMAA, DMAA and arsenobetaine determined in seaweed.	Arsenic monitored at 193.7 nm; Power 1.1 kw; Coolant 18 $lmin^{-1}$; nebuliser 0.5 $lmin^{-1}$. Best separation with Nucleosil $N-(CH_3)_3$ and 0.05 M phosphate buffer.	147
Column - Nucleosil - $NH(CH_3)_2$, 250 x 4 mm i.d., plus guard column with same material. Mobile phase - 2mM ammonium dihydrogen phosphate and 5mM ammonium acetate, pH 4.6, switched to 80 mM ammonium dihydrogen phosphate, pH 6.9. Both delivered at 1.4 ml min^{-1}.	Arsenite, arsenate, selenite and selenate as aqueous standards.	Arsenic monitored at 228.8 nm; 1.05 kw power; whole system under computer control. Sensitivity was insufficient for analysis of biological samples.	148
Column - Bio-Rad Aminex A27 anion exchange resin, 100 x 8 mm cartridge. Mobile phase - 100% water to 0.5 M ammonium carbonate linear gradient. Column held at 45°C by a water bath. Flow rate 1 ml min^{-1}.	Arsenite, arsenate, MMAA and DMAA in calf serum culture medium.	Arsenic monitored at 197 nm, 1.25 kw power; coolant 14.5 $lmin^{-1}$, auxiliary 0.4 $lmin^{-1}$; nebuliser 0.5 $lmin^{-1}$; viewing height 15mm.	149

Chromatography	Sample	Comments	Ref
Column - Hamilton PRP-x100 anion exchange, 250 x 4.1 mm i.d. Mobile phase - 10 mM ammonium carbonate and 2.5 mM sodium sulphate, pH 8.2, flow rate 2 ml min^{-1}.	Arsenite, arsenate, MMAA and DMAA in urine and synthetic solutions containing high chloride concentrations.	Arsenic monitored at 228.8 nm; 0.9 kw power; nebuliser 1.0 $lmin^{-1}$, viewing height 14-18 mm. A model is proposed to explain the effect of chloride on peak splitting.	150
Column - Partisil 5 ODS-3, 250 x 4.6 mm i.d. Mobile phase - 5mM tetrabutyl-ammonium phosphate, flow rate 0.7 ml min^{-1}.	Arsenite, Arsenate, MMAA, DMAA and benzenearsonic acid in shale oils and coals.	Arsenic monitored at 193.7 nm; 1.8 kw power; 17 $lmin^{-1}$ coolant; auxiliary 0.5 $lmin^{-1}$ nebuliser 0.2 $lmin^{-1}$; auxiliary nebuliser flow 0.6 $lmin^{-1}$. A direct injection nebuliser was used to improve detection limits. The HPLC eluate was split so only 15% passed to the nebuliser.	151
Column - Nucleosil 10SA cation exchanger or Nucleosil 10SB anion exchanger packed into 250 x 4.6 mm i.d. column. Mobile phase - anion exchanger: 20 mM phosphate buffer, pH 7.0, 1.0 ml min^{-1} cation exchanger: 100 mM pyridine - formate buffer pH 3.1, 1.0 ml min^{-1}.	Arsenite, arsenate, MMAA, DMAA, arsenobetaine and arsenocholine in shell-fish.	Arsenic monitored at 193.7 nm, 1.2 kw power; viewing height 16 mm. Best results obtained with anion exchanger.	152

ml min^{-1}. Obviously long run times are common, often of greater than two hours. The repeated firings of the furnace means it is not uncommon for cuvettes to fracture. Some reported HPLC-GFAAS couplings are detailed in Table 2.3. Additionally Weiss et al. (165) have described the application of HPLC-GFAAS to determining arsenic species in fossil fuels and Irgolic et al. (166) has included HPLC-GFAAS amongst other strategies for determining arsenic in water supplies.

In Table 2.3 references (158; 161; 162) are not true coupled techniques as fraction collection following column chromatography was used. However, they have been included for completeness.

2.3.5 High Performance Liquid Chromatography-Inductively Coupled Plasma-Mass Spectrometry

This coupling is becoming increasingly popular and is likely, where affordable, to be one of the speciation techniques of the future. No complicated interfacing is required, HPLC flow and ICP-MS uptake rates are compatible and high sensitivity to all arsenic species is attainable. Another major advantage of the ICP-MS, and one of the reasons it was used in preference to GFAAS later in the study is that being a real-time detector continuous chromatograms are given. Thus calculation of results is both easier and more reliable since all of the signal arising from the analyte is recorded. Additionally the reported sensitivity of HPLC-ICP-MS couplings (70; 167; 168)

Table 2.3 HPLC-GFAAS for the Determination of Arsenic Species

Chromatography	Sample	Comments	Ref
Column - Lichrosorb 10 μm C_{18} RP, 250 x 4.6 mm i.d. Mobile phase - Methanol at 0.12 ml min^{-1}	Triphenylarsine as aqueous standard plus tin, mercury and lead species.	A PTFE flow through cup was used in the 'pulse' mode or in the 'survey' mode the eluent was collected and analysed via the autosampler. Arsenic monitored at 193.7nm; Dry - 80°C, 15s; Atomise - 2700°C, 5s. 10-50 μl samples.	156
Column - 10 μm Bondapak C_{18} RP. Dimensions not given. Mobile phase - 95/5/6 H_2O/ acetonitrile/acetic acid with 5 mM heptanesulfonic acid, or 2/3/0.6 H_2O/acetonitrile/acetic acid with 5 mM dodecylbenzenesulphonate, stepped to 100% acetonitrile after 17 minutes. All flow rates 0.5 ml min^{-1}.	Mixed standards of inorganic arsenic, arsenocholine and arsenobetaine.	An interface for Zeeman effect GFAAS was constructed which consisted of a pneumatically actuated slider injection valve with 40 μl loop, under automatic sequence control. Run time approximately 30 minutes.	157
Column - AG50W-X8 cation exchange column, 250 - 300 x 10 mm i.d. Eluant - 30 ml of 0.2M trichloroacetic acid followed by 70 ml of 1M ammonium acetate. System pressurised to reduce analysis time.	Arsenite, arsenate, MMAA and DMAA in river water and sediments.	Samples analysed off-line by fraction collection. Dry - 105°C, 30s; ash - 1000°C, 25s; atomise - 2600°C, 7s. 10 μl injections. L.O.D. = 2$\mu g l^{-1}$.	158

Chromatography	Sample	Comments	Ref
Column - 10 μm Bondapak C_{18} RP, 300 x 4 mm i.d. and 10 μm strong anion or cation exchange, 250 x 3.2 mm i.d. Mobile phase - aqueous acetate buffers were used with the ion exchange columns and water/ methanol mixtures plus tetra-heptylammonium nitrate with the C_{18} column, all at 0.5 ml min^{-1}.	Arsenite, arsenate, MMAA and DMAA in soil extracts and drinking water.	The autosampler method described in (156) was used, as was the interface method described in (157). Problems were encountered with calibration, due to differing atomisation efficiencies of the species. Typical run time was 60 mins. L.O.D. approx. 5 $\mu g l^{-1}$.	159
Column - Glass Dionex anion exchange, 250 x 3 mm i.d. with Dionex guard column, 150 x 3 mm i.d. Mobile phase - 80/20 water/ methanol gradient eluted to 85/15 20 mM ammonium carbonate /methanol, both at 1.2 $ml min^{-1}$.	Arsenite, arsenate, MMAA and DMAA in soil extracts.	The column was coupled to the furnace by an automatic sampling unit consisting of a PTFE flow-through cup. LOD was approx. 250 $\mu g\ l^{-1}$. 20 μl samples were used. Run time approximately 35 min. Dry - 110°C, 8s; ash - 1200°C, 7s; Atomise - 2500°C, 8s; cool - 20s.	160
Column - Dowex 50W-X8 and Al-X8 resins. Cation exchange column was 160 x 10 mm and the anion exchange was 100 x 10 mm. Eluate - Each species required separate elutor system.	Arsenite, arsenate, MMAA and DMAA in aqueous standards.	Each species was collected and analysed by GFAAS separately. Arsenite was determined by difference.	161

Chromatography	Sample	Column	Ref
Column - 9 cm of Bio-Rad AGi-X8 anion exchange resin and 26 cm of Bio-Rad AG50 W-X8 cation exchange resin in a 350 x 10 mm i.d. column. Eluate - 50 ml of 6mM trichloroacetic acid (TCA) then 8 ml of 200 mM TCA at 2 ml min^{-1} then 55 ml of 1.5 M ammonium hydroxide then 50 ml of 0.2 M TCA each at 6 ml min^{-1}.	Arsenite, Arsenate, MMAA and DMAA in lake waters and sediment interstitial water.	Samples were analysed by GFAAS following fraction collection. Good resolution of species in the samples was obtained. Detection limit was 10 $\mu g\ l^{-1}$. Injection volume was 10-100 μl.	162
Column - Bio-Rad Aminex A-27 anion exchange resin in cartridge. Mobile phase - water gradient eluted to 100% 0.2M ammonium carbonate, both at 1.2 ml min^{-1}.	Arsenite, arsenate, MMAA, DMAA and trimethylarsine oxide in fruit and vegetables.	A PTFE flow-through cup under autosampler control was used for interfacing. Dry - 160°C, 7s; ash - 1250°C, 7s and atomise 2400°C, 5s. All 5 species separated in under 30 mins.	118
Column - 3-5 μm Hypersil ODS RP column, 250 x 4 mm i.d. Mobile Phase - isocratic elution with 1.8 x 10^{-5} M sulphuric acid.	Arsenite, arsenate, MMAA and DMAA in soil pore water.	A commercial GFAAS was modified so that a syringe controlled by a solenoid and nitrogen pressure could be mounted on the face plate. A nitrogen lance was used to increase cooling rate. Dry - 175°C, 10s; ash - 270°C, 5s; atomise - 1900°C, 5s. The chromatography was found to be unreliable.	21

Chromatography	Sample	Comments	Ref
Column - 10 μm Lichrosorb RP-1, ODS, 250 x 4.6 mm i.d. Mobile phase - 1mM Hexadecyltrimethylammonium bromide, pH 9.5, at 0.4 ml min^{-1}, switched to 6/4/1 H_2O/methanol/ acetonitrile at 0.5 ml min^{-1}.	Arsenite, arsenate, MMAA and DMAA in synthetic river water.	The interface was described in ref. 157 and full construction details are in ref. 51. The four species were separated inside 50 mins. LOD was 15 ng As for 100 μl injection.	163
Column - 3-5 μm Hypersil ODS, RP column. Mobile phase - 0.1M ammonium formate.	Arsenite, MMAA and DMAA in aqueous standards.	The computer controlled interface described in (21) was used. This paper principally describes all the electronics and software commands necessary to construct the interface.	164

mean that it is suitable for environmental analysis without recourse to preconcentration. The major practical difficulty is an isobaric overlap at 75 u between monoisoptopic ^{75}As and $^{40}Ar^{35}Cl$. Reported HPLC-ICP-MS couplings are summarised in Table 2.4.

2.3.6 Other Coupled Techniques

The techniques described in the sections above are not the only approaches to arsenic speciation e.g. Maruo et al. (170) described the application of microbore HPLC with ultraviolet detection for determining organoarsenic acids. The lack of publications on a technique is usually an indication that it is esoteric in its applications, or limited in sensitivity for real samples. Techniques where a mass spectrometer is the final detector have considerable potential as they have high sensitivity and offer unequivocable species identification. The determination of arsenocholine by pyrolysis GC-MS (75) and arsenocholine and arsenobetaine by fast atom bombardment tandem mass spectrometry (74) have been reported.

Table 2.4 HPLC-ICP-MS for the Determination of Arsenic Species

Chromatography	Sample	Comments	Ref
Column - 5 μm Econosphere C_{18}, 250 x 4.6 mm i.d. Mobile Phase - 5% Methanol, 5mM PIC-A, at 1.0 ml min^{-1}.	Arsenite, arsenate, MMAA and DMAA plus other elements as aqueous standards.	Stainless steel tube used to interface column with ultrasonic nebuliser. Power - 1.2 kw; coolant - 13 $lmin^{-1}$; auxiliary - 0.8 $lmin^{-1}$; nebuliser - 1.2$lmin^{-1}$. Arsenite and MMAA unresolved. Detection limit below 200 pg for all species.	167
Column - Pharmacia Pep RPC 5/10, RP. Mobile phase - 5/95 methanol/ 10mM phosphoric acid, pH 2.5, delivered at 1 ml min^{-1}.	Roxarsone in chicken meat.	The interface was direct coupling of the column outlet to the ICP nebuliser. Roxarsone was separated from other unidentified arsenic species and chloride. Instrumental parameters not given.	112
Column - 5 μm Pierce C18 cartridge, 300 x 4.6 mm. Mobile Phase - 10 mM sodium dodecyl sulphate, containing 5% methanol and 2.5% glacial acetic acid, flow rate 3 ml min^{-1}.	Arsenite, arsenate, MMAA, DMAA, arsenobetaine and arsenocholine in dogfish reference material.	Interfacing by direct coupling between column outlet and nebuliser. Power - 1.4 kw; data collected by single ion monitoring at 75 m\z. LOD (arsenobetaine) = 300 pg as arsenic.	70
Column - 5 μm Adsorbosphere anion exchange, 250 x 4.6 mm i.d., plus guard and saturation column. Mobile Phase - 15mM ammonium dihydrogen phosphate/ 2mM ammonium acetate containing 30% methanol, pH 7.5.	Arsenite, arsenate, MMAA and DMAA in urine.	Coupling as above. 2% oxygen bled into the nebuliser to decrease carbon build-up. Power 1.5 kw; coolant-17 $lmin^{-1}$; auxiliary - 0.5 $lmin^{-1}$, nebuliser - 0.65 $lmin^{-1}$. An aluminium sampler cone was required to overcome pitting and blocking. LOD between 20-40 pg. Problems encountered with chloride interference.	104

Chromatography	Sample	Comments	Ref
Column - Asahipak GS220 gel-permeation, 500 x 7.6 mm, or Intersil ODS-2, RP, 250 x 4.6 mm. Mobile Phase - various, all using tetraethyl or tetramethyl ammonium hydroxide.	Fifteen arsenic species including arsenite, arsenate, MMAA, DMAA, arsenobetaine and six different arsenosugars in urine.	Coupling as above. Power - 1.3 kw; coolant - 13 $lmin^{-1}$; auxiliary - 0.5 $lmin^{-1}$; nebuliser - 0.78 $lmin^{-1}$. LOD approx 20-50 pg As.	168
Column - various, including C_{18}, anion exchange and cation exchange. Mobile Phase - Various, for anion exchange and for anion and cation ion pairing.	Arsenite, arsenate, MMAA, DMAA and arsenobetaine in a dogfish reference material.	Coupling as above. Power 1.4 kw; coolant - 14 $lmin^{-1}$; auxiliary - 2.0 $lmin^{-1}$; nebuliser - 0.9 $lmin^{-1}$. Paper is a comparison of various separation regimes, and their suitability for ICP-MS.	169

3. SEPARATION OF ARSENIC SPECIES BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

3.1 Introduction

A recent survey of the literature (131) indicates HPLC, rather than GC, separations of arsenic species are favoured. This is because HPLC, unlike gas chromatography, does not require some species to be derivatised before entering the column. A range of HPLC arrangements can be used: reverse phase (157), ion-pairing agents (159), anion exchange (103) etc. The separation often involves switching between two mobile phases (163) or gradient elution (149) to separate the chemically different arsenic species. Low et al. (171) suggested the possibility of switching between two columns, one packed with octadecylsilane (ODS) to separate the organic species, and one packed with an ion exchanger to separate the inorganic species. This study focussed initially on this two column method. Later an ion-exchange resin that had previously been used in the Polytechnic South West laboratories (21) was investigated.

3.2 Materials and Methods

For chromatographic optimisation an HPLC-AAS system that had previously been used for monitoring tributyltin (172) was used. The main feature of the arrangement was a vented capillary that balanced the uptake rate of the AAS with the lower flow rate of the HPLC system. The column packings used were:

Benson strong anion exchange (SAX) resin, 7-10 μm (Benson Co., Reno, NV, USA),
Partisil SAX, 5 μm, and
Hypersil ODS, 5 μm (Whatman, Maidstone, Kent, UK).

The Benson resin was slurry packed into a 125 x 4 mm i.d. stainless steel column and the Partisil and Hypersil were packed into 250 x 4 mm stainless steel columns. A variety of mobile phases was used, composed of solutions containing mixtures of Aristar potassium sulphate, AnalaR ammonium carbonate, AnalaR ammonium dihydrogen orthophosphate, AnalaR ammonium acetate, AnalaR acetic, hydrochloric, nitric, phosphoric and sulphuric acids and Aristar ammonia (all BDH Chemicals, Poole, Dorset, UK).

Two HPLC pumps were used. A Pye Unicam PU4010 (Philips Analytical, Cambridge, UK) with a PTFE switching valve (Omnifit, Cambridge, UK) and a Waters 6000A (Waters Associates Inc., Mass., USA). A Rheodyne 7125 (Rheodyne Inc., Cotati, California, USA) injection valve was used for sample introduction. A Pye Unicam SP9 atomic absorption spectrometer (Philips Analytical, Cambridge, UK) was one of the instruments used for arsenic detection. In addition a VG Plasmaquad 2 ICP-MS (VG Elemental, Winsford, Cheshire, UK) was used, fitted initially with a Meinhard nebuliser but later with an Ebdon high solids nebuliser (PSA Ltd, Sevenoaks, Kent, UK) to overcome minor problems of salt deposition around the orifice. The following standards were

supplied by Prof. K. Irgolic of Texas A & M University: arsenobetaine, arsenocholine and trimethylarsine oxide. Disodium methanearsonate was donated by Dr A Howard of Southampton University. Dimethylarsinic acid was obtained from Sigma (Sigma Chemical Co., Poole, Dorset, UK) and AnalaR grade disodium hydrogen arsenate and sodium arsenite were purchased from BDH (BDH Chemical Co., Poole, Dorset, UK).

3.3 Experimental

3.3.1 Experiments with the Twin Column System

A block diagram of the instrumental arrangement is shown in Figure 3.1.

Initially, a number of different mobile phases of differing ionic strength and pH were investigated, including ammonium dihydrogen orthophosphate, 0.02-50 mM, pH 6.0; 1 x 10^{-4}% sulphuric acid, pH 4.2; potassium hexacyanoferrate (II), 0.04-5 mM, pH 8.0; and ammonium acetate, 0.05-5 mM, pH 7.2. It was observed that the best results were obtained with ammonium dihydrogen phosphate adjusted to pH 6.0 with ammonia. Some typical chromatograms and operating conditions are shown in Figures 3.2 and 3.3. From these results it was apparent that isocratic elution was not ideal. Low concentrations of phosphate were required to separate arsenite, MMAA, DMAA and arsenobetaine. At concentrations above 1 mM phosphate the resolution between arsenite and MMAA was lost. At concentrations below 0.01 mM

Diagram of Instrumental Arrangement

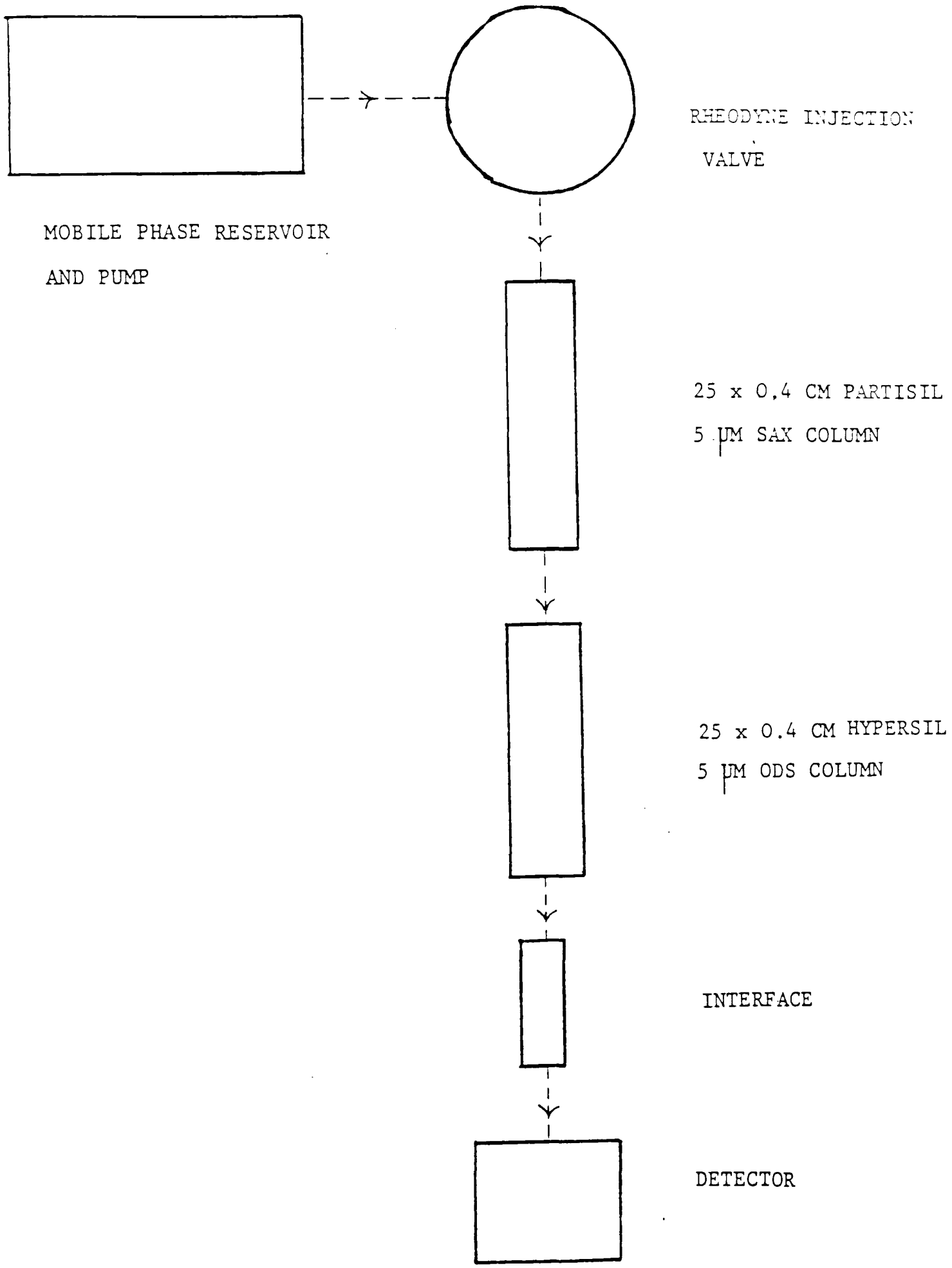


Figure 3.2 Separation of a 250 ul mixture of 5 Arsenic Species. Mixture contained 50 mg As l^{-1} of each species. Mobile Phase 0.04 mM $NH_4H_2PO_4$, pH 6.0; flow rate 2.0 ml min^{-1}; monitored at 193.7 nm. Bandpass 1nm; damping 2s; background correction off.

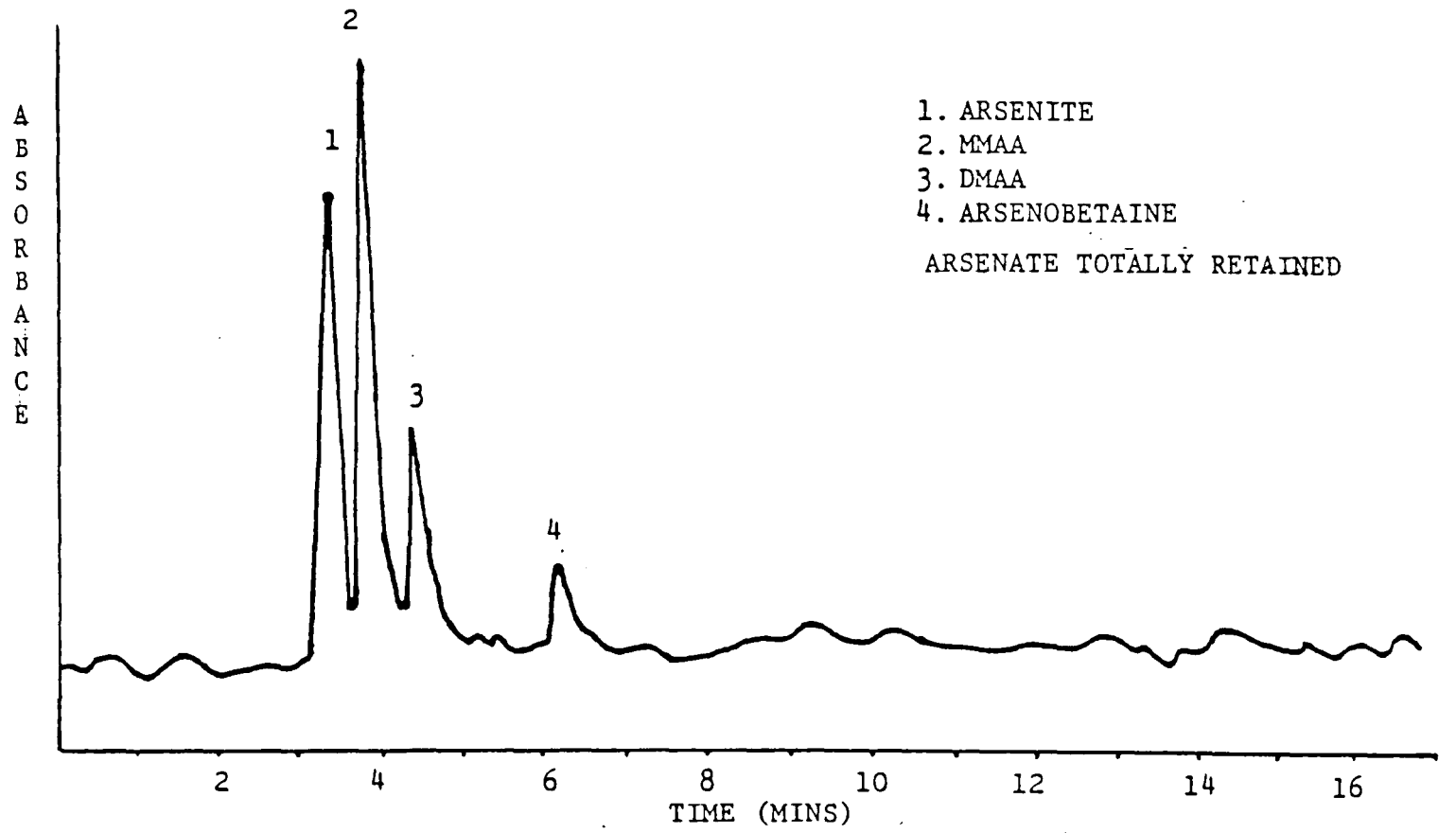
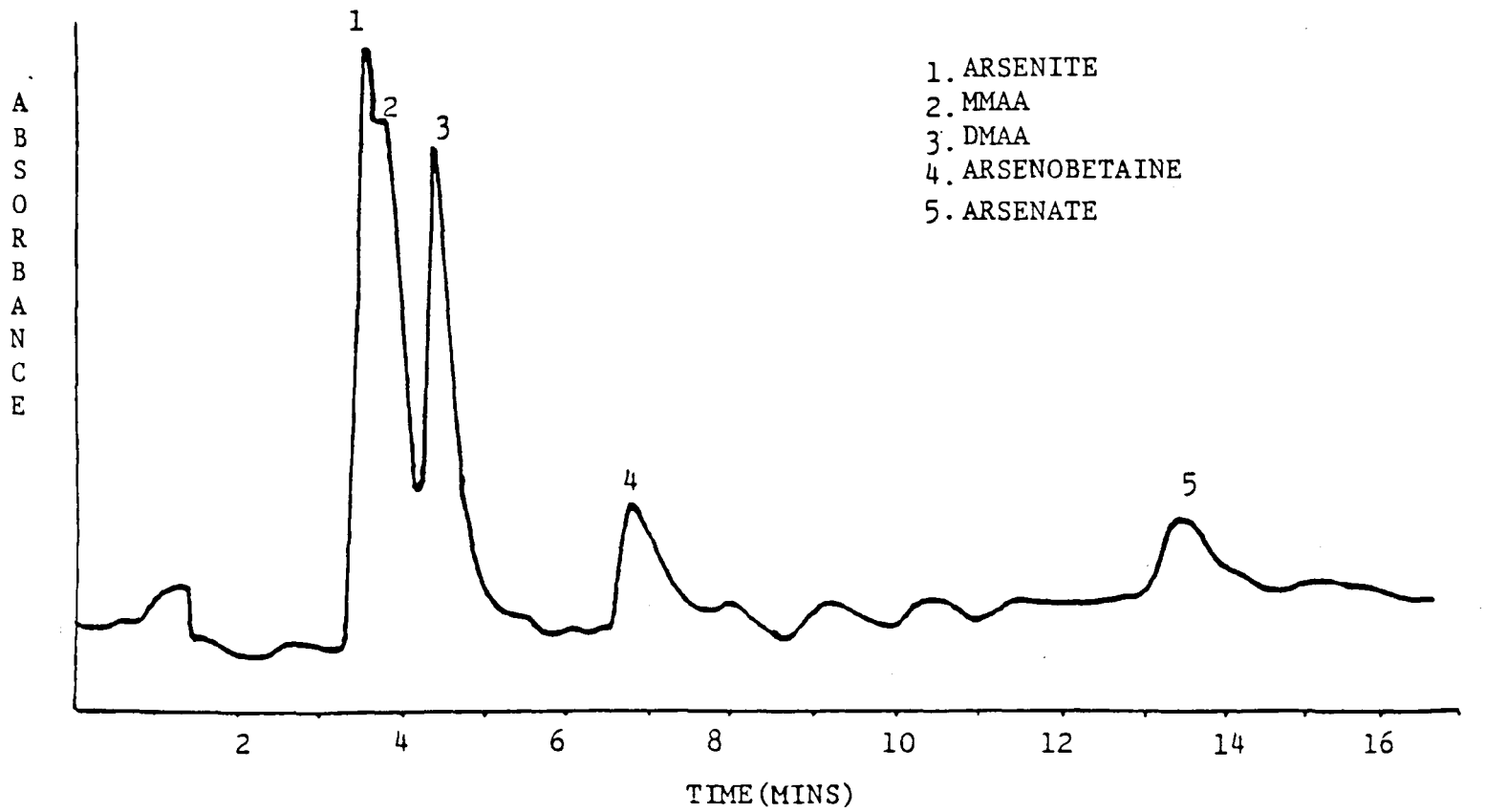


Figure 3.3 Separation of a 250 ml mixture of 5 Arsenic species. Mixture contained 50 mg As l^{-1} of each species. Mobile phase 20 mM $NH_4H_2PO_4$, pH 6.0. Other conditions as Figure 3.2.



phosphate the MMAA was retained because the ionic strength of the mobile phase was too low to elute the species. Concentrations above 10 mM $NH_4H_2PO_4$ were required to elute the strongly retained arsenate, the most acidic species. Thus a step gradient was required from low concentration - to separate the arsenite, MMAA, DMAA and arsenobetaine - to a high concentration to elute the arsenate. A number of such gradients were investigated and a step gradient from 0.08 mM $NH_4H_2PO_4$, pH 6.0, to 20 mM $NH_4H_2PO_4$, pH 6.0, was found to produce an acceptable separation over a timescale of 30 minutes. However this avenue of approach was not fully investigated due to a number of difficulties. The poor stability of silica-based anion exchange packings has previously been reported (103). It was found that after several weeks use efficiency rapidly deteriorated, reproducibility was lost and the column needed to be repacked. Also phosphate as mobile phase would have presented difficulties in conjunction with the detectors under consideration. Interfacing with ICP-MS would have given problems with deposition around the nickel cones (104) and with GFAAS the phosphate would have given severe background problems due to an interference from molecular phosphate absorption at the arsenic wavelength (173). Thus the study was switched to the resin-based exchanger.

3.3.2 Experiments with Benson Anion Exchange Resin

The Benson resin was packed into a 125 x 4 mm i.d. column which was inserted into the arrangement in place of the twin columns. Work began by replicating the studies of Haswell

et al. (44) with carbonate and dilute sulphuric acid mobile phases. A sample chromatogram is shown in Figure 3.4. The slope of the baseline was an artefact arising from the slotted tube atom trap not reaching thermal equilibrium. Perseverance with this mobile phase would undoubtedly yield better results. However the problem was that mixing between the mobile phases leads to gas evolution. As the mobile phase is pressurised the evolved carbon dioxide remains in solution. However on the recovery stroke of the pistons the mobile phase will degas CO_2, leading to seal damage.

A different approach to the mobile phase was required. Alkaline sulphate mobile phases were chosen. At pHs above 3 only SO_4^{2-} is present. With only one counter-ion present (unlike phosphate) the dynamics of the separation are easier to understand. Sulphate is the strongest eluent anion so elutes all the strongly held species without recourse to pH steps. These are undesirable as long equilibration periods are then required between injections. At pHs above 9.2 all the species, except possibly arsenobetaine, are ionised and have affinity for the column. This makes the separation easier to manipulate. A typical separation is shown in Figure 3.5. The column was equilibrated for approximately 15 minutes on 1mM K_2SO_4, pH 10.5 and switched on injection to 50 mM K_2SO_4, pH 10.5. The mobile phase in the connecting tubing eluted the first four species before the higher concentration mobile phase swept through and eluted the arsenate. The order of elution can be tentatively assigned

Figure 3.4 Separation of 5 Arsenic species using step-elution. 175 µl injection of a mixture containing 100 mg As l^{-1} of each species. Mobile phase was 1 mM NH_4HCO_3 pH 9.0, stepped to 18 mM H_2SO_4 pH 3.0. Flow rate 1.5 ml min^{-1}; 193.7 nm; no background correction; bandpass 1 nm; damping 2s.

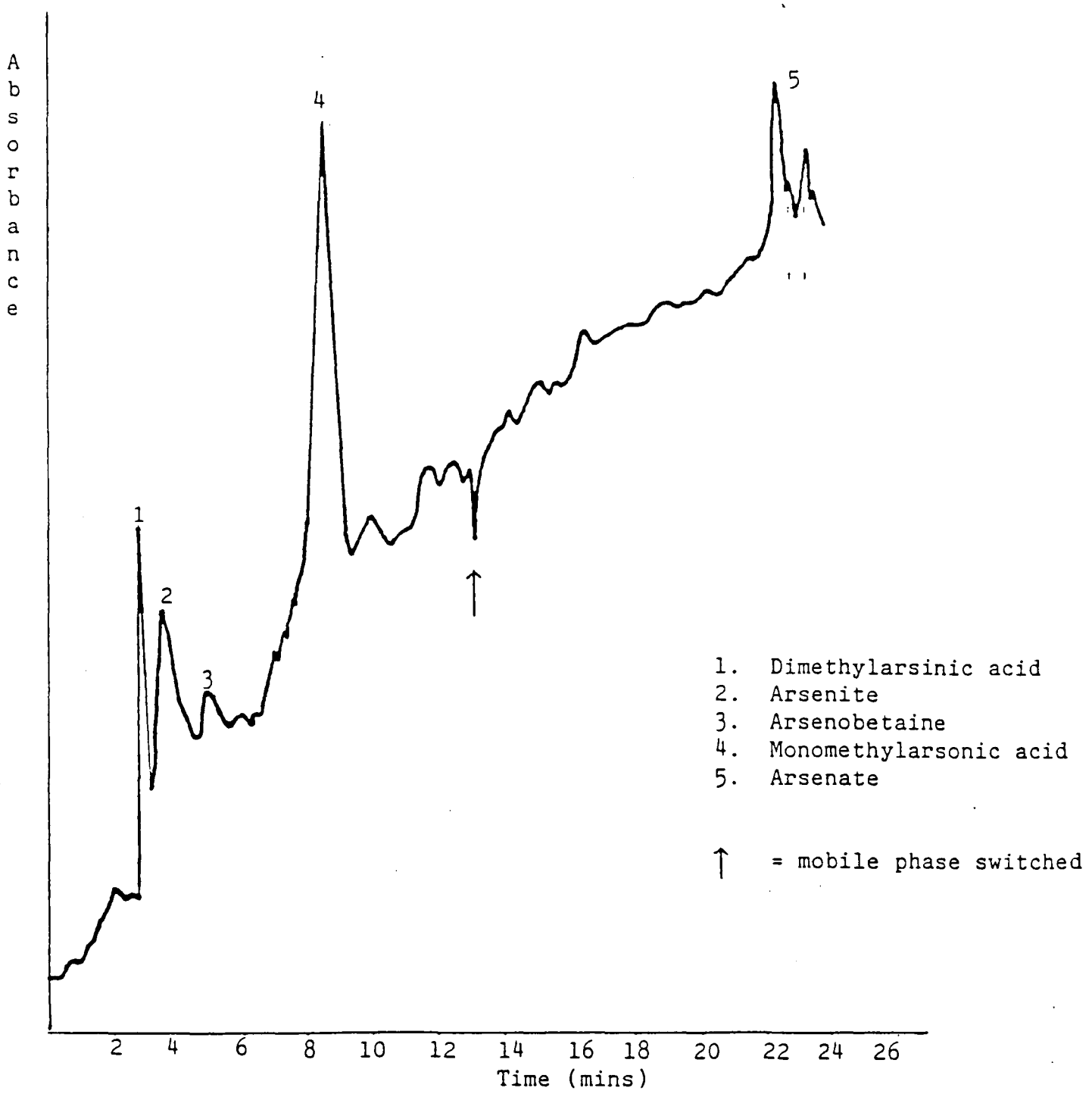
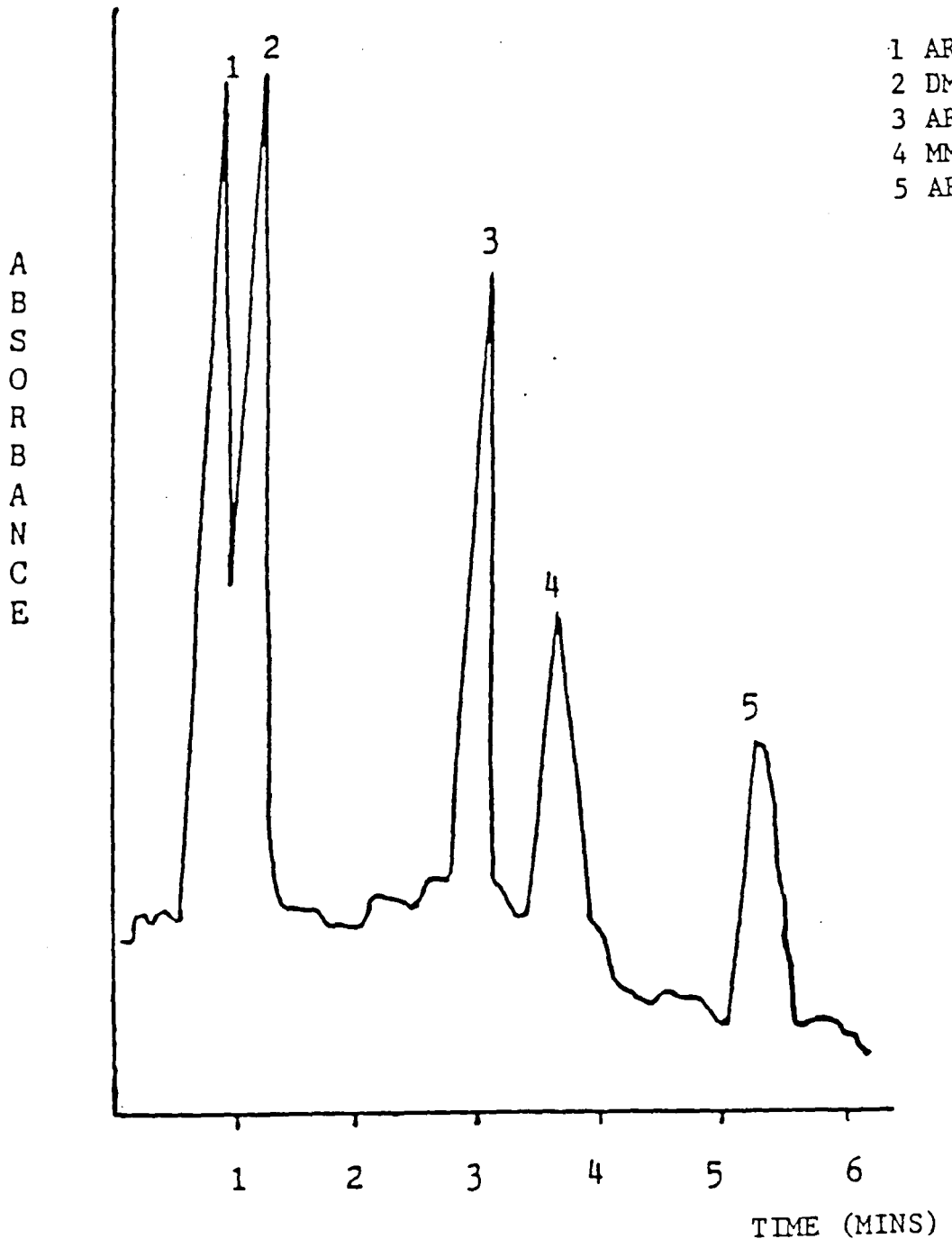


Figure 3.5 Separation of a 175 µl mixture of 5 Arsenic Species using a sulphate mobile phase

Mixture contained 100 mg As l^{-1} of each species. Column equilibrated with 1 mM K_2SO_4, pH 10.5, and switched on injection to 50 mM K_2SO_4, pH 10.5. Flow rate 1.5 ml min^{-1}. All other conditions as Figure 3.2.



to differences in pK_a of the various species. Arsenobetaine was probably undissociated, had no affinity for the column and passed straight through. MMAA and arsenate are the most acidic in character with a pK_{a1} of 4.26 and 2.25 respectively and a pK_{a2} of 8.25 and 7.25 respectively. Thus arsenate eluted last and MMAA eluted penultimately. The behaviour of arsenite and DMAA was somewhat anomalous as with pK_{a1} of 9.23 and 6.25 respectively arsenite was expected to elute first. Why this did not occur is unknown although the size and polarity of the molecules were probably significant.

Using these conditions excellent results were obtained. Reproducibility data is given in Table 3.1. Good reproducibility of retention times was a prerequisite for species identification and this was achieved. High sensitivity was not yet important as a more sensitive detector would be used for the real samples. Thus the poor reproducibility for the MMAA, which arose from the difficulty experienced manually measuring such small peak heights, was not considered critical.

With the development of a satisfactory separation concluded, a suitable high sensitivity element-specific detector was required. Preliminary studies began by constructing a thermospray, based on the design of Nygren et al, (174), to interface the HPLC system to a modified GFAAS.

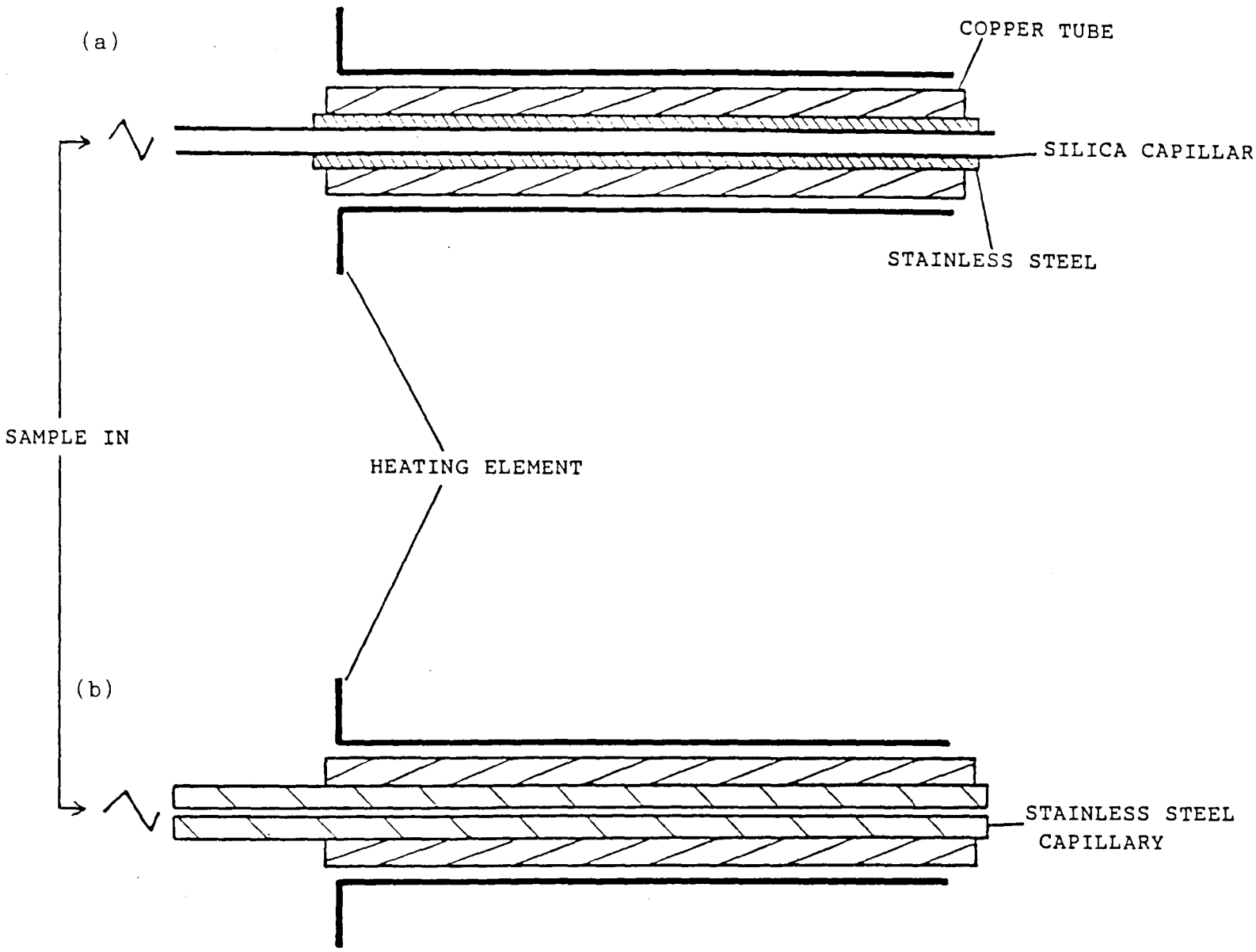
Thermosprays have recently become increasingly popular for

Table 3.1 **Reproducibility of Peak Height Measurements and Retention Times.** Each value is the mean of at least 8 injections.

Species	Mean Peak Height (mm)	Relative Standard Deviation (%)	Mean Retention Time (s)	Relative Standard Deviation (%)
Arsenite	28	11	138	6
Arsenate	12	10	324	2
MMAA	11	15	198	5
DMAA	37	5	50	5
Arsenobetaine	37	3	30	6

interfacing liquid chromatography to mass spectrometry and for both this application and coupled HPLC-GFAAS the main features are the same. The HPLC solvent containing the dissolved sample passes through a central silica or stainless steel capillary, externally heated, which in the design pursued in this study was performed by the heating element from a soldering iron. The heating causes the solvent to be rapidly vaporised forming a jet of small droplets similar in nature to an aerosol. In LC/MS the analyte is ionised through a charge exchange mechanism with a salt e.g. ammonium acetate, which is added to the mobile phase. This is not necessary in HPLC-GFAAS, the purpose of the thermospray being simply to deliver the vaporised sample to the cuvette in a continuous stable stream. The cuvette is held at the analyte atomisation temperature and as the aerosol is blown into the hot cuvette the droplets are further desolvated and the analyte element atomised. The GFAAS will respond only to the element, i.e. arsenic, that absorbs at the selected wavelength and if a chart recorder is connected normal chromatograms are obtained. The elimination of the drying and ashing stages means continuous chromatograms are given. Two designs were manufactured and are shown in Figure 3.6. To give adequate resistance to temperatures of up to 2000°C the final link between thermospray and furnace was a glassy carbon tube (Ringsdorff Werke GmbH, Bonn, FRG) which was fitted to the end of the copper tube. The furnace door of a Pye Unican SP9 GFAAS was adapted to allow passage of the thermospray and the cuvette

Figure 3.6 Thermospray Designs for Interfacing HPLC to GFAAS.
(a) with a fused silica capillary tube (0.32 mm internal diameter)
(b) with a stainless steel capillary tube (0.2 mm i.d.)



injection hole was enlarged to house the open end of the glassy carbon tube. The initial design incorporating a 0.32 mm i.d. silica capillary was found to suffer from pulsing, a problem that was overcome by using the 0.2 mm i.d. stainless steel capillary. This design was found to generate stable, evenly dispersed spray at flow rates up to 0.3 ml min^{-1}, flow rates that are compatible with millibore HPLC columns. However at this stage the studies were terminated as Plymouth Polytechnic took delivery of an ICP-MS, and HPLC-ICP-MS couplings were deemed likely to produce superior results to HPLC-GFAAS.

3.4 Coupled High Performance Liquid Chromatography - Inductively Coupled Plasma-Mass Spectrometry

For the majority of these experiments the PU4010 pump was replaced by the Waters 6000A pump. The external switching valve of the former was found to be awkward to use and prone to leaks when compared with the Waters pump which has an in-built valve for switching between mobile phases.

The HPLC system was interfaced to the ICP-MS instrument by means of a short length of Tigon tubing which connected the stainless steel column outlet capillary to the PTFE uptake capillary of the ICP-MS nebuliser. The column eluate was forced into the nebuliser by the forward pressure of the pump. Typical operating conditions are given in Table 3.2.

Initial work focussed on achieving reproducible signals for standards and it was found that the precision of peak area

Table 3.2 Instrumental Parameters for the HPLC-ICP-MS Coupling

HPLC Parameters	
Mobile phase	Equilibration on 1mM K_2SO_4, pH 10.5. Switched on injection, or after 3 mins, to 50mM K_2SO_4, pH 10.5.
Flow rate	1.5 ml min^{-1}
Injection volume	175 μl
Run time	671 s
ICP-MS Parameters	
Nebuliser gas flow	0.72 l min^{-1} (Meinhard) or 0.85 l min^{-1} (Ebdon)
Coolant gas flow	14 l min^{-1}
Auxiliary gas flow	0.8 l min^{-1}
Forward power	1.5 kW
Reflected power	<10 W

Data acquisition mode Survey scan. Local mass set to 75 u.

and retention time measurements were 3.8% and 5.0% respectively. No problems were encountered with the coupling and conventional ICP-MS operating conditions could be used.

One area of concern was an increase in peak width due to:

(i) the uptake capillary being approximately 60 cm long,

(ii) dispersion in the Scott double-pass spray chamber used, which had a volume of 110 ml.

The normal spray chamber was replaced by one built in our laboratories (175) based on the design of Kempster et al. (176). The spray chamber is shown in Figure 3.7. It was used in conjunction with a "mini-Ebdon" nebuliser (PSA Ltd, Sevenoaks, Kent, UK). A further improvement in performance was based on previous observations in our laboratories that the Benson resin gives a more efficient separation at elevated temperatures, typically 60°C (177). Thus the column was submerged in a thermostatted 60°C water bath. The dramatic improvements in peak shape given by these two approaches are evident from Figure 3.8. The reduced volume spray chamber decreased width, but unfortunately led to tailing of the peak, probably as a result of droplet accumulation in the two bends through which the aerosol passed prior to entering the plasma or because the tailing from the dead volume of the capillary tubing interface is

Figure 3.7 Reduced Volume Spray Chamber

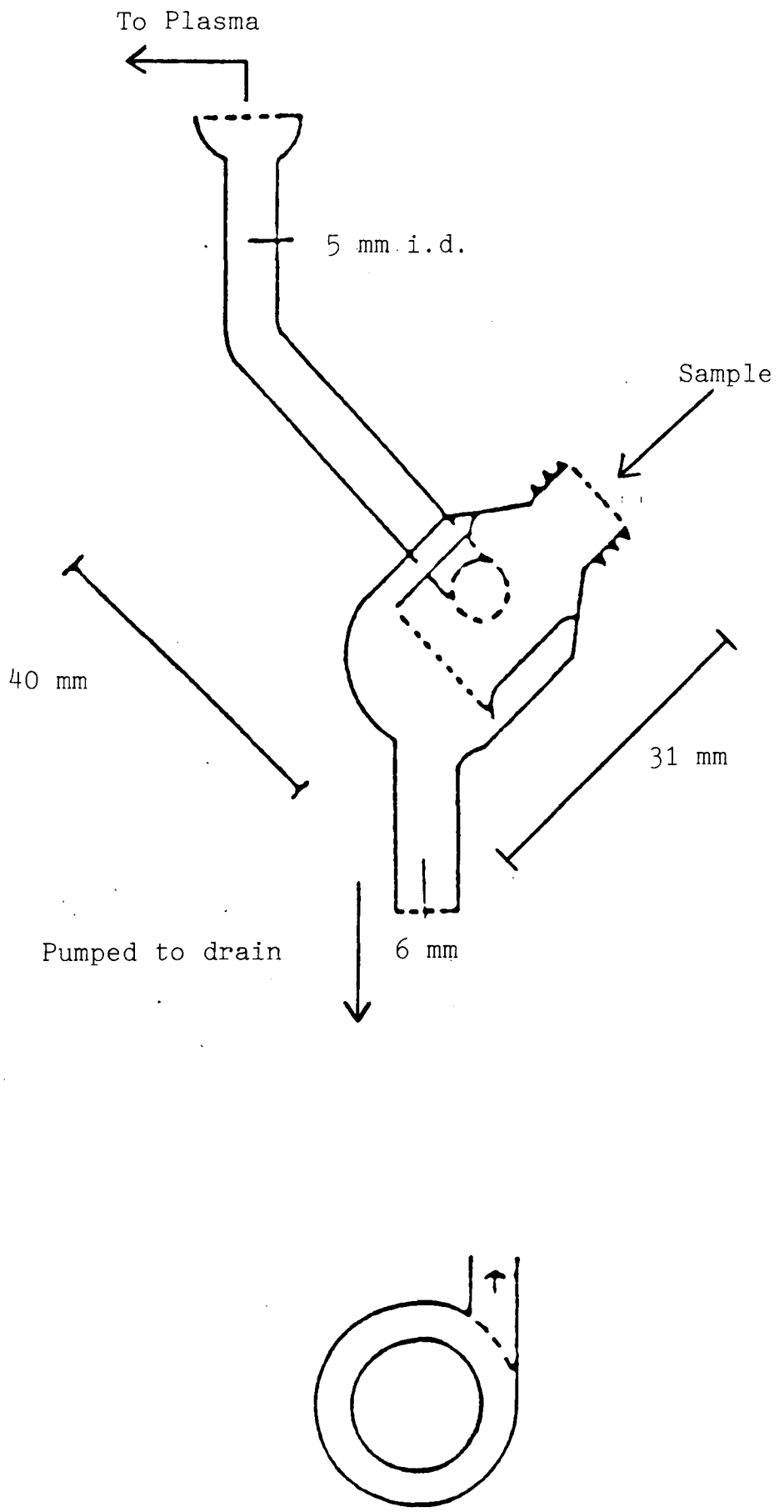
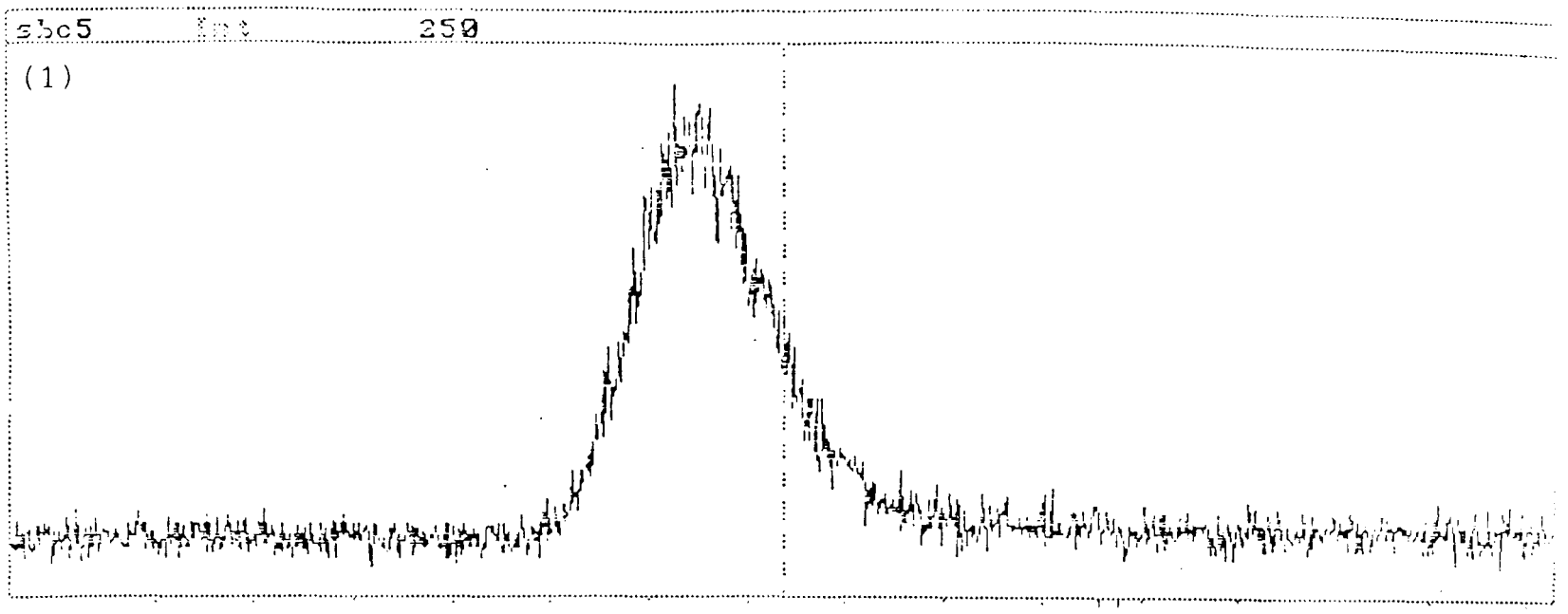
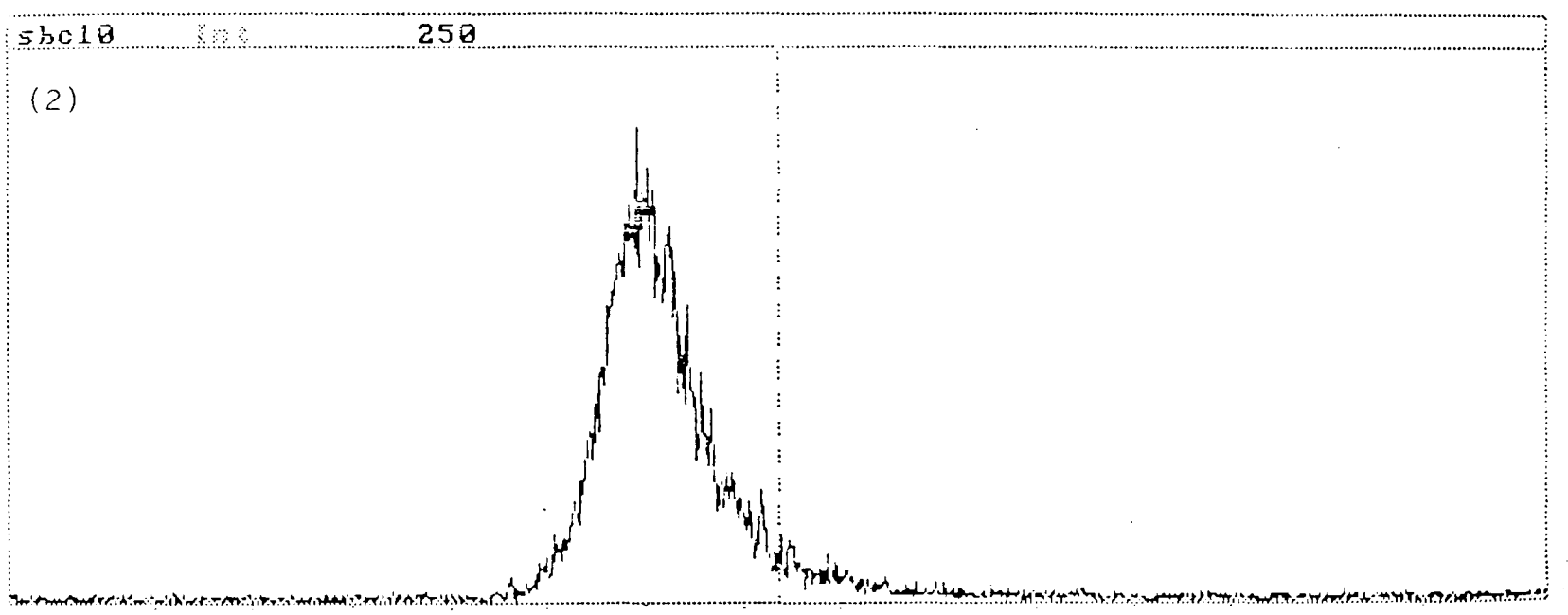
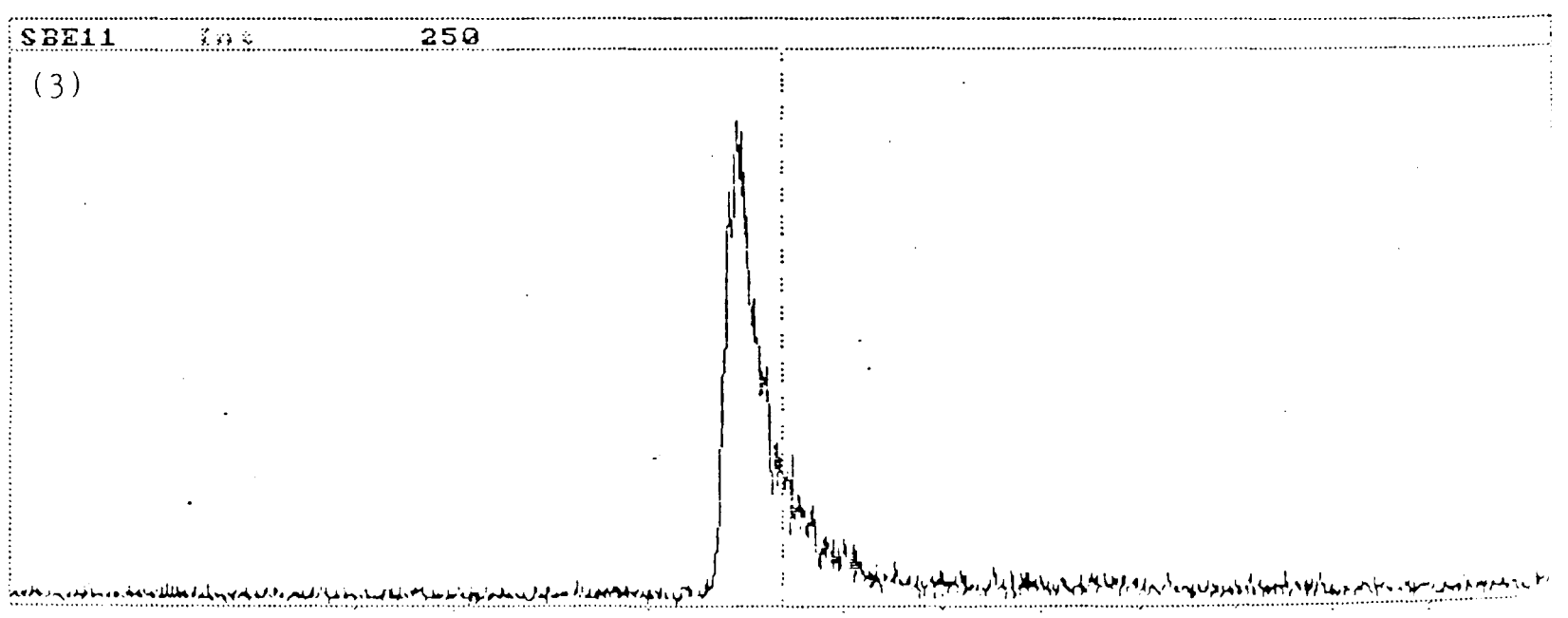


Figure 3.8 Improvement in (1) normal peak shape by using (2) a reduced volume (11 ml) spray chamber and (3) immersing the column in a 60°C water bath







now more pronounced. Heating the column removed the fronting on the peak and improved the tailing to a lesser extent. As a result of these findings the column was held at 60°C for all subsequent studies. The reduced volume spray chamber has considerable potential but a design incorporating a smooth, curved connection between spray chamber and torch is required, before it can be used on a routine basis.

3.5 Quantification of Species

As supplied the Plasmaquad 2 is not designed for use as an HPLC detector, but by careful manipulation of operating conditions this problem can be overcome and time resolved data acquired. Using the survey scan mode a mass range that avoids the skipped scan regions, (*i.e.* the regions 10-18, 27-42 and 79-81 u where the large signals from entrained gases, polyatomic ions and argon dimers would damage the detector if not excluded from data collection), must be selected *e.g.* 85-240 u. Only one sweep can be allowed, with a very long dwell time, typically 1.6×10^5 μs. Obviously the longer the dwell time the greater the signal. The number of channels must then be selected - 512, 1024, 2048 or 4096 - to give the required run time which in this example would be 167, 335, 671 or 1342 seconds. The run time must be chosen carefully as once a scan has begun it cannot be stopped prematurely without loss of all data acquired during that run. When the data has been copied to the disc there are three ways of obtaining quantitative data:

(a) Peak area method. By moving the cursor the "digitise peak function" can be used to integrate all the counts within the cursor boundary. Unfortunately the background beneath the peak is also included. Therefore an area of background of the same peak width must be selected, digitised and subtracted from the total counts. This method is unsatisfactory if the background slopes, or is not uniform.

(b) Automatic heights method. By reducing the cursor to its smallest jump step the number of counts in the channel at the peak maxima can be measured. A number of channels from the background can then be sampled, and the average counts subtracted from the peak maxima. The limitation of this method is it is open to subjectivity in respect of where the maxima lies and which part of the background to choose. Usually the background immediately before the peak is sampled.

(c) Manual heights method. The full scale deflection of each printout can be adjusted to a standard value, say 1000. Thus each analysis consisting of standards and samples can be set to the same value and the heights measured manually after printout.

Investigation of the calibration curves given by solutions of aqueous standards indicated method (c) gave the best

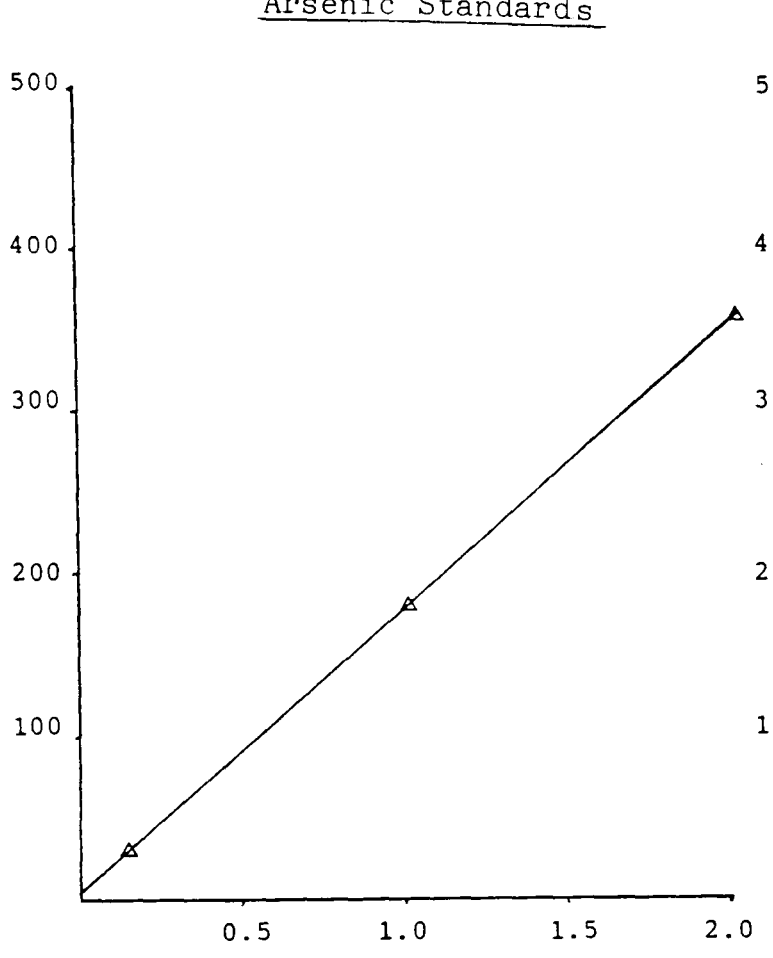
calibration i.e. the curve intercepted the origin and a high correlation coefficient was calculated for the calibration.

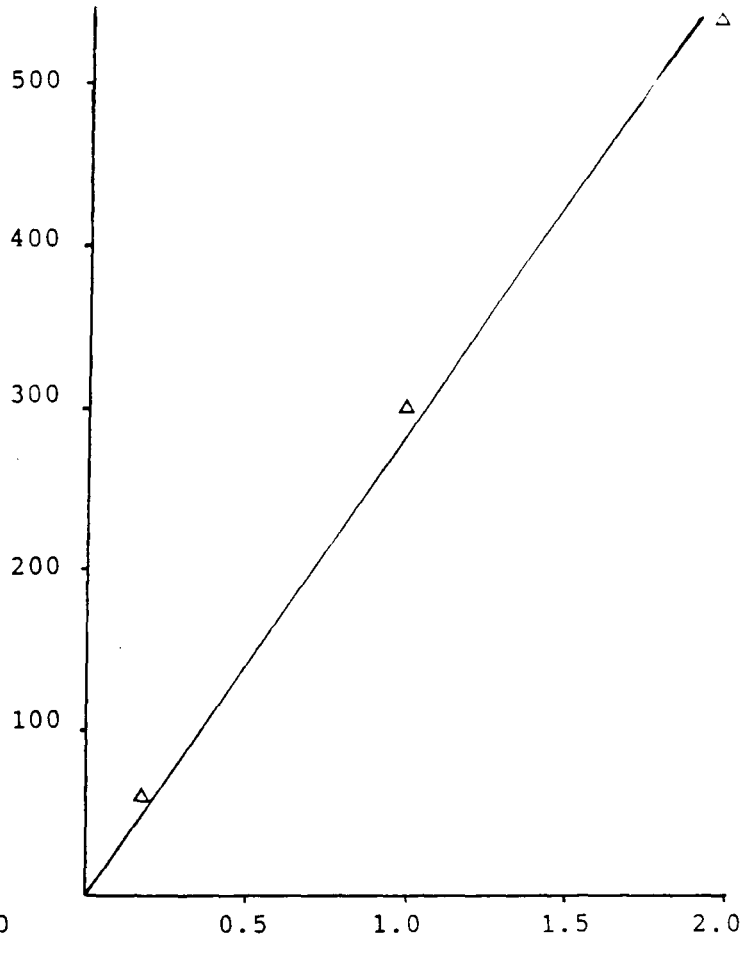
Thus method (c) was adopted for the analyses. Figure 3.9 shows the calibration curves given by mixed standards of arsenobetaine, arsenite, arsenate, MMAA and DMAA at 0.2, 1.0 and 2 $\mu g\ ml^{-1}$.

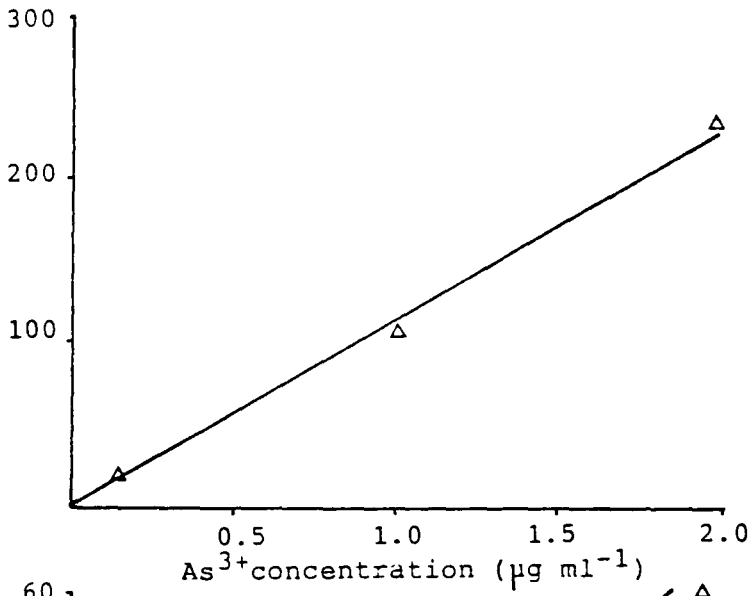
During the course of the study VG Elemental supplied a dedicated chromatography software package known as Time Resolved Acquisition, TRA, (VG Elemental, Winsford, Cheshire, UK). Although this was very useful for qualitative work it suffered a number of limitations including (a) the peak area method described above was used for quantification, (b) the hard disk could only accommodate approximately seven runs and (c) at least three floppy disks were required to hold one scan.

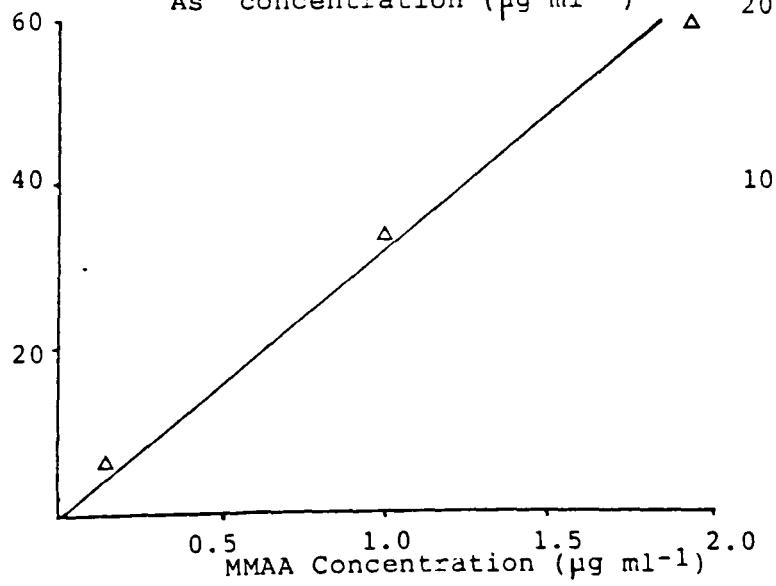
Therefore for the study of plant materials and fish the manual heights method was used. For the dietary trial a modification to the ICP-MS was performed which allowed the Single Ion Monitoring package (VG Elemental, Winsford, Cheshire, UK) to be used to collect data. The features of this package are (a) retention times of each peak are printed and (b) the area of the peak to be integrated can be selected by adjustment of the cursor. This allows contributions from the background to be avoided. Furthermore as only data for a single mass is collected large memory space is not essential for data storage.

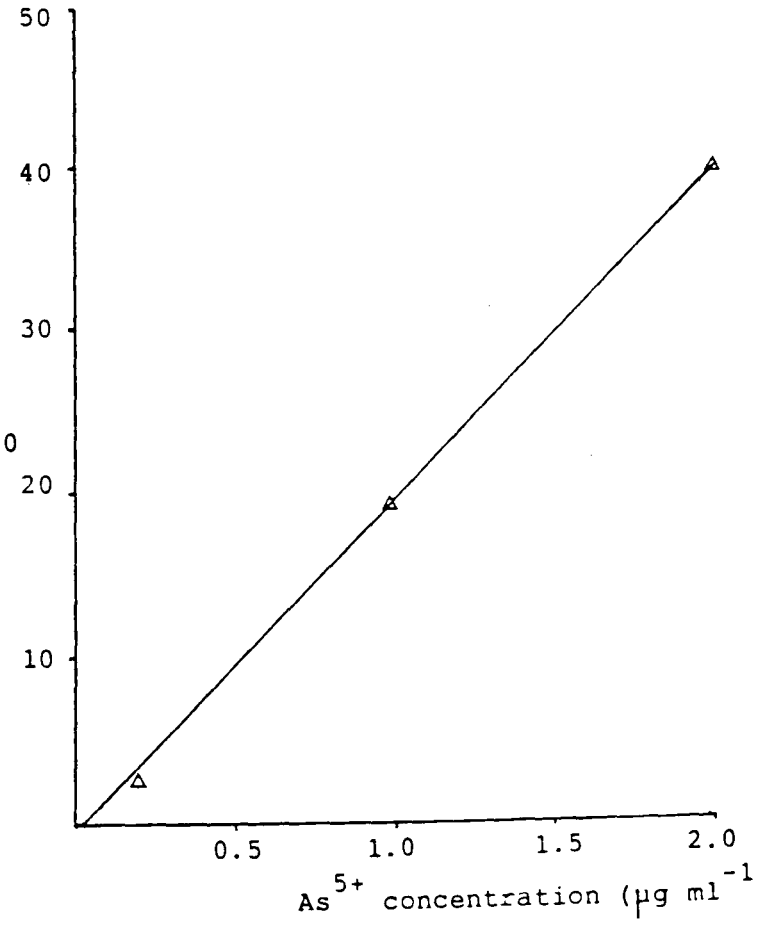
Figure 3.9 Calibration Curves for Solutions Containing five Arsenic Standards











To summarise, the HPLC-ICP-MS system developed for arsenic speciation comprised a 125 x 4 mm i.d. column packed with Benson strong anion exchange resin, interfaced to an ICP-MS by a short length of Tigon tubing which coupled the nebuliser uptake capillary to the HPLC column outlet. The column was held at 60°C in a water bath. The mobile phase used was 1mM K_2SO_4, pH 10.5, switched to 50mM K_2SO_4, pH 10.5. Quantitative data was obtained either by manual peak height measurement of the hard copy of the chromatograms or by using proprietary software packages. This arrangement was used for the subsequent determination of arsenic species in plants, fish and human urine.

4. DETERMINATION OF ARSENIC IN SOIL AND PLANT MATERIALS

4.1 Introduction

In the study of trace element concentrations in plants the soil levels are highly relevant. Soil concentrations have implications for aquatic life as arsenic may be leached from the soil into rivers and estuaries. Mean arsenic concentrations in the Earth's crust are reported to be 2 mg kg^{-1} (178). Concentrations in normal soils range from 0.1-55 mg kg^{-1} (18). These levels vary depending on the geological base, soil type and anthropogenic input e.g., the use of arsenical herbicides.

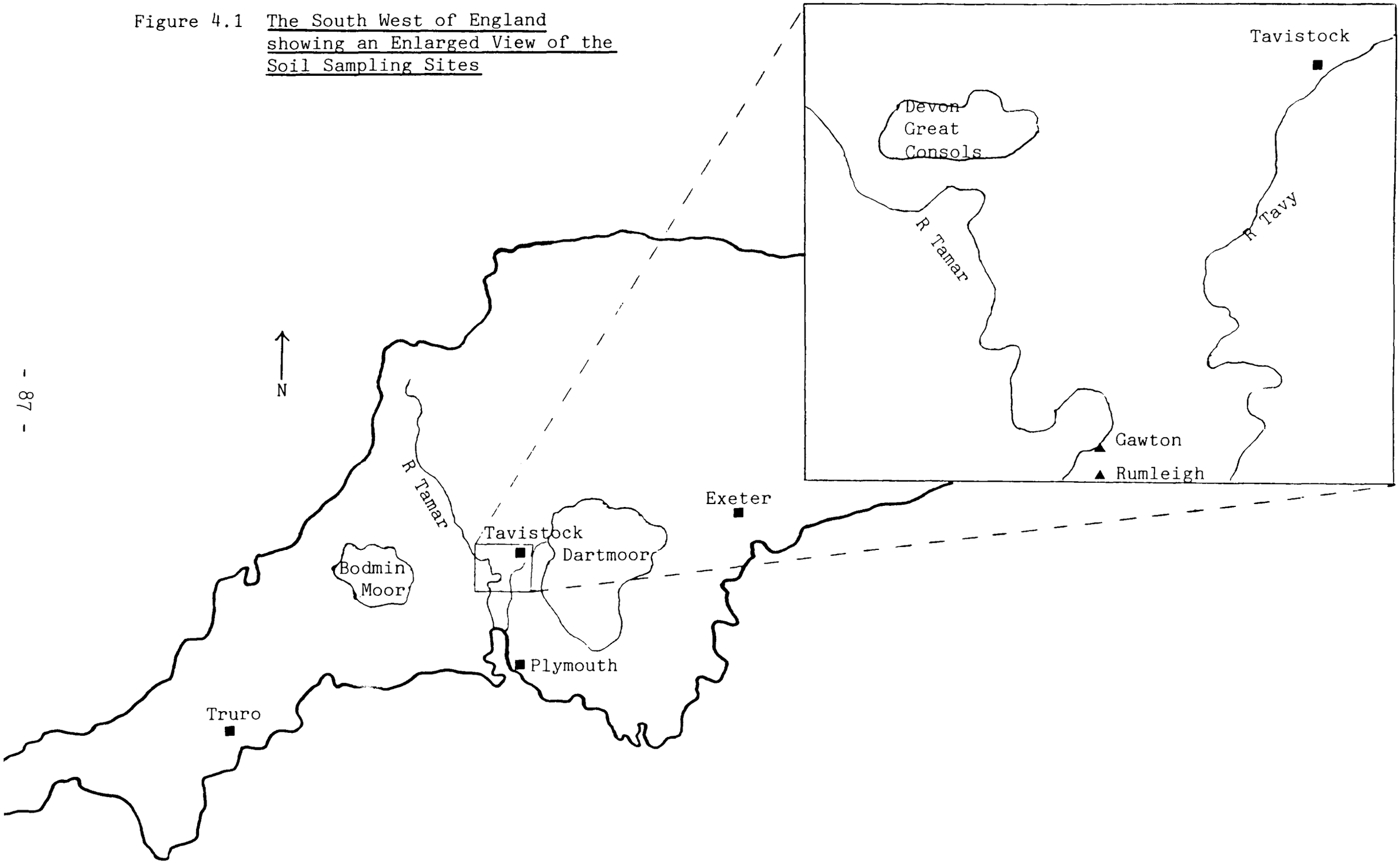
For this study a range of arsenic containing soils were required on which to grow crops and thus assess plant uptake and potential dietary risk. Soils were collected from Devon Great Consols (DGC) and Gawton mines from the Tamar Valley where large arsenic deposits were known to exist. To act as a reference, soil was also collected from the Polytechnic field station at Rumleigh, a site not associated with excessively high soil levels of arsenic. The sites of soil collection are shown in Figure 4.1.

4.2 Determination of Arsenic in Soil

4.2.1 Materials and Methods

Soil samples from various depths and locations were collected from Devon Great Consols, Gawton and Rumleigh. The samples were dried at 65°C in a soil drying cabinet and sieved through 2mm and 180 μm sieves. Between 0.9-1.1 g of

Figure 4.1 The South West of England showing an Enlarged View of the Soil Sampling Sites



the resulting fine powder was weighed into a digestion tube and 15 ml of concentrated AnalaR nitric acid (BDH Chemicals, Poole, UK) added. The samples were digested for four hours using a digestion block (Tecator, Bristol, UK) and the following programme:

0 - 30 minutes - 40°C
30 - 60 minutes - 75°C
60 - 90 minutes - 110°C
90 - 120 minutes - 145°C
120 - 240 minutes - 145°C

After cooling the digests were filtered and diluted to 100 ml with Milli-Q double de-ionised water (Millipore Corporation, Bedford, MA, USA). This digestion procedure has previously been described by Haswell (21).

Initial results were obtained using a continuous flow hydride generator (PSA Ltd, Sevenoaks, Kent, UK) and an AAS instrument (SP9, Philips Scientific, Cambridge, UK). Operating conditions are given in Table 4.1. Later analyses were performed using direct aspiration and flame AAS. A direct current plasma-atomic emission spectrometer, DCP-AES, (Spectraspan IV, ARL, Crawley, West Sussex) was used for quality control checks. Arsenic standards were prepared fresh daily by dilution of a 1000 mg l^{-1} stock solution of AnalaR grade arsenic trioxide (BDH Chemicals, Poole, UK). Acid washed glassware was used throughout.

Table 4.1 Operating Conditions for Soil Analysis

Hydride Generation	
Rise Time	10s
Decay Time	10s
Argon Purge	500ml min^{-1}
Reducing Agent	4% sodium borohydride in 0.1M sodium hydroxide
Acid	6M Hydrochloric Acid

Atomic Absorption Spectrometer	
Wavelength	193.7 nm
Lamp current	9 mA
Band Pass	0.5 nm
Air	5.0 l min^{-1}
Acetylene	1.5 l min^{-1}
Background	Correction On

4.2.2 Results and Discussion

The results obtained are given in Table 4.2. The results for Rumleigh were similar to the values reported by others (21, 22). The concentrations from the mine areas were very high, in some cases in excess of one percent w/w, but were not totally unprecedented (179).

After the first few analyses hydride generation was deemed unnecessary as the test solutions required considerable dilution prior to analysis. In general the hydride generation results were lower than those obtained by AAS which may indicate an interference, from copper or iron in the soil, on the hydride generation process. Certainly soil from the selected areas is known to be high in copper (21). For both methods the agreement between duplicates is reasonable and the precision of measurement good for each sample.

The levels around each site are very variable, depending on the actual position and depth at which each sample was taken. This is understandable at DGC because the spoil heaps are on a very exposed site and dust from the spoils is likely to be blown across and around the mine area. Thus it can be predicted that the surface layers will have the highest concentration. The results support this premise. As expected, high concentrations of arsenic were found on flues and mine buildings through which arsenic vapour had passed during smelting, leading to arsenic trioxide accumulation on the walls.

Table 4.2 Results of Soil Analysis

Soil Origin and Depth	Hydride Generation -AAS (mg kg^{-1})	RSD %	Flame -AAS (mg kg^{-1})	RSD %
Rumleigh	90	2.3	-	-
Depth 8"	100	0.0	-	-
Gawton	6100	2.1	6900	1.2
451,688 5"	6200	3.1	-	-
Gawton, 451,	5500	0.0	-	-
688 15"	5300	1.3	5800	4.3
Gawton,	14300	1.3	17100	1.6
452,689 9"	15200	3.7	17100	0.7
Gawton	36600	1.3	47500	5.5
Flue Deposits	54100	3.4	47500	5.4
Gawton	5500	2.0	5200	2.8
453,689 12"	5500	3.7	-	-
Gawton	8700	2.5	9000	1.0
453,689 2"	9200	0.0	-	-
[1]DGC 429,729 12"	600	0.8	500	10.6
DGC 429, 729	-	-	400	8.2
2-4"	-	-	600	3.0
DGC 429,	-	-	100	9.3
729 4"	-	-	ND	-
DGC 429,	-	-	ND	-
729, 18"	-	-	ND	-
DGC 431,	-	-	700	3.3
731 Surface	-	-	700	3.2
DGC 431,	-	-	200	14.4
731, 10"	-	-	200	8.6
DGC 430,	-	-	20700	0.6
737 0-2"	-	-	21500	3.9
DGC 430,	-	-	6900	9.1
737 6-8"	-	-	4500	7.5
DGC	-	-	12000	6.2
Lower Dump	-	-	10100	4.9
DGC Flue	-	-	18600	3.6
Deposits	-	-	18100	2.3
DGC Coating from	-	-	16800	0.9
building surface	-	-	16200	1.8

[1] DGC = Devon Great Consols ND = Not Detectable

The quality of results was maintained by standard additions and the use of an independent technique, DCP-AES, to analyse some of the samples. The results, in percent w/w, for DGC soil, map reference 430, 737, depth 0-2", are given below:

Sample	AAS	DCP	Standard Addition
(1)	2.1	2.2	-
(2)	2.2	2.0	2.4

The agreement between independent techniques indicates a reasonable level of confidence can be placed in the accuracy of the results.

It was concluded that with such a range of natural arsenical soils available these would be used for crop production rather than adding specific arsenic compounds to low arsenic soils.

4.3 Determination of Total Arsenic in Fruit and Vegetables

4.3.1 Introduction

A range of fruit and vegetables were grown on high arsenic soils. Primary studies focused on the total uptake of arsenic by the plants. A range of soils were used to give normal levels, moderate contamination and high contamination (a 'worst case' situation). The moderate contamination soil was used to ensure there would be samples available if the high level crop failed.

4.3.2 Materials and Methods

Two soil samples were taken from sites at Devon Great Consols (DGC) known to have high arsenic concentrations. A sample was taken at Rumleigh Field station. The samples were analysed as previously described. A range of plants were then grown on the DGC soil, a soil consisting of one part DGC:nine parts Rumleigh soil, and on Rumleigh soil.

The crops grown were stonehead cabbage, crimson globe beetroot autumn mammoth leek, white lisbon spring onions, King Edward potatoes, Cambridge strawberries, broccoli, carrots, lettuce and tomatoes. Soil samples were removed from the pots after approximately one month of growing and analysed for arsenic as before. On maturity the plants were harvested and frozen. When required for analysis they were defrosted in their closed containers at room temperature. This prevented condensation problems. Samples were taken for digestion and determination of moisture content, which was performed by drying 1g sub-samples for four hours at 105°C. However, as the oven drying led to some of the samples caramelising this protocol was later changed to taking the frozen samples, freeze drying (Edwards Super Modulyo, Edwards High Vacuum, Crawley, Sussex), grinding by acid washed pestle and mortar and storage in a dessicator prior to analysis.

Samples were washed and then digested by:

(a) directly weighing 0.5g of wet sample (only the edible

parts were analysed) into PTFE inserts, adding 3 ml of Aristar HNO_3 (BDH Chemicals, Poole, UK) and placing the inserts into steel digestion bombs which were oven heated at 145°C for four hours. After cooling the resulting clear solutions were spiked with indium (Aldrich AAS Standard, Aldrich Chemical Co., Milwaukee, USA) and diluted to 25, 50 or 100 ml with double de-ionised water to give a final indium concentration of 100 $\mu g l^{-1}$;

(b) the frozen plants were cleaned by washing with tap water prior to freeze drying. After drying and grinding approximately 100-200 mg of the resulting powder was weighed accurately into a PTFE digestion bomb. Aristar HNO_3 (2-3 ml) was added, the bomb lid loosely replaced and the samples left to pre-digest overnight. They were then digested in a household microwave oven (Toshiba ER-761E, Toshiba Company, Japan) using the following programme:

(1) 5 minutes - warm (setting 2)
(2) 5 minutes - roast (setting 7)
(3) 5 minutes - full power (setting 9)

Step 3 was only performed if the sample had not been adequately digested. Between each step the samples were cooled and vented to prevent excessive pressure build up. The practice of microwave digestion has been

comprehensively reviewed by Kingston and Jassie (180). The final solution was spiked with indium to give a concentration of 100 $\mu g\ l^{-1}$ In following dilution.

Samples prepared by method (a) were analysed by hydride generation-AAS, (HGAAS), using the conditions previously described (Table 4.1) or by ICP-MS, using the conditions displayed in Table 4.3. The samples prepared by method (b) were analysed by ICP-MS, and also by GFAAS (Thermo-Electron Video 12 Atomic Absorption Spectrometer with 665 Furnace, Thermo-Electron Ltd., Warrington, Cheshire). The GFAAS operating conditions are given in Table 4.4.

4.3.3 Results and Discussion

The two soil samples collected at DGC were found to have arsenic concentrations of (a) 2.3 ± 0.5% and (B) 1.2 ± 0.3% dry weight. Sample B was used for the pot trials. After a month's growing the undiluted soil was found to have an arsenic concentration of 1.35% dry weight and the 1:9 diluted soil had an arsenic concentration of 800 mg kg^{-1} dry weight. At this stage samples of the soils were sent to the Agricultural Development and Advisory Service, Starcross, Devon and the results of their analyses are shown in Table 4.5. The nitrate and ammonium levels were low in all samples. In addition the high arsenic soil had a very low pH and potassium concentration.

Only strawberries flowered and fruited on the high arsenic soil although the potato seeds developed tubers. The

Table 4.3 ICP-MS Operating Conditions for Plant Analysis

Forward power	1.35-1.5 kW
Reflected power	<10 W
Nebuliser gas	0.8 l min^{-1}
Auxiliary gas	1.0 l min^{-1}
Coolant gas	13-14 l min^{-1}
Channels	1024
Sweeps	400
Dwell time	80 μs
Monitored masses	75, 77 and 115 u
Typical sensitivity	2 x 10^6 counts/mg kg^{-1} In

Table 4.4 Graphite Furnace Operating Conditions for Plant Analysis

Lamp current	5 mA
Bandpass	2 nm
Wavelength	193.7 nm
Injection volume	30 μl
Matrix modifier	1000 mg l^{-1} nickel nitrate

	Ramp(s)	Hold(s)	Temperature(°C)
Dry	5	15	110
Ash	10	30	870
Atomise	0	5	2700

Smith-Heiftje background correction and wall atomisation were used.

Table 4.5 Results of Analysis of Soil Parameters

Sample	Sample Type	pH	Lime Requirement Tonne per Hectare Ground Limestone		Phosphorous		Potassium		Magnesium		Nitrate Nitrogen	Ammonium Nitrogen
			Permanent Grass, hops and fruit	Arable general horticulture crops glass house	mg l^{-1}	Index	mg l^{-1}	Index	mg l^{1}	Index	mg kg^{-1}	mg kg^{-1}
High Arsenic Soil	Clay loam	5.1	7	13	91	5	67	1	96	2	8.1	7.6
Diluted Soil	Clay loam	7.5	0	0	114	6	304	3	196	4	1.5	2.2
Rumleigh Soil	Clay Loam	7.9	0	0	86	5	133	2	84	2	8.3	2.5

All analyses performed by the Agricultural Development and Advisory Service, Starcross, Devon.

leaves showed evidence of chlorosis. The broccoli, carrots and tomatoes failed to grow at all. Beetroot, cabbage, leeks, potatoes, spring onions and strawberries grew on the diluted soil, although the cabbages, leeks and spring onions were stunted. The heart of the cabbage was very small.

The crops, along with some plants from Rumleigh that were available, were analysed for total arsenic concentration. The results are given in Table 4.6 and the moisture content in Table 4.7.

The preliminary analyses were performed by HGAAS and a number of problems were encountered. As the samples were heated in nitric acid most of the arsenic was oxidised to As^{5+}. This has a low hydride forming efficiency and thus it was necessary to add potassium iodide to reduce the arsenate to arsenite. Unfortunately the addition of potassium iodide to the potatoes and carrots caused the formation of a black precipitate, probably due to reaction between starch and iodine. The garlic and rhubarb digests turned red-violet on addition of KI which is indicative of the reaction between iodine and amylopectin (181), one of the components of starch. Arsenic may have been lost in the precipitate or during the subsequent filtration to remove the precipitate. In addition the sensitivity of the HGAAS system suffered in comparison with the other techniques, being of the order of 5 $\mu g\ kg^{-1}$ compared to 1 $\mu g\ kg^{-1}$ for GFAAS and 0.1 $\mu g\ kg^{-1}$ for ICP-MS. Also, it is far more difficult to

Table 4.6 Arsenic Concentrations in Plant Materials.
All results in mg As kg^{-1} dry weight

Sample	Analytical Method		
	HGAAS(1)	GFAAS(2)	ICP-MS(2)
Rumleigh			
Onion	<1.0	-	<0.2
Whitecurrent	<1.0	-	<0.2
Garlic	<1.0	-	0.2
Raspberry	<1.0	-	<0.2
Carrots	<0.3	<0.6	0.4 ± 0.1
Rhubarb	<0.1	<0.3	1.0 ± 0.1
Leaf Beet	1.1	-	-
Diluted DGC Soil			
Peeled Potato	<0.25	<0.6	<0.2
Unpeeled Potato	3.0	1.5	18.0 ± 0.8
Unpeeled Beetroot	4.0 ± 0.6	3.3 ± 0.6	3.6 ± 0.3
Strawberry	-	1.1 ± 0.4	0.9 ± 0.2
Cabbage	0.5	<0.6	1.7 ± 0.1
Spring Onion	0.3	-	-
Leeks	2.2 ± 0.2	-	2.4 ± 0.2
Undiluted DGC Soil			
Peeled Potato	-	4.3	3.5 ± 0.1
Unpeeled Potato	-	60.7	73.4 ± 3.0
Strawberry	-	4.8 ± 0.1	4.7 ± 0.2

(1) Prepared by method (a)

(2) Prepared by method (b)

Table 4.7 Moisture Content of Plant Materials.
All results in % w/w.

Rumleigh Soil	
Onion	86, 87
Whitecurrent	79, 81
Garlic	58, 59
Raspberry	88, 87
Carrots	75, 85
Rhubarb	93, 95
Leaf Beet	83, 85
Diluted DGC Soil	
Unpeeled potato	69, 70
Unpeeled beetroot	84, 87
Strawberry	88, 86
Cabbage	86, 86
Spring Onion	75, 79
Leek	80, 81
Undiluted DGC Soil	
Unpeeled Potato	77, 78
Strawberry	90, 90

representatively sample wet samples relative to freeze-dried samples which can be bulked and ground to a small particle size.

Notwithstanding these minor difficulties it was still possible to determine arsenic in the plantstuffs and as all the results were below 5 $\mu g\ kg^{-1}$ it can be concluded no dietary risk was presented by these crops.

The results for the GFAAS analyses and those by ICP-MS are in some cases in good agreement e.g. strawberries, carrot, beetroot. However for some of the other samples the results are much less satisfactory, such as high peeled potato, cabbage and particularly the unpeeled potato from the diluted soil, which shows variation between the results for all three analytical methods. Several explanations are possible:

(a) arsenic may have been lost in the precipitation of the starch-iodine complex giving an erroneous value;

(b) the arsenic is associated with the peel which proved difficult to grind and may have been distributed unevenly though the sample. It is worth noting that the smaller samples, e.g. strawberries, produced the better results. They were easier to grind into a homogeneous sample. A previous report (119) has indicated that arsenic is associated with potato peel;

(c) the ICP-MS result takes into account a mathematical correction (182) for $ArCl^+$ interference which is not entirely satisfactory and may have given a false value although a positive error of the magnitude observed is unlikely. This interference correction will be discussed in more detail in Section 6.2.

Explanation (b) is supported by the finding that on peeling the potatoes better agreement between the methods is achieved. Arsenic, either in the peel or in the soil on the peel, lead to the high values for the unpeeled potato on the undiluted DGC soil. The plants were not exhaustively cleaned prior to analysis as this is unlikely to occur in the average kitchen. Inadequate cleaning may have been responsible for the high beetroot and leek results.

The levels in the unpeeled potato from undiluted soil are slightly worrying and certainly might pose a dietary risk if unpeeled potatoes are not strenuously cleaned prior to consumption or if the practice of preparing wines, spirits and snacks from potato peel continues. The fact that the strawberries fruited on the high arsenic soil is also slightly worrying although the levels are not a serious cause for concern. The FAO/WHO recommends a maximum daily intake of 3.5 mg of arsenic for a 70 kg individual. If 200 g of these strawberries were eaten the daily intake would still only be 96 μg.

The results are in good agreement with previous findings for arsenic in plants (95, 115, 119, 183) e.g. reported values for cabbage are 0.19-0.34 mg kg^{-1} (119), dry matter, d.m., <0.01-0.02 mg kg^{-1} fresh weight, f.w., (95) and 0-2.0 mg kg^{-1}, f.w. (183) compared to the findings in this study of between 0.5-1.7 mg kg^{-1}, d.m. Indeed in a comprehensive review of arsenic in fruit and vegetables (183) there were no results for untreated samples greater than 8 mg kg^{-1} and the highest value was 83 mg kg^{-1} in treated potato peelings, findings which are in accordance with the outcome of this study.

Having analysed the plants for their total arsenic content they were then re-analysed to determine the arsenic species present.

4.4 The Determination of Arsenic Species in Fruit and Vegetables

4.4.1 Introduction

The results of the preceding study indicated only a small percentage of arsenic in soil is taken up by plants. To get a more accurate picture of the dietary risk posed by arsenic it is necessary to establish what species are present in the plant i.e. are toxic species predominant? To this end two extraction procedures were applied to the plant materials and the extracts analysed by HPLC-ICP-MS.

4.4.2 Materials and Methods

The first requirement was to develop an extraction procedure

that removed the arsenic species unaltered from the plant. In a visit to Plymouth, Francesconi (184) reported that methanolic extraction removed over 90% of arsenic from fish. A similar percentage was removed from freeze-dried dogfish by a chloroform/methanol extraction procedure described by Beauchemin et al. (70). O'Neill and Bancroft (119) observed that the extraction efficiency was much lower for many plant materials, e.g. 10-15% for potatoes. They also investigated water, 0.1M sodium hydroxide, 6M hydrochloric acid, chloroform/methanol and trichloroacetic acid, but ultimately selected methanol as the extracting solution. Pyles and Woolson (118) used a methanol/chloroform/water extraction procedure that gave recoveries between 15-100%. For this study a water extraction and a methanol/chloroform extraction based on the method of Beachemin et al. (70) were performed. The procedures are described below:

(a) Water Extraction

1-2 g of the freeze dried plant were accurately weighed into clean beakers, 10 ml of double de-ionised water added and the mixture sonicated for four hours. The resulting sludge was then centrifuged at 3000 rpm for 20 minutes. The supernatant was gravity filtered into a round bottomed flask, rotary evaporated and redissolved in 1 ml of water. 175 μl aliquots of this solution were then analysed by the HPLC-ICP-MS method described in Section 3.4, in some cases after a further filtration.

(b) Chloroform/Methanol Extraction

1 g of the freeze dried plant was weighed into a clean plastic centrifuge tube and 10 ml of Aristar methanol and 5 ml of Aristar chloroform (both BDH Chemicals, Poole, Dorset) added. The tubes were sealed and sonicated for 30 minutes. The mixture was then centrifuged for 10 minutes at 2000 rpm and the supernatant decanted into a 100 ml separating funnel. The extraction was repeated on the residue. To the combined supernatants 10 ml of Aristar chloroform and 10 ml of double de-ionised water were added and the funnel shaken vigorously. The funnel was left to stand to allow phase separation. The chloroform layer was run-off, rotary evaporated and redissolved in 2 ml of water. Similarly the methanol/water layer was rotary evaporated to dryness and redissolved in 2 ml of water. Aliquots (175 μl) of both extracts were analysed by the previously described HPLC-ICP-MS method. Sub-samples (1 ml) of the extracts were removed for analysis by GFAAS. The operating conditions are given in Table 4.4.

Additionally using method (b) five separate samples of peeled potato from the undiluted soil were analysed to assess the homogeneity of the sample.

Throughout the extractions appropriate blanks were performed, and recovery monitored by spiking the samples with known concentrations of arsenic species.

4.4.3 Results and Discussion

Early analyses of the products of the extraction showed no arsenobetaine was present so a slight modification to the mobile phases, namely reducing the pH to 6.0, was undertaken to improve the chromatography for the inorganic species. The results are shown in Table 4.8. No arsenic species were detected in the blanks or the chloroform layer from extraction (b). An example of a blank, a standard and a plant extract are given in Figure 4.2. The 'Int' value given in Figure 4.2a is the full scale deflection in area counts per seconds (acps). Thus the scale in Figure 4.2b has been enlarged 40 times which accounts for the high apparent noise. The hump prior to the arsenate peak was chloride breaking through the column. The TRA software was used throughout these surveys to monitor 77 u and ensure that no $ArCl^+$ was contributing to the arsenic peaks. It could not be used for quantitative analysis however as after approximately seven runs it became necessary to delete chromatograms to provide more space on the hard disc.

Overall the results are rather unsatisfactory. Extraction efficiency ranged from about 10% for potatoes and strawberries to better than 100% for the leeks. In general the aqueous extraction gave better results than the methanolic extraction but presented considerable practical

Table 4.8 Arsenic Species Extracted from Fruit and Vegetables in mg kg^{-1} of As (dry weight)

Low(‡) Potato	Unpeeled		Peeled
Species	Extraction(a)	Extraction(b)(1)	Extraction(b)(1)
DMAA	0.05	0.05 ± 0.01	<0.02
Arsenite	0.07	0.15 ± 0.03	<0.02
MMAA	<0.05	<0.05	<0.05
Arsenate	0.06	<0.05	<0.05

(1) These extracts had a total arsenic concentration of <0.06 mg kg^{-1} (by GFAAS)

High(*) Potato	Unpeeled		Peeled
Species	Extraction(a)	Extraction(b)(1)	Extraction(b)(2)
DMAA	0.11	0.05 ± 0.01	<0.02
Arsenite	3.8	1.03 ± 0.23	0.12 ± 0.01
MMAA	<0.05	1.27 ± 0.23	<0.05
Arsenate	0.33	0.36 ± 0.10	0.10 ± 0.03

(1) This extract had a total arsenic concentration of 2.8 ± 0.01 mg kg^{-1} (by GFAAS)

(2) This extract had a total arsenic concentration of <0.06 mg kg^{-1} (by GFAAS)

Low Cabbage		
Species	Extraction(a)	Extraction(b)
DMAA	<0.02	0.06 ± 0.01
Arsenite	0.41 ± 0.02	0.41 ± 0.02
MMAA	<0.05	0.34 ± 0.08
Arsenate	0.2 ± 0.03	0.05 ± 0.01

(‡) Low refers to samples from the diluted soil

(*) High refers to samples from the undiluted soil

Table 4.8 (continued) **Arsenic Species Extracted from Fruit and Vegetables in mg kg^{-1} of As (dry weight)**

Rumleigh Carrots		Low Leek
Species	Extraction(b)	Extraction(a)
DMAA	0.72 ± 0.07	0.88 ± 0.3
Arsenite	0.1 ± 0.02	1.11 ± 0.01
MMAA	0.43 ± 0.09	<0.05
Arsenate	<0.05	1.4 ± 0.01

Rumleigh Rhubarb	
Species	Extraction(b)
DMAA	0.18 ± 0.05
Arsenite	0.05 ± 0.01
MMAA	0.19 ± 0.03
Arsenate	<0.05

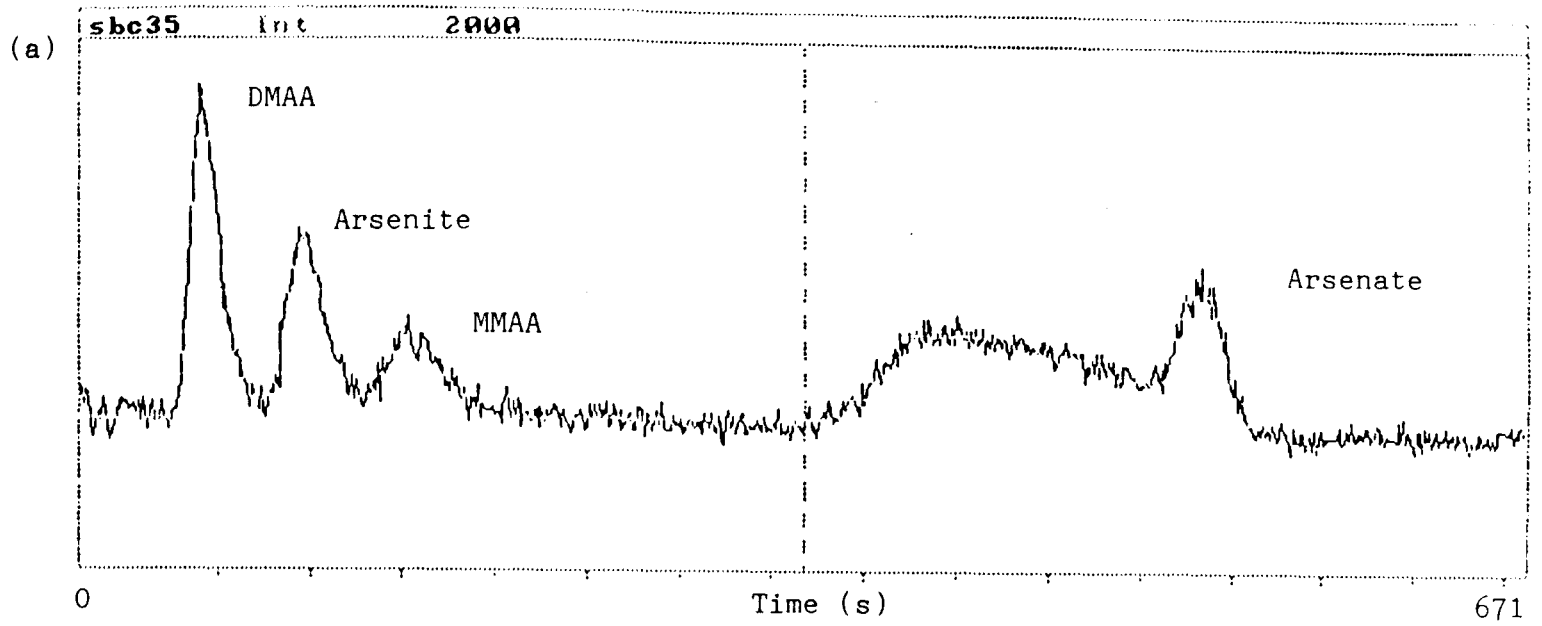
Low Beetroot	Unpeeled	Unpeeled
Species	Extraction(b)	Extraction(b)
DMAA	0.31 ± 0.09	<0.02
Arsenite	0.1 ± 0.02	0.1 ± 0.02
MMAA	<0.05	<0.05
Arsenate	0.09 ± 0.01	0.29 ± 0.02

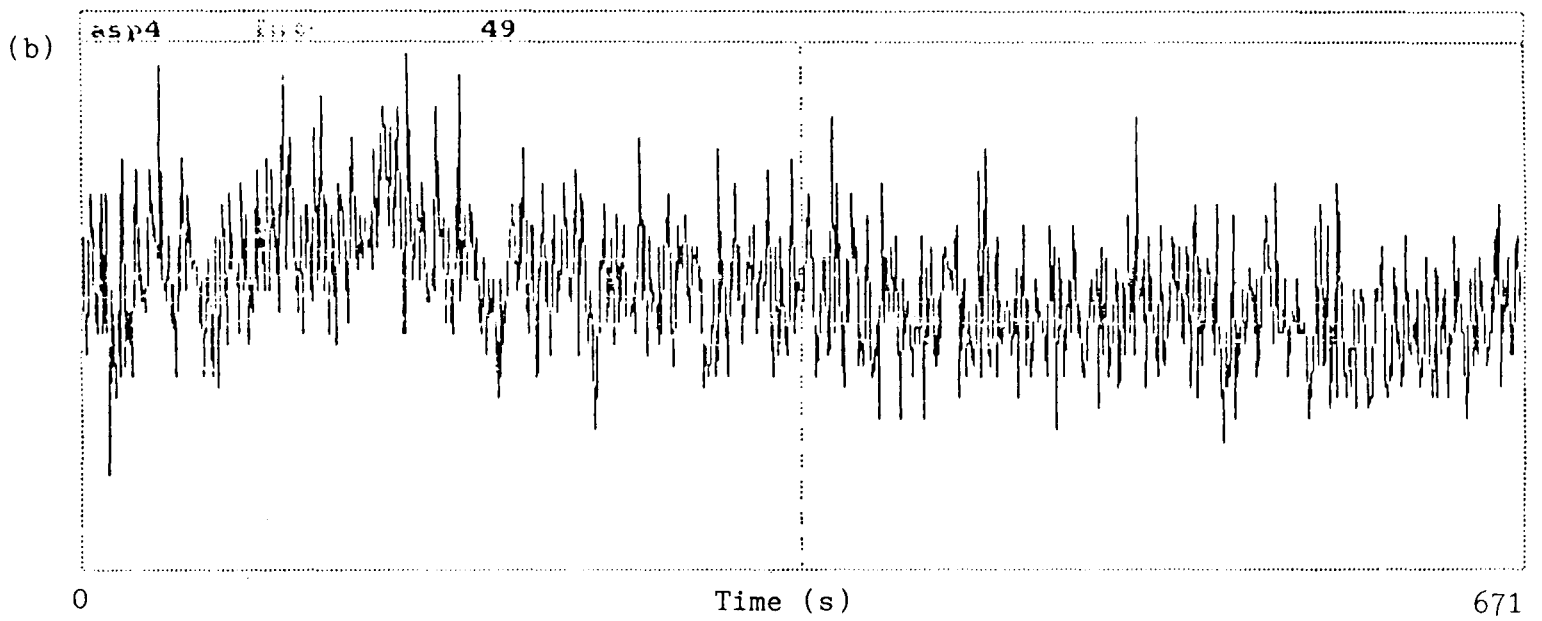
The extracts had total arsenic concentrations of <0.06 $mgkg^{-1}$

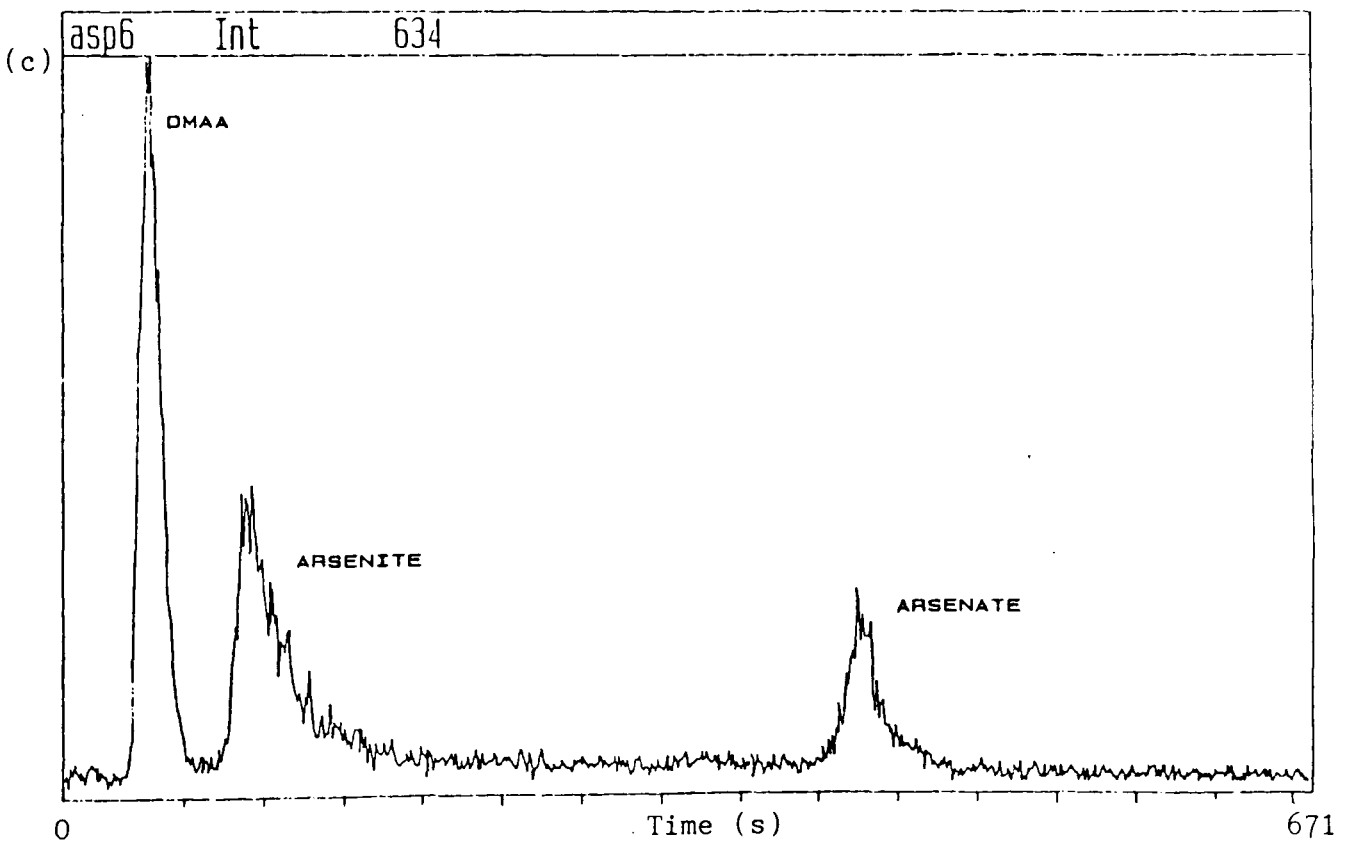
Low Strawberry		High Strawberry
Species	Extraction(b) (1)	Extraction(b) (2)
DMAA	0.04 ± 0.01	0.06 ± 0.01
Arsenite	0.08 ± 0.01	0.21 ± 0.01
MMAA	<0.05	<0.05
Arsenate	0.04 ± 0.01	0.3 ± 0.1

(1) This extract had a total arsenic concentration of 0.11 ± 0.05 mg kg^{-1} (by GFAAS)
(2) This extract had a total arsenic concentration of 0.11 ± 0.05 mg kg^{-1} (by GFAAS)

Figure 4.2 (a) Mixed standard containing 100 mg As l^{-1} of each species
(b) Extraction(a) reagent blank and
(c) Arsenic species in Leek using extraction (a)







difficulties. To prevent excessive dilution of the sample only small volumes of extracting solution were added. However, as might be predicted, the addition of an aqueous solution to a freeze-dried sample serves only to rehydrate the sample and give a very unmanageable 'slush'. This was particularly evident when the trypsin digestion, which will be described in Chapter 5, was applied to some of the samples. Even ultracentrifugation and repeated filtration failed to give a clear sample and when this was analysed the recoveries were lower than for the other methods.

Recoveries of arsenic species spiked into the plant species were between 70-80%. This gives an indication that nothing in the sample is interfering significantly with the determination, but does not give an indication of the extractability of the species as it cannot be spiked into the cell. The difference in results between the plants and fish for method (b) is presumably a function of the difference in cell wall structure between plants and animals. Higher extraction efficiencies might be possible if appropriate enzymes are applied to the plants, e.g. amylases to attack the starch in potatoes and cellulases to degrade the plant cell wall.

The highest recorded result, 3.8 mg kg^{-1}, was for arsenite in the unpeeled 'high' potato, using method (a). Lower recoveries were recorded using method (b), although the concentration of MMAA increased, and still lower recoveries

were found when the potatoes were peeled. These results contradict the findings of Pyles and Woolson (118) who recovered 90% of the total arsenic in potato peel, of which more than a third was present as arsenate. They postulated that the remaining arsenic was present as an unidentified organic species. In contrast to the present study DMAA and arsenite were absent in their crops. It is unlikely that there were any unidentified species in our methanol\chloroform\water extraction as the sum of the individual species equated well with the total arsenic in the extract found by GFAAS. However as all the other GFAAS results appear to be low, probably as a result of concomitant extraction of phosphate, (173), the absence of further arsenic species cannot be ruled out unequivocally. The results for both the high and low potato again demonstrate that the majority of the arsenic is either in the peel or in the soil not washed from the potatoes when they were cleaned.

Interestingly, those plants that contained the highest levels of methylated species were those in which the edible portion was in direct contact with the soil i.e. potatoes, carrots, leeks. The species recovery for both leeks and carrots were much higher than their total arsenic concentration and again this was probably due either to the limitations of the method used for determination of total arsenic or to soil mixed in with the sample when it was extracted. Certainly leeks are extremely difficult to clean.

Pyles and Woolson (118) observed that since no MMAA was present in the soil but it was recovered in some of the plants it may be a plant metabolite. In a previous examination of the soils used in this study Haswell (21) found methylated arsenic species thus it is difficult to speculate on the presence of metabolites, although it is worth noting that when the potatoes were peeled, i.e. all sources of contamination removed, no methylated species were recovered. However Haswell found no evidence of DMAA in soil pore water, a species which was in relative abundance in some of the plants, such as the leeks. In future work the scrupulous cleaning of plants prior to analysis would give an indication whether methylated arsenic species are a product of the plants metabolism or a result of soil contamination. Additionally, in future studies the nature of arsenic species in the soil pore water and the soil matrix should be determined and correlated with the arsenic species found in the plants.

Soil contamination cannot be blamed alone for the variability of the results. The repeat extractions of the potato from the undiluted soil showed a high variation for both arsenite and arsenate. The results are given in Table 4.9. This sample came from a different population to that in Table 4.8 and the biological variation between samples is clearly demonstrated.

Table 4.9 Replicate Extractions of a Peeled Potato from the Undiluted Soil. Results in mg As kg^{-1} (dry weight)

Sample	Arsenite	Arsenate
1	0.37 ± 0.02	0.22 ± 0.05
2	0.44 ± 0.03	0.22 ± 0.03
3	0.54 ± 0.05	0.16 ± 0.02
4	0.51 ± 0.05	0.31 ± 0.03
5	0.58 ± 0.06	0.22 ± 0.05
$\bar{x}$ ± sd	0.49 ± 0.08	0.23 ± 0.05

O'Neill and Bancroft (119) observed a difference in the arsenite/arsenate ratio in root and non-root crops, with arsenite predominating in non-root crops. In this study arsenite predominated in most crops, being in a 9:1 excess in extract (a) of the unpeeled high potato. This has slightly worrying implications for human health as arsenite is the more toxic species, despite the actual concentration being too low to exceed the FAO/WHO recommended daily maximum arsenic intake.

4.5 Conclusions

The HPLC-ICP-MS method described in Chapter 3 has been applied to plant foodstuffs grown on soils containing various concentrations of arsenic. Both methylated and inorganic species were quantified in the plants, with arsenite being the predominant inorganic species. The extraction efficiencies were generally low, and a better extraction method needs to be developed, possibly based on enzyme action. With one exception the concentration of the arsenic species were below 1.5 mg kg^{-1} d.m., and, therefore, represent no dietary risk.

The only dietary risk is posed by unpeeled potatoes from the undiluted DGC soil. The high arsenic levels presumably arose from soil collected on the peel and therefore the risk can be eliminated by careful cleaning of the potatoes .

Most crops failed to grow, or grew in a stunted fashion, on the soils containing elevated levels of arsenic. Such

stunted crops are quite unsuitable for retail sale and so any potential risk is further reduced.

Thus it can be concluded that provided the varieties studied are washed, these food crops grown on soils containing high arsenic concentrations present little or no threat to human well-being.

5. THE DETERMINATION OF ARSENIC AND ARSENIC SPECIES IN FISH

5.1 Introduction

Fish and marine-based products are the major source of arsenic in the human diet (12; 53; 63; 95; 110). Since the turn of the century (52; 53) fish and shellfish were known to contain relatively high levels of arsenic, being in excess of 1 mg kg^{-1}. In 1926 Chapman (53) proposed that the arsenic compound in lobster was a low toxicity organic molecule, although it was not until 1977 that Edmonds et al. (57) identified the molecule as arsenobetaine, a compound that is now known to be widely distributed in marine organisms (57-72). Although the total arsenic concentration in fish and shellfish from UK waters has been determined (95), no attempt has been made to identify the nature of the arsenic species present. The following chapter addresses this problem. The chapter also describes the results when an enzymatic digestion procedure, based on the action of trypsin, was applied to fish, the purpose of the experiment being to assess if toxic arsenic species become bioavailable during human digestion.

5.2 Materials and Methods

Duplicate samples of 'local' cod, Atlantic cod, dab, haddock, sole, mackerel, plaice and whiting were purchased from Plymouth Fish Market. The vendor prepared the fish as he would for a normal customer. In the laboratory each fish was cut into small pieces and freeze dried (Edwards Super

Modulyo, Edwards High Vacuum, Crawley, Sussex). The weight loss on drying of each fish was recorded. The dry fish were then ground in a pestle and mortar, thoroughly mixed and sub-sampled for analysis. The bulk of the dried sample was stored in a dessicator until required.

Total arsenic was determined by taking between 0.1-0.2 g of the dry sample accurately weighed into a PTFE digestion bomb, and adding 2 ml of Aristar nitric acid (BDH Chemicals Ltd, Poole, Dorset). The samples were left to pre-digest overnight and were then digested in a domestic microwave oven (Toshiba, Model No. ER-761E, Plymouth, England) using the following programme:

Setting (2)	Warm	5 min
Setting (7)	Roast	5 min
Setting (9)	Full Power	3-5 min

The samples were then diluted to 10 or 100 ml with double de-ionised water (Millipore Corp., Bedford, MA, USA), which was used throughout. The samples and matrix matched standards were spiked at 100 $\mu g l^{-1}$ indium (Aldrich AAS standard, Aldrich Chemical Co., Milwaukee, USA) and analysed by ICP-MS using the conditions previously described. A dogfish reference material (DORM-1, Canadian Research Council, Ottawa, Canada) was simultaneously analysed to maintain quality control. Arsenic species in the fish were determined following two different extraction procedures,

method (1) being a variation on the method previously described by Beauchemin et al. (70) and method (2) being based on an enzymatic digestion described by Dean et al. (112) and used by the Food Science Laboratory at Norwich. The details of both methods are given below.

Method (1) Approximately 1.0 g of the dry sample was accurately weighed into a glass beaker, 20 ml of methanol and 10 ml of chloroform added (both Aristar, BDH, Dorset) and after covering, the samples were sonicated for an hour. The samples were then transferred to centrifuge tubes and centrifuged for 10 min at 2500 rpm. The supernatants were transferred into a 100 ml separating funnel and the process repeated on the remaining pellet. Chloroform (20 ml) and water (20 ml) were then added to the combined supernatants in the separating funnel and it was shaken vigorously. The funnel was left to stand to allow phase separation. The chloroform layer was run off and the water\methanol layer filtered (Whatman 541 filter paper, Whatman, Maidstone, Kent, UK) into a 100 ml round-bottom flask. The solvent was rotary evaporated and the samples redissolved in 10 ml or for plaice, 20 ml. The samples were then filtered (0.45 μm, Millipore Corp., Bedford, MA, USA) into plastic storage bottles prior to analysis by HPLC-ICP-MS. The samples were refrigerated at 4°C if there was a delay in analysis. Recovery experiments and determinations of arsenic in the solid residue and the chloroform layer were also performed on some of the samples. The results are presented in Section 5.3.

Method (2) Approximately 1 g of each sample was accurately weighed into a plastic centrifuge tube, along with 100 mg of trypsin (Sigma Chemical Co., Poole, Dorset). Ammonium carbonate solution (20 ml, 0.1M, AnalaR, BDH, Poole) was added, the tube sealed and placed in a shaking water bath for 4 hours at 37°C. The samples were then ultracentrifuged at 11000 rpm for 20 min (MSE Highspeed 18, MSE Scientific Instruments, Crawley, Sussex). The samples were then filtered and refrigerated prior to analysis by HPLC-ICP-MS. As with method (1) recovery tests were performed and some of the residues analysed for total arsenic concentration.

Method (2) is based on the work of Crews et al. (185; 186), later modified by Dean (112), on the development of an extraction method which mimicked human digestion and thus gave an indication of the bioavailability of elemental species. The method of Dean et al. (112) was chosen in preference to that of Crews et al. because the latter is relatively complex and time consuming and because reported recoveries (185) of a variety of trace elements from fish varied between 0-55%, being on average less than 18%. Dean et al. (112) noted that the recovery of the arsenic species, roxarsone from chickens, rose from 3-15% to 44-112% when trypsin digestion was used rather than the two stage enzymolysis described by Crews et al. (185; 186).

In addition to the procedures already described total

inorganic arsenic (As_i) was determined in some of the extracts by HGAAS using the conditions detailed in Table 5.1. Prior to analysis the samples were made 10% (w/v) with respect to potassium iodide.

5.3 Results and Discussion

The moisture contents of the various fish are shown in Table 5.2. In most cases the results for two separate fish are presented and these are represented by (1) and (2) in the table. All the fish, with the exception of mackerel, contained greater than 70 percent water. The lower result for the mackerel may be related to the 'oily' nature of the fish. This characteristic will be discussed later.

The results for extraction methods (1) and (2) are presented in detail in Tables 5.3 and 5.4. The total arsenic concentration in pairs of fish of the same species were similar to one another, the only exception being lemon sole. It is interesting that the two cod specimens, one coming from the Atlantic, the other from waters around Plymouth, had markedly different arsenic concentrations in their tissues. Other authors (63; 69) have also noted that the arsenic content of the same species of fish varies with the locality at which they were caught. This may be taken as tenuous evidence in support of Edmonds and Francesconi's (43) theory that arsenobetaine does not arise within higher marine animals endogenously, but is derived from microbial action upon algae within sediments releasing arsenobetaine to the water column. If this theory is correct then fish

Table 5.1 Operating Conditions for Hydride Generation Atomic Absorption Spectrometry

Wavelength	193.7 nm
Bandpass	1 nm
Damping	2 s
Lamp current	9 mA
Air flow	5 l min^{-1}
Acetylene flow	1.5 l min^{-1}
Reducing agent concentration	1% $NaBH_4$ (m/v) in 0.1M sodium hydroxide
Acid concentration	4M HCl

Background correction and scale expansion on

Table 5.2 Moisture Content of Selected Fish (in % w/w)

Fish		Moisture
Atlantic cod		85.6
'Local' cod		86.1
Dab	(1)	73.8
	(2)	72.9
Haddock		77.3
Lemon sole	(1)	75.8
	(2)	77.2
Mackerel	(1)	59.6
	(2)	62.6
Plaice	(1)	81.4
	(2)	74.3
Whiting	(1)	69.6
	(2)	75.5

Table 5.3 Arsenic Species Extracted by Method 1. All Results in mg As kg^{-1} dry weight

Sample	Total Arsenic	AB	DMAA	Arsenate	As_i	Total As in Residue	Total As in MeOH/H_2O phase	Total As in $CHCl_3$ phase
DORM-1	17.8 ± 0.7	15.1 ± 0.6	NP	NP	NP	NP	NP	NP
Atlantic cod	12.8 ± 0.8 14.6 ± 0.6	13.2 ± 0.6	<0.3	<0.5	NP	0.6 ± 0.01	1.0 ± 0.1	0.09 ± 0.01
Local cod	25.5 ± 1.1 28.7 ± 0.2	31.2 ± 0.8	<0.3	<0.5	NP	1.3 ± 0.01	22.5 ± 0.8	0.3 ± 0.03
Dab (1)	28.3 ± 0.4	26.9 ± 1.9	<0.3	<0.5	2.3	NP	NP	NP
Dab (2)	30.7 ± 0.2	27.5 ± 1.8	<0.3	<0.5	<0.2	NP	NP	NP
Haddock	57.2 ± 3.4 67.1 ± 2.1	59.3 ± 2.1	<0.5	<1.0	NP	2.5 ± 0.04	53.4 ± 1.1	<0.05
Lemon sole(1)	172.9 ± 2.6	145.6 ± 12.8	<0.5	<0.5	4.5	NP	NP	NP
Lemon sole(2)	149.6 ± 15.1	102.6 ± 19.4	<0.5	<0.5	6.8	NP	NP	NP
Mackerel (1)	4.4 ± 0.9	1.1 ± 0.3	0.5 ± 0.1	<0.5	NP	0.5 ± 0.03	1.5 ± 0.8	0.08 ± 0.01*
Mackerel (2)	3.5 ± 0.3	1.0 ± 0.1	0.3 ± 0.1	1.1 ± 0.8	NP	0.5 ± 0.02	2.0 ± 0.2	0.08 ± 0.01*
Plaice (1)	196.1 ± 3.5	187.3 ± 20.3	<0.5	<1.0	4.8	NP	NP	NP
Plaice (2)	183.1 ± 1.6	102.9 ± 13.6	<0.5	<1.0	4.2	NP	NP	NP
Whiting (1)	15.9 ± 0.5	10.4 ± 1.8	<0.3	<0.5	1.2	NP	NP	NP
Whiting (2)	16.4 ± 0.2	9.8 ± 0.7	<0.3	<0.5	<0.2	NP		

Certificate Values for DORM-1: Arsenobetaine = 15.7 ± 0.8 mg kg^{-1}
Total Arsenic = 17.7 ± 2.1 mg kg^{-1}

NP = Not Performed
AB = Arsenobetaine
DMAA = Dimethylarsinic acid

* These results should be regarded as "water-leachable" arsenic

Table 5.4 Arsenic Species Extracted by Method 2
All results in mg As kg^{-1} dry weight

Sample	Total As	AB	DMAA	Arsenate	Total As in Residue	Total As in Extract
DORM-1	17.8 ± 0.7	16.1 ± 0.4	NP	NP	NP	NP
Atlantic cod	12.8 ± 0.8 14.6 ± 0.6	13.5 ± 0.9	<0.5	<1.0	2.3 ± 0.1	13.2 ± 0.6
Local cod	25.5 ± 1.1 28.7 ± 0.2	26.9 ± 1.2	<0.5	1.0 ± 0.4	3.7 ± 0.1	29.6 ± 2.0
Dab (1)	28.3 ± 0.4	40.0 ± 5.7	<0.5	2.4 ± 0.3	NP	NP
Dab (2)	30.7 ± 0.2	41.4 ± 0.4	<0.5	<1.0	NP	NP
Haddock	57.2 ± 3.4 67.1 ± 2.1	50.0 ± 2.1	<0.5	<1.0	15.7 ± 0.2	68.0 ± 3.0
Lemon sole(1)	172.9 ± 2.6	183.2 ± 14.1	<0.5	<1.0	NP	NP
Lemon sole(2)	149.6 ± 15.1	77.0 ± 0.2	<0.5	<1.0	NP	NP
Mackerel (1)	4.4 ± 0.9 3.8 ± 0.1	0.8 ± 0.1	0.4 ± 0.1	0.2 ± 0.1	2.8 ± 0.2	2.1 ± 0.2
Mackerel (2)	3.5 ± 0.3	0.2 ± 0.01	0.4 ± 0.1	0.3 ± 0.1	1.4 ± 0.2	2.3 ± 0.1
Plaice (1)	196.1 ± 3.5	106.0 ± 19.0	<0.5	<1.0	NP	NP
Plaice (2)	183.1 ± 1.6	77 ± 5.0	21.6 ± 2.3	<1.0	NP	NP
Whiting (1)	15.9 ± 0.5	12.1 ± 0.2	<0.5	1.3 ± 0.2	NP	NP
Whiting (2)	16.4 ± 0.2	15.8 ± 1.3	<0.5	<1.0	NP	NP

NP = Not Performed

Certificate Values for DORM-1: Arsenobetaine = 15.7 ± 0.8 mg kg^{-1}
Total Arsenic = 17.7 ± 2.1 mg kg^{-1}

AB = Arsenobetaine DMAA = Dimethylarsinic acid

from different areas are likely to contain different concentrations of arsenobetaine as the products of microbial action will vary from site to site. The flatfish surveyed in this study i.e. dab, plaice, sole, were found to contain the highest levels of arsenic. This adds further weight to Edmonds and Francesconi's theory as all of these fish are bottom feeders and are highly carnivorous feeding on the bivalves and molluscs which filter feed on the sea beds. Of course it is also possible that the variation in arsenic concentration of the two cod specimens may be related to age or health status. The weight of the two specimens was approximately the same.

The arsenic concentration in some of the fish was very high *e.g.* in plaice in excess of 180 mg kg^{-1}. However these concentrations are not unprecedented. Luten *et al.* (69) found the concentration of arsenic in plaice from various locations in the North Sea to be in the range 3-166 mg kg^{-1}. The study values are higher than those reported by the Ministry of Agriculture Fisheries and Food (95) for plaice from UK coastal waters who found the arsenic content of fresh plaice to vary between 1-20 mg kg^{-1}. The arsenic content of the Lemon Sole also expected to be high as Luten *et al*. (61) have reported values of 24.9-31.4 mg kg^{-1} fresh weight which correlates well with the findings of this study, approximately 150-173 mg kg^{-1} dry weight. The results for this study for cod, dab and haddock agree well with the MAFF report for UK waters (95). The mackerel

results, 3.5- 4.4 $mgkg^{-1}$ dry weight, are in good agreement with a number of surveys of fish from Scottish waters (95), the results for which lay in the range 0.2-1.6 $mgkg^{-1}$ fresh weight. The results of this study for cod, haddock, mackerel and sole are all similar to the results for the same fish, caught in the Atlantic, reported by Lawrence et al. (63).

Using both extraction methods the observed major arsenic species in the cod specimens, dab, haddock, lemon sole, plaice and whiting was arsenobetaine. Using the methanol/chloroform and trypsin method arsenobetaine comprised nearly 100 percent of the arsenic in both the cod samples, haddock and the second whiting which was trypsin extracted. Using the described extraction methods no arsenite or MMAA was identified in any of the extracts. In the chloroform phases from method (1) no individual species were identified except for 350 ± 100 ng kg^{-1} of arsenobetaine from the local cod.

In the methanol/water phases extracted by method (1) the total arsenic determined by ICP-MS was less than the sum of the individual species. This was probably because the methanol in the samples, approximately 5 percent v/v, suppressed the arsenic signal and gave low recoveries. The indium signal was unaffected suggesting indium is not a good internal standard for this application. Attempts to matrix match the samples proved fruitless as the samples had started to undergo microbial degradation. Methanol was not

spiked into the standards in the first instance as the exact amount in the samples is not known due to evaporative losses.

Using method (1) the most interesting results were obtained for the mackerel. The sum of the individual species, the arsenic in the extracted residue and the arsenic in the chloroform phase was less than the total arsenic determined in the original fish. This was almost certainly because, mackerel being an oily fish, it contains a significant proportion of its arsenic lipid bound. This arsenic would be extracted into the chloroform layer. Only 50 percent of the original mackerel sample, i.e. 0.5 g, was left following extraction compared to approximately 90 percent, i.e. 0.9 g, of the cod and haddock samples. After evaporation of the chloroform from the separated chloroform layer a viscous orange solid was left. This solid proved insoluble in water, which was decanted and analysed by ICP-MS to give the figure in the "total arsenic in $CHCl_3$ phase" column in Table 5.3, which should be more accurately described as "water-leachable" arsenic. Attempts to digest the solid with nitric acid proved unsuccessful as the sample charred. The characterisation of the arsenic species in the chloroform fraction would prove an interesting area of future study. If some of the arsenic in mackerel was lipid-bound it would explain why low recoveries of arsenic were recorded for mackerel using method (2). Trypsin, a protease, would be unable to break down such a compound.

Since the sum of the individual species was less than the total arsenic in the extract, e.g. 1.4 and 2.1 mg kg^{-1} respectively for mackerel 1, this might indicate that some of the lipid-bound arsenic was extracted but remained bound to the column. During the fish analyses the column had to be periodically reversed and flushed to overcome pressure build-up resulting from the accumulation of material on the column. The discrepancy between the sum of the arsenic species and the total arsenic determined by ICP-MS for lemon sole, plaice and whiting may be indicative of the fish containing arsenic in a lipid-bound form. However if this was so then the same species of fish might be expected to give similar results which was clearly not the case for lemon sole and plaice. The failure to determine arsenic in the residues and chloroform layers of all the extracts was an oversight which should not be repeated in any future work. Using extraction method (1) DMAA was identified only in the mackerel at levels which would be insignificant to the consumer. The presence of 1.1 mg kg^{-1} of arsenate in the second mackerel is slightly more worrying although the high standard deviation should be noted.

The results obtained for total inorganic arsenic in the solutions extracted by method (1) are surprising since no arsenite or arsenate was detected in the samples by HPLC-ICP-MS. It is possible that the inorganic arsenic arose from microbially mediated degradation of more complex arsenic species in the extract. The HGAAS analyses were performed a number of days after the HPLC-ICP-MS work and it

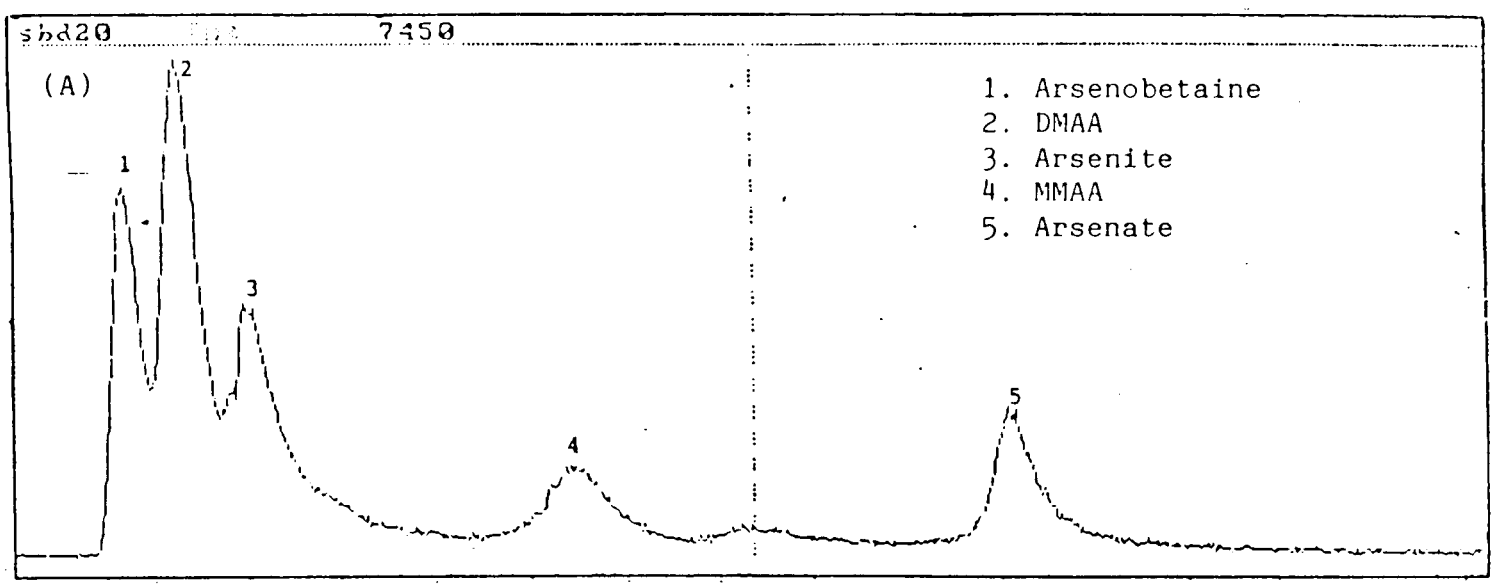
was subsequently observed that the fish extracts began to show signs of mould growth within 3-4 days of preparation, even if stored in a refrigerator. Total inorganic arsenic could not be determined in the trypsin extracts due to the formation of an insoluble precipitate in the gas-liquid separator of the hydride generator.

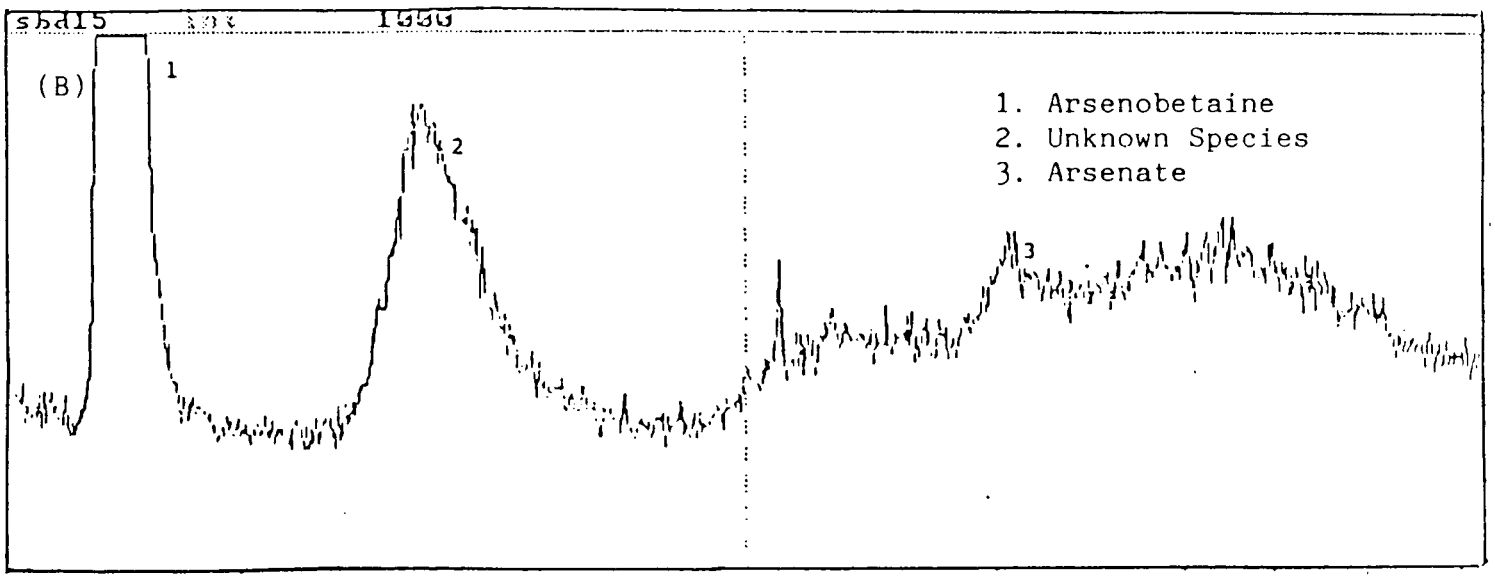
The results for the trypsin extractions are more difficult to explain than the methanol\chloroform extractions. It might be predicted that the former method would give higher extraction efficiencies than the latter as the protease would break-down the lipid\protein cell membrane and release the cell contents. This hypothesis is supported by the results for whiting and DORM-1 which gave higher recoveries with method (2). However both plaice, both mackerel and one of the lemon sole samples gave lower recoveries in the extracts. As previously discussed this may be related to the composition of the fish tissues. The result for the haddock again indicates that arsenobetaine was the predominant species in the extract. The result for total arsenic in the extract may be erroneously high as this value compared to that for the arsenic remaining in the extracted residue gives a result in excess of that for total arsenic by microwave digestion-ICP-MS. However the sum of the individual arsenic species and the arsenic remaining in the extracted residue is in accordance with the value for total arsenic in the fish.

The results for the dab samples are unsatisfactory because the arsenobetaine results were in excess of 130% of the total arsenic figure. The result is further complicated by the presence in the second dab of an unidentified arsenic species (see Figure 5.1). There is no obvious explanation for these results and the dab is clearly a fish worthy of extra study.

Method (2) is based upon the action of trypsin and therefore gives an insight into the bioavailability of arsenic species. Reassuringly trypsin did not degrade the arsenobetaine into toxic inorganic species, an observation also made by Chapman (53) when studying lobsters. However it should be noted that the action of trypsin on one of the plaice apparently degraded arsenobetaine to DMAA to give a concentration of 21.6 mg kg^{-1} which if true is a worrying result. However the plaice extracts were not analysed immediately and it is quite possible that the sample may have begun to undergo microbial degradation. The trypsin digests appeared to be a particularly good medium for microbial growth and the apparent microbial conversion of arsenobetaine to simpler arsenic species in fish extracts is an especially interesting result. This phenomena should be studied further. Why this occurred in one sample and not another is unclear. Trypsin action also produced an unidentified arsenic compound in one of the dab specimens. Research effort should be directed towards identifying this species. The levels of arsenate in the fish samples were not unacceptably high as the results are not expressed as

Figure 5.1 (A) HPLC-ICP-MS Chromatogram of Mixed Arsenic Standard.
(B) HPLC-ICP-MS Chromatogram of Methanol/Chloroform Extract from Dab 1 Showing the Presence of an Unidentified Species.





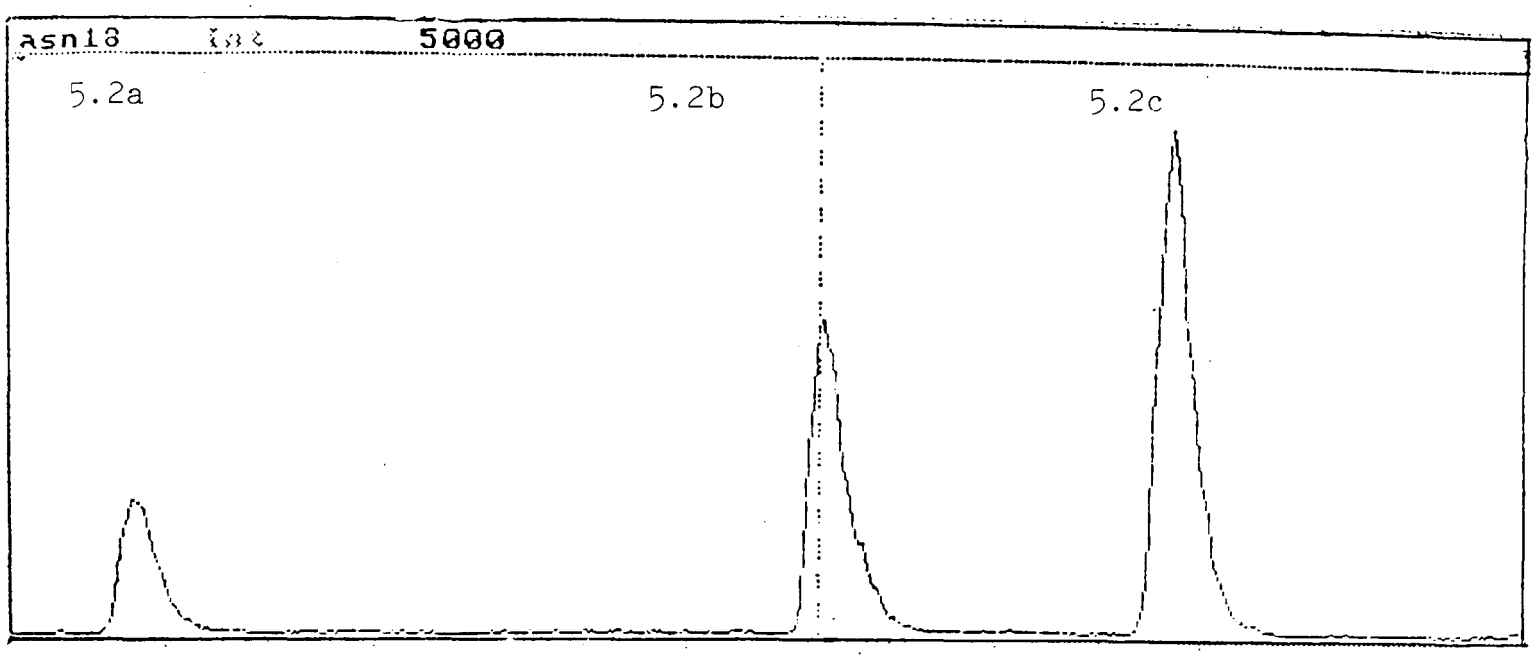
fresh weight.

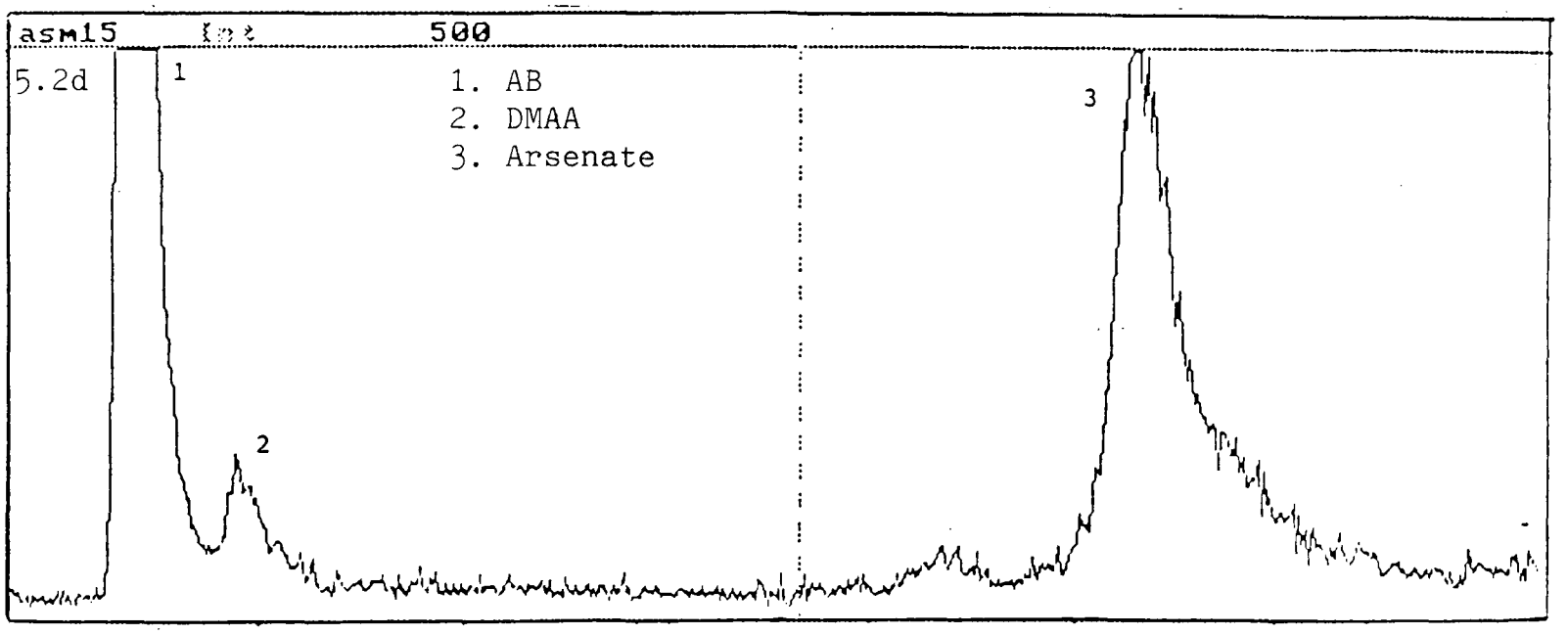
Throughout the fish study considerable effort has been expended to maintain quality control. The experimental results for the fish reference material, DORM-1, were in good agreement with the certificate values for both total arsenic and arsenobetaine. The slightly higher value for arsenobetaine by method (2), in comparison to the published value probably arose as the latter value was for arsenobetaine extracted by method (1). The recoveries of individual species spiked onto plaice samples extracted by method 1 varied between 80 percent for arsenobetaine to 108 percent for arsenate. The latter recovery was later recognised to stem from arsenate in the mobile phase accumulating on the column. Standard addition analysis of plaice 1 gave a recovery of 190 mg kg^{-1} dry weight, or 98 percent. The recovery of spikes to whiting 1 extracted by method (2) varied between 85 percent for arsenobetaine to 115 percent for arsenate. Unfortunately the recovery of spikes from every fish species was not performed which in retrospect was unwise as the results in the tables clearly demonstrated a wide range of extraction efficiencies between different fish types. Peak identity was confirmed by co-injection of standards, some results of which are shown in Figure 5.2.

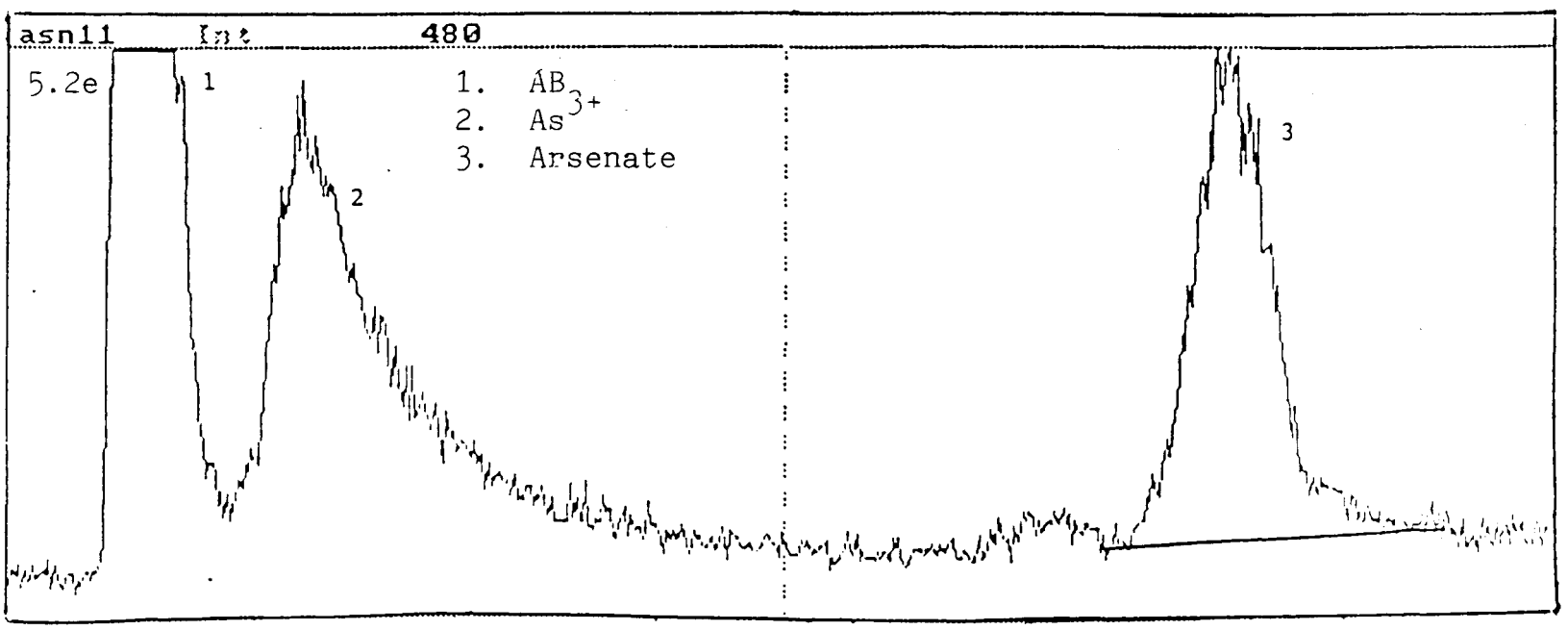
To summarise, the results of this survey show that in the fish analysed, with the exception of mackerel, the

Figure 5.2 HPLC-ICP-MS Chromatograms of Methanol/Chloroform Extracts of Fish

5.2a Plaice Extract
5.2b Plaice Extract plus 2mg l^{-1} AB Standard
5.2c Plaice Extract plus 4mg l^{-1} AB Standard
5.2d Plaice extract plus DMAA standard
5.2e Lemon Sole plus arsenite standard







predominant arsenic compound was non-toxic arsenobetaine. The toxic inorganic species, arsenite and arsenate, were negligible or below the limits of detection in all of the fish analysed. Since these fish are amongst the most popular fish eaten these are significant results when assessing dietary risk. The trypsin extraction which partially mimics the human digestive mechanism did not lead to an increase in the toxic inorganic species although disturbingly high levels of DMAA were given by one of the plaice samples possibly as a result of microbial degradation. Effort should be directed at assessing the extent of microbial conversion of arsenobetaine in fish extracts. The high rate of microbial attack probably stems from the incubation at 37°C which the extracts underwent. Furthermore an unknown arsenic species was detected in one of the dab samples. Further effort should be directed to assessing whether these results are replicated in other edible fish species.

6. THE DIETARY TRIAL

6.1 Introduction

A dietary trial was performed, with the assistance of the Catering School of Plymouth College of Further Education (CFE), to assess the affect on arsenic speciation of human metabolism. To this end volunteers were recruited and fed a series of set meals prepared at the CFE. They were asked to collect, and record the volume of, all urine samples passed during and after the meals. Full details of the design and results of the trial are given in the following sections. Preceding these sections, details of a method developed for the determination of total arsenic in urine are given.

6.2 The Determination of Arsenic in Human Urine by Inductively Coupled Plasma-Mass Spectrometry

6.2.1 Introduction

Since the development of ICP-MS one of the limiting features has been polyatomic ion interferences (182; 187), usually in the region of the spectrum below 80 u. The polyatomic ion, $^{40}Ar^{35}Cl^{+}$ is obviously a particular problem because it complicates the determination of monoisotopic arsenic, ^{75}As, in samples with a high chloride content e.g. urine. A number of approaches to overcoming this interference have been proposed. These include the co-precipitation of the chloride with silver (188), mathematical correction based on the $^{40}Ar^{37}Cl^{+}$ signal (182), chromatographic separation of the arsenic and chloride (189; 190) and hydride generation

ICP-MS (191) in which the arsenic and chloride are separated using a membrane gas liquid separator. None of these methods are particularly convenient, therefore arsenic is usually determined either by hydride generation or graphite furnace atomisation with atomic absorption spectrometry, notwithstanding the drawbacks of these techniques discussed in Chapter Two.

Recently Evans and Ebdon (192) described two methods to overcome polyatomic ion interference. In the first method small volumes of an organic solvent such as propanol were added to samples and standards. The second approach involved bleeding small flows of a molecular gas, either oxygen or nitrogen, into the argon nebuliser gas. They found that these methods reduced polyatomic interferences on ^{75}As and ^{77}Se to a substantial degree.

For the analysis of arsenic in urine samples, it was decided to investigate the suitability of the nitrogen addition method as it is both the simplest approach, and it avoids the problems associated with the introduction of organic solvents to the ICP-MS (193).

6.2.2 Materials and Methods

The ICP-MS results were obtained using a VG Elemental Plasmaquad 2 (VG Isotopes, Winsford, Cheshire, UK) which is designed to admit low flows of a second gas into the nebuliser gas via a so-called 'organic solvents kit'. The

instrument was fitted with an Ebdon V-groove high solids nebuliser (PSA Ltd., Sevenoaks, Kent, UK). Full operating conditions are given in Table 6.1. During the method development two reference materials were analysed: Seronorm reference urine (Nycomed AS, Oslo, Norway) and Sargasso seaweed (NIES No. 9, National Institute of Environmental Standards, Onogawa 16-2, Iloraki, Japan). Arsenic recovery from solutions of Aristar sodium chloride (BDH Chemicals, Poole, Dorset, UK) was also determined. All standards were prepared fresh from 100 mg As l^{-1} stock solutions of sodium arsenite (AnalaR grade, BDH). All standards and samples were spiked at 100 μg l^{-1} indium (Aldrich Chemical Co., Milwaukee, USA) and made up to volume with 2% Aristar nitric acid.

The Seronorm urine was prepared by 1:9 dilution with 2% nitric acid, and spiking with indium to a final concentration of 100 μg In l^{-1}. The Sargasso seaweed samples were prepared by digesting approximately 0.2 g of the sample in microwave digestion bombs (Savillex Corp., Minnesota, USA) containing 3 ml of nitric acid (Aristar, BDH). The following microwave (Toshiba ER-761E, Toshiba Co., Japan) protocol was followed:

Predigestion at room temperature	Overnight
Low power	5 minutes
Medium power	3 minutes

The bombs were refrigerated at 4°C and then vented between

Table 6.1 ICP-MS Operating Conditions for Nitrogen Addition

Forward power	1.6-1.8 kW
Reflected power	20 W
Ar nebuliser gas flow rate	0.82 l min^{-1}
N_2 nebuliser gas flow rate	0.03 l min^{-1}
Auxiliary gas flow rate	1.0 l min^{-1}
Coolant gas flow rate	14 l min^{-1}

100 μg l^{-1} indium was used as the internal standard

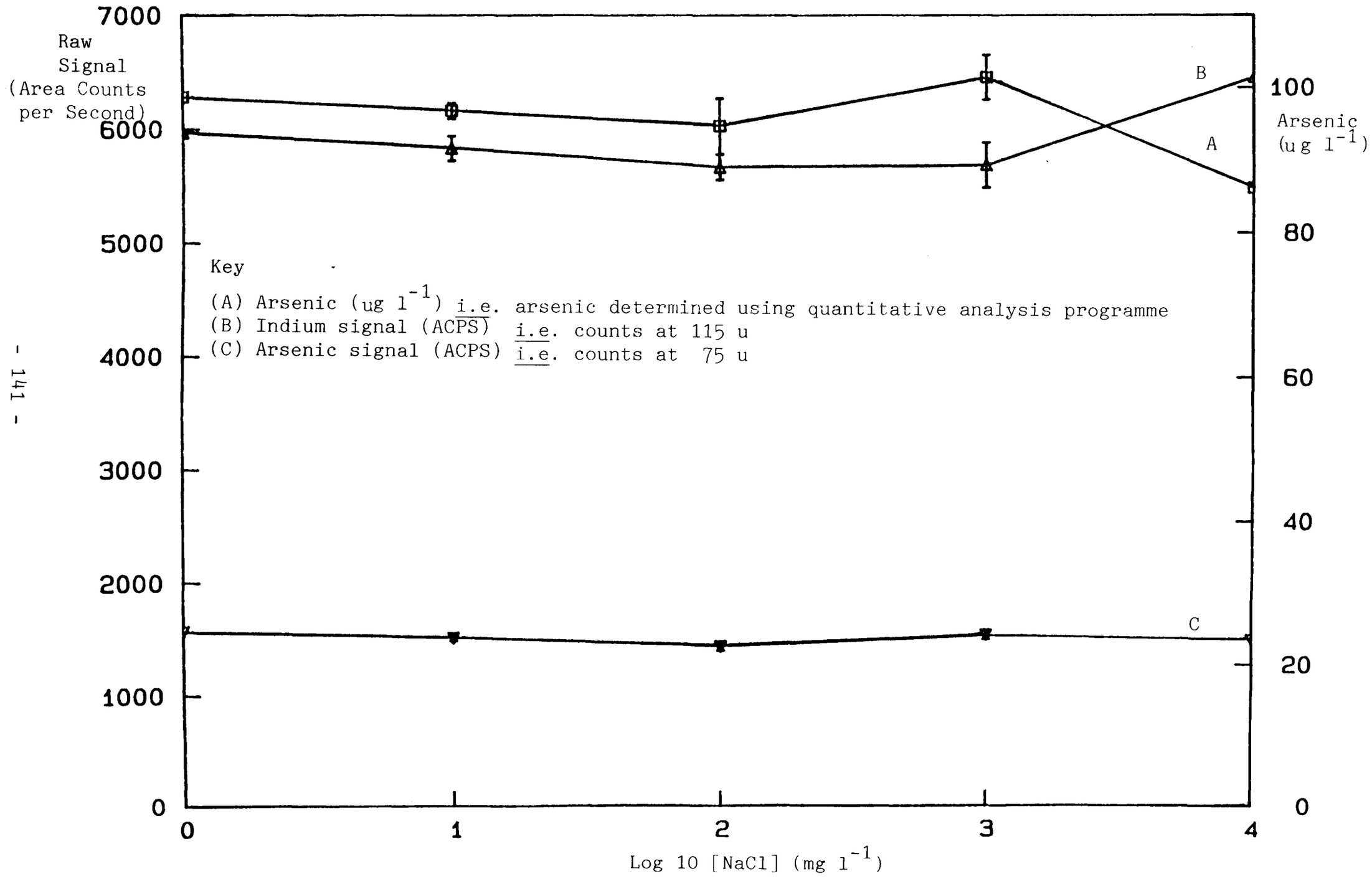
each stage. The digest was diluted with 2% nitric acid to give a final arsenic concentration below 50 $\mu g\ l^{-1}$ and spiked with indium.

6.2.3 Results and Discussion

6.2.3.1 Synthetic Sodium Chloride Solutions

To examine the validity of the experimental protocol investigations began to determine, using N_2 addition-ICP-MS, the apparent concentration of 100 $\mu g\ l^{-1}$ arsenic spikes in solutions containing 0, 10, 100 and 10,000 mg l^{-1} of sodium chloride. The sodium chloride solutions were prepared so that they could be diluted with 2% nitric acid in the same manner as the urine samples. The results are shown in Figure 6.1. The arsenic concentration was determined using the ICP-MS quantitative analysis software whilst simultaneously the raw counts for arsenic and indium were recorded to assess whether there were any enhancement, suppression or transport effects. At sodium chloride concentrations of 0, 10, 100 and 1000 mg l^{-1} arsenic quantification was within 5% of the theoretical value (plot (A) on Figure 6.1). Only at a concentration of 10,000 mg l^{-1} NaCl was a significant interference observed. This was surprisingly a result of an enhanced indium signal; up to this level the indium signal had fallen gradually which is consistent with decreased nebulisation efficiency as the dissolved salts content increased. These results indicate that chloride, i.e. as $^{40}Ar^{35}Cl$, had little effect upon the arsenic signal. This was confirmed by the signal at 77 u,

Figure 6.1 Effect of Increasing Chloride Concentration on Arsenic and Indium Signals with N_2 Addition -ICP-MS



i.e. that which arose from $^{40}Ar^{37}Cl$, not exceeding 20 acps for any of the analysed solutions. These results indicate that urine samples, containing up to 10,000 mg kg^{-1} sodium chloride, or 1 percent dissolved salt, should yield results within 15 percent of the correct value. Such high dissolved salt loadings yield other complications as the ICP-MS instrument will not tolerate such high dissolved solids on a continuous basis. Flow injection methods may, however, be used in such cases of high salt concentration.

6.2.3.2 Results for Reference Materials

The reference seaweeds and urines were analysed using normal operating procedures. The lenses, nebuliser gas flow and torch position were adjusted to give maximum response for ^{75}As. This was usually found to lie in the region 200-300,000 acps/mg As l^{-1}. The results for the urine and seaweed are given in Table 6.2. This Table also compares the values for total arsenic in the CRM Sargasso samples with and without nitrogen addition to the carrier gas. It is apparent that the certified chloride content of these samples, 5.1 percent chloride, produced a high positive bias (an approximately 30% increase above the certified arsenic value) when nitrogen gas was absent. When N_2 addition was used good agreement between certified and experimental values was obtained. The chloride levels in the urine were not certified, but normal levels are known to be in the high mg l^{-1} range (194). Again using N_2 addition excellent agreement between certified and experimental results was observed. The possible small positive bias is well within

Table 6.2 Results for the Determination of Arsenic in Reference Urine and Certified Reference Seaweed

Sample	Certified Concentration As	Concentration of As with N_2 Addition	Concentration of As without N_2 Addition
NIES Sargasso No. 9			
		Arsenic content mg kg^{-1}	
1	115 ± 9	106 ± 11 (1)	148 ± 10
2	115 ± 9	111 ± 12	150 ± 19
3	115 ± 9	103 ± 8	146 ± 8
4	115 ± 9	121 ± 11	148 ± 9
Mean	115 ± 9	110 ± 3.3 (2)	148 ± 3.1
Seronorm Urine			
		Arsenic content $\mu g\ l^{-1}$	
1	200 (3)	201 ± 6	ND
2	200	214 ± 17	ND
3	200	219 ± 16	ND
4	200	195 ± 10	ND
Mean	200	207 ± 4.3	

ND = Not Determined

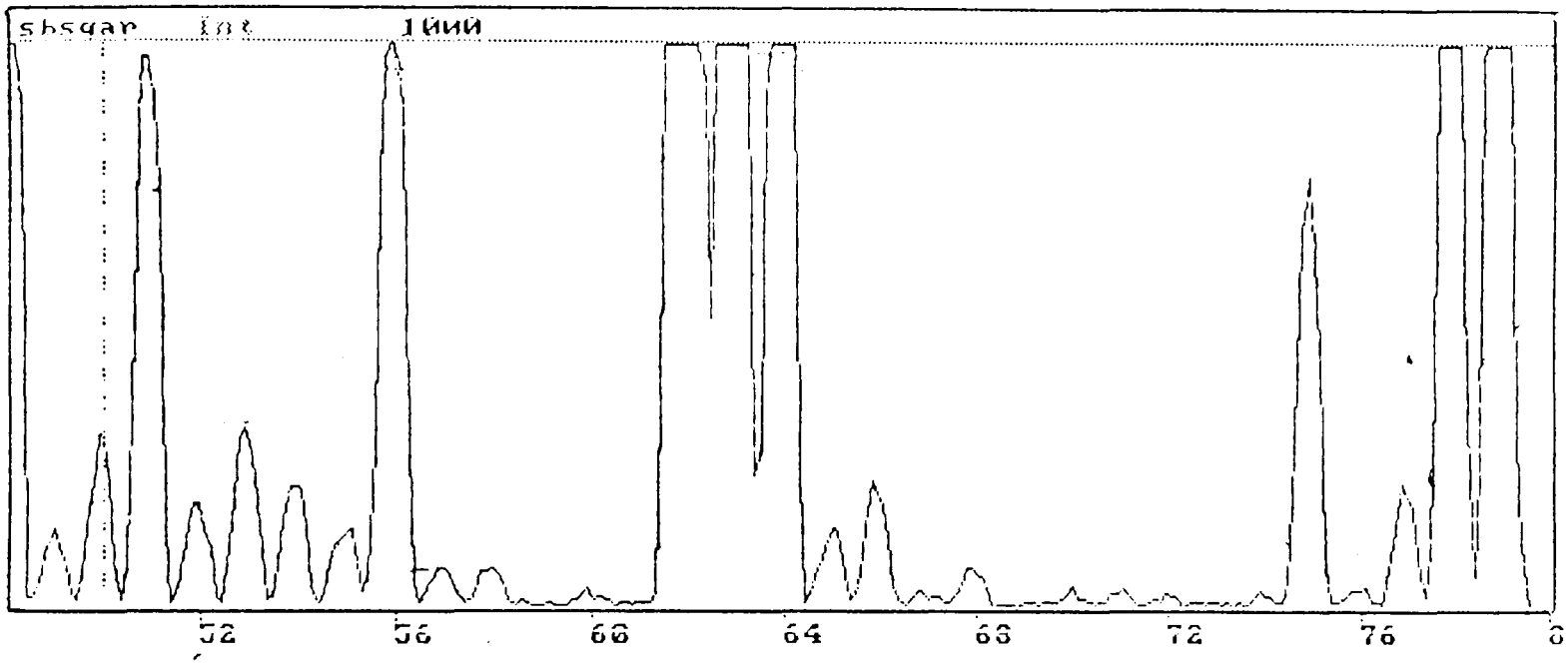
(1) ± range is one standard deviation
(2) Standard error of the mean value
(3) Range is 176-208 $\mu g\ l^{-1}$

the combined range of uncertainties. Recently Mulligan et al. (195) described a similar sample preparation procedure for the determination of trace elements in urine. The determination of arsenic was compromised by $^{40}Ar^{35}Cl^{+}$ which gave a positive interference. The results of this study clearly indicate that if N_2 addition is used these problems can be overcome. Thus the method described above was used for the subsequent determination of total arsenic in all the urine samples collected during the trial.

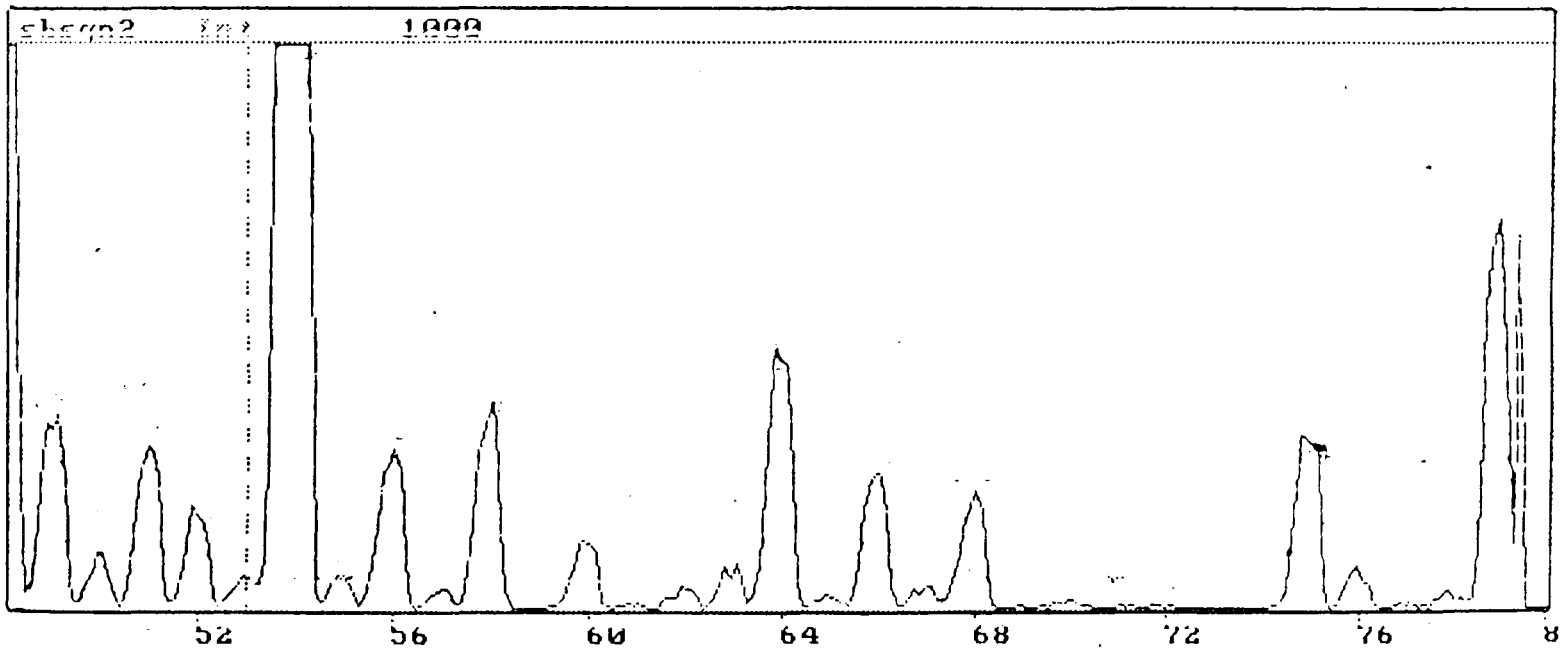
The mechanism that removes the $^{40}Ar^{35}Cl^{+}$ ion is not fully understood. Figure 6.2 shows the extent to which N_2 addition modifies the mass spectrum in the range 48-80 u for a urine sample. The elimination of $^{40}Ar^{35}Cl^{+}$ is confirmed by the absence of a peak at 77 u, corresponding to $^{40}Ar^{37}Cl^{+}$. The increase in the peak seen at mass 49 u may be attributed to the presence of $^{14}N^{35}Cl^{+}$ which suggests that the peak at 51 u may in part be due to $^{14}N^{37}Cl^{+}$. Certainly the presence of nitrogen decreases the ClO^{+} and ArO^{+} peaks (the former interfering with vanadium at mass 51, the latter with iron at mass 56) but with a subsequent large increase at mass 54 from $^{40}Ar^{14}N^{+}$. One possibility for this modification is that the nitrogen containing polyatomics are more readily formed. Alternatively N_2 addition may decrease the ionisation temperature of the plasma altering the energetics of the plasma and hence formation of certain polyatomics. Research towards elucidating the mechanisms is clearly a worthwhile area of further study.

Figure 6.2 ICP-MS Spectrum of Urine (a) Without and (b) With N_2 Addition

(a)



(b)



6.3 Design of the Dietary Trial

6.3.1 Introduction

There are several approaches to dietary trials, such as the use of duplicate studies or alternatively diary methods. The merits of these approaches, and the organisation of dietary trials, have been discussed in the literature (196-198). For this study a variation on the duplicate meals method was adopted whereby the meals were prepared by the catering students at the CFE rather than by the subjects themselves. This ensured that everyone ate the same food and the same sized portions and also substantially reduced the number of meals to be analysed. Set meals were provided at luncheon and dinnertime on the first two days of the trial. The subjects were asked to refrain from eating any breakfast, which was normal for eight out of ten volunteers. Each subject was provided with a diary in which to record the time of passing and volume of each urine sample from the moment they arose on the first day of the trial to the moment they went to bed five days later. In the diary details of all liquid intake during the five days of the trial were recorded, as were details of the food eaten on days 3-5. The importance of not eating fish prior to or during the trial was impressed upon the volunteers.

Ten volunteers were recruited, nine male and one female, and were designated subject A-I. Subject B was the female. Subject D was the only regular smoker. The participants all expressed themselves to be fit and healthy during and prior

to the trial. Eight of the subjects were members of the research community at Plymouth and two were undergraduates. Research students were preferred to undergraduates as they did not require financial inducement to participate and because it was felt they would approach the trial in a responsible fashion. All of the participants were between 21-30 years of age.

The trial commenced on Wednesday 22 November 1989 and terminated at midnight on Sunday 26 November 1989. Prior to commencement the participants were supplied with dry, acid washed, polyethylene bottles and measuring cylinders in which to collect and store their urine. They were also given indelible marker pens with which to label the sample bottles. Arrangements were made for collection or deposit of the samples so that they could be analysed as promptly as possible.

6.3.2 Experimental

The meals were prepared at the CFE with ingredients purchased from normal retail suppliers. Additionally leaf beet, spring onions, leeks and potatoes were grown at Rumleigh on soil containing 500 mg kg^{-1} arsenic, and supplied to the caterers to incorporate into the meals. The importance of washing the vegetables was stressed. All the Rumleigh vegetables were severely stunted, but could still be bulked with the 'normal' vegetables used by the CFE. It had been hoped to use Rumleigh strawberries in the trial but

these failed to fruit. The meals prepared by the students are detailed in Table 6.3, along with the date and time of consumption. A dessert had been intended for lunch on 22.11.89, but was spoilt during preparation. The fish meals were chosen to allow flaking and mixing of the sample during preparation. Unfortunately, this was not done and the subjects were served individual fillets. Twelve servings of each meal were prepared, ten for consumption and two for analysis. On return to the laboratory the components of the meals were weighed, the meals sampled in the appropriate ratios, pureed in a domestic blender and subsampled for total arsenic determination by microwave digestion ICP-MS. The puree was then frozen. The fish-based meals were subsequently freeze dried, ground and extracted by the two methods described in detail in Chapter 5. The chicken-based meals and the leek soup similarly were freeze-dried, ground, trypsin extracted and speciated by HPLC-ICP-MS.

The urine samples were analysed in the following fashion. Immediately upon receipt 1 ml was subsampled, spiked with indium to a final concentration of 100 μg In l^{-1} and diluted to 10 ml with 2% nitric acid. The sample was then analysed by nitrogen addition-ICP-MS as described in section 6.2. Some of the initial samples were analysed undiluted. Coincident with each batch of urine samples a Seronorm reference urine (Nycomed AS, Oslo, Norway) was run as a quality control measure. Following determination of total arsenic the urine sample was frozen. For subsequent analysis selected urine samples were defrosted at room

Table 6.3 Set Meals Prepared for the Dietary Trial

1 pm 22.11.89 Lunch

Pea Soup
Poached cod/salad/oven chips

7 pm 22.11.89 Dinner

Cheese fritters with tomato sauce
Chicken in mushroom sauce/cabbage/rice
Pineapple gateaux

1 pm 23.11.89 Lunch

Leek and potato soup
Plaice with leaf beet/green beans/unpeeled potatoes
Strawberry fool

7 pm 23.11.89 Dinner

Tomato salad with vinaigrette
Chicken and noodles/saute potatoes
Rice pudding

temperature, filtered (Whatman No. 541, Whatman, Maidstone, Kent), and then subsampled for a repeat determination of total arsenic by N_2 addition-ICP-MS, as previously described. The undiluted urine was also analysed by HPLC-ICP-MS for the determination of arsenobetaine, MMAA, DMAA arsenite and arsenate. The Benson column and chromatographic conditions described in Chapter 3 were used. MMAA, DMAA, arsenite and arsenate were all found to be below the detection limit of HPLC-ICP-MS and thus were determined by hydride generation - cryogenic trapping - atomic absorption spectrometry (HG-CT-AAS). This method has been described by Howard and Arbab-Zavar (133) and has been used in the laboratories at Plymouth for a number of environmental studies (24; 25). A PSA hydride generator (PSA Ltd., Sevenoaks, Kent) was used in conjunction with a Philips SP9 atomic absorption spectrometer (Philips Analytical, Cambridge, UK). Operating conditions are given in Table 6.4. Using this method arsenite and arsenate are quantified as total inorganic arsenic (As_i). HG-CT-AAS was not performed for subjects B, D and H because of the constraints of time and because it was obvious that DMAA, MMAA and As_i represented only a tiny fraction of the urinary arsenic species.

6.4 Results of the Dietary Trial

6.4.1 Total Arsenic and Arsenic Species in the Set Meals

Total arsenic in the set meals is shown in Table 6.5. As expected the principal arsenic burden lay with the fish

Table 6.4 **Standard Operating Conditions for Hydride Generation-Cryogenic Trapping - Atomic Absorption Spectrometry**

Sodium borohydride concentration	1.0 % m/v
Hydrochloric acid concentration	0.5 M
N_2 purge gas flow rate	0.8 l min^{-1}
Delay time	5.0 s
Analysis time	180.0 s
Memory time	10.0 s
Trap temperature	-196.0 °C
Wavelength	193.7 nm
Lamp current	9.0 mA
Flame	Air/acetylene - fuel lean
Scale expansion	On
Background correction	Off

Table 6.5 **Total Arsenic in the Set Meals.**
(Units are $\mu g\ kg^{-1}$ fresh weight.)

Food Sample		Result
Pea Soup		<18
Poached cod/salad/chips	(1)	1290 ± 13
	(2)	4783 ± 130
Cheese fritters/tomato sauce	(1)	<90
	(2)	<90
Chicken/mushroom/cabbage/rice	(1)	<86
	(2)	<90
Pineapple gateaux	(1)	<95
	(2)	<90
Leek and potato soup	(1)	347 ± 5.8
	(2)	<90
Plaice/leaf beet/potatoes	(1)	1089 ± 92
	(2)	1132 ± 37
Strawberry fool	(1)	<90
	(2)	<92
Tomato Salad/vinaigrette	(1)	<84
	(2)	<93
Chicken/noodles/potatoes	(1)	<100
	(2)	<91
Rice pudding	(1)	<81
	(2)	<98

The figures (1) and (2) refer to separate individual meals.

meals. The variations in limits of detection was because of the variation in sample size, which lay between 0.4-0.6 g, and because the ICP-MS multiplier was ageing and giving arsenic detection limits of 3-5 $\mu g\ l^{-1}$. The results also indicate problems with the sampling regime, as there was considerable variation between results for the duplicate cod meals and also the leek soup. Thus the leek soup, cod and plaice meals were freeze dried and ground in their entirety. This procedure was also followed for the chicken based meals as later in the trial it was desired to speciate the arsenic in the chicken. The results for total arsenic in the freeze dried duplicate meals are shown in Table 6.6. The results for the fish meals indicate that this sample preparation regime gives more precise results, the maximum relative standard deviation being approximately 10 percent. Unfortunately the results also show the problem caused by not flaking and mixing the fillets i.e. that there was a considerable variation in individual arsenic content between the two cod fillets. To determine the total arsenic input during the trial the mean of these values was taken, but the limitations of this approach are substantial. The results for the chicken meals and leek soup were compromised by the arsenic content in the digests being close to the detection limit, which in this instance was 0.1 $\mu g\ l^{-1}$, and because the vacuum line was vented during freeze-drying which resulted in much of the leek soup sample being lost. The results demonstrate that on a fresh weight basis very little arsenic was assimilated from the chicken. On the basis of the preceding results and the weights of the

Table 6.6 **Total Arsenic in Selected Set Meals Determined using N_2 Addition - ICP-MS and Following Freeze Drying**
All results in mg kg^{-1}

Cod/salad(1)	Cod/salad(2)
4.7 ± 0.1	18.4 ± 0.5
4.7 ± 0.1	19.2 ± 0.7
3.9 ± 0.3	19.0 ± 0.2
5.1 ± 0.2	18.5 ± 0.3
5.2 ± 0.4	19.4 ± 0.3
$\bar{x}$ ± s.d. = 4.7 ± 0.5	$\bar{x}$ ± s.d. = 18.9 ± 0.4

Plaice/leaf beet/potatoes(1)	Plaice/leaf beet/potatoes(2)
5.8 ± 0.1	5.1 ± 0.1
5.6 ± 0.1	4.7 ± 0.1
6.1 ± 0.3	4.9 ± 0.5
7.0 ± 0.3	4.5 ± 0.1
5.5 ± 0.3	4.9 ± 0.3
$\bar{x}$ ± s.d. = 6.0 ± 0.6	$\bar{x}$ ± s.d. = 4.8 ± 0.2

NBS 1574 total mixed diet*	0.95 ± 0.11
Chicken/noodles (1)	0.02 ± 0.04
Chicken/noodles (2)	<0.01
Chicken/cabbage/rice(1)	0.12 ± 0.04
Chicken/cabbage/rice(2)	0.15 ± 0.03
Leek soup (1)	0.23 ± 0.04
Leek soup (2)	0.31 ± 0.06

* reference value = 0.924 ± 0.344 mg kg^{-1} dry weight

The figures (1) and (2) refer to individual separate meals.

food portions it can be calculated that the total arsenic consumed from the set meals was 1.43 mg, 1.1 mg on the first day, 0.33 mg on the second day. If it was concluded that the high cod value i.e. cod/salad/chips (2), was a freak result, particularly in view of the all previous findings that plaice contain more arsenic than cod then cod/salad/chips (1) could be used as the basis of the calculations in which case the total arsenic intake from the set meals was 0.8 mg, 0.47 mg on the first day.

Most of the meals contained too little arsenic for speciation to be feasible. However, all the fish meals could be speciated, using the methanol/chloroform and trypsin extraction procedures described in detail in Chapter 5. The extracts were then analysed by HPLC-ICP-MS, the results of which are shown in Table 6.7.

The results in Chapter 5 indicated that the principal arsenic species in a range of edible fish was arsenobetaine, and the results in Table 6.7 reinforce those findings. Indeed the results for the trypsin extraction of the plaice meals demonstrate that arsenobetaine was the only arsenic species observed. The trypsin extraction gave the higher recoveries for the meals and the reference material, in all cases exceeding 80 percent of the total arsenic found by N_2 addition-ICP-MS. Plaice/beet (1) was spiked with 1 mg l^{-1} of arsenobetaine and extracted with the MeOH\CH_3Cl and

Table 6.7 **Summary of Extraction Results for the Fish Meals**
All results in mg As kg^{-1} dry weight

(i) $MeOH/CH_3Cl$ Extraction

	Total As[a]	AB	DMAA	MMAA	Arsenate	Total[c]
DORM-1[b]	16.0 ± 0.6	14.8 ± 2.5	-	-	-	12.0 ± 1.1
Cod/salad(1)	4.7 ± 0.5	1.3	<0.08	<0.08	<0.08	1.3
Cod/salad(2)	18.9 ± 0.4	8.5 ± 2.4	<0.15	<0.15	<0.15	7.8 ± 0.4
Plaice/beet(1)	6.0 ± 0.6	3.3	<0.16	<0.16	<0.16	3.2 ± 0.2
Plaice/beet(2)	4.8 ± 0.2	3.0 ± 0.3	<0.08	<0.08	<0.08	2.9 ± 0.2

(ii) Trypsin Extraction

	Total As[a]	AB	DMAA	MMAA	Arsenate	Total[c]
DORM-1[b]	16.0 ± 0.6	17.1 ± 2.5	-	-	-	17.8 ± 0.7
Cod/salad(1)	4.7 ± 0.5	3.9	<0.52	<0.52	<0.52	3.7 ± 0.3
Cod/salad(2)	18.9 ± 0.4	17.9	<0.48	<0.48	<0.48	14.9 ± 0.5
Plaice/beet(1)	6.0 ± 0.6	6.3	<0.51	<0.51	<0.51	6.3 ± 0.3
Plaice/beet(2)	4.8 ± 0.2	4.9	<0.48	<0.48	<0.48	5.0 ± 0.4

(a) Total arsenic determined by microwave digestion N_2 addition-ICP-MS
(b) DORM-1 certificate values - total arsenic: 17.7 ± 2.1
arsenobetaine: 15.7 ± 0.8 (in $MeOH/H_20$)
(c) Total arsenic in the extract, determined by N_2 addition-ICP-MS

AB = arsenobetaine
DMAA = dimethylarsinic acid
MMAA = monomethylarsonic acid

trypsin methods, the recoveries being 66 and 81 percent respectively. The sum of the individual species tallies with the total arsenic in the extract in all cases except the trypsin extraction of cod/salad (2) and for both extractions applied to the reference material, although for the trypsin extract the difference lies within the standard deviation of the result.

Unexpectedly using the normal HPLC-ICP-MS conditions the arsenite did not come off the column. Reducing the pH of the mobile phase to 7.0 caused the arsenite to elute and it was possible to ascertain that arsenite was below the same levels as the arsenate in the meals. This problem disappeared when the column was reversed and flushed, which suggests arsenite was binding to debris at the column head.

The results in Table 6.7 demonstrate that the arsenic consumed from the set meals was almost exclusively in the form of non-toxic arsenobetaine. The chicken/rice dishes and the leek and potato soup were also extracted and speciated by the described methods, but no quantifiable species were present, although there was evidence of DMAA in the methanol\chloroform extract of the soup at a level below the detection limit of 20 $\mu g\ kg^{-1}$.

6.4.2 Total Arsenic and Arsenic Species in Urine

The number, volume, arsenic content and time of passing of the urine samples from all of the volunteers are given in

Tables 6.8-6.17. Some samples were not handed in and gaps have been left in the tables for these. Occasionally the time noted for the samples differed in the diary and on the bottle. The time on the bottle was taken as the correct one. Subject E handed in a sample that was not recorded in the diary. This was designated E13A. Time zero refers to the time of the first meal, i.e. 13.00 hrs on 22.11.89. These results were all obtained by N_2 addition-ICP-MS prior to freezing. A Seronorm reference urine was run with each batch of samples and in almost every instance gave a result within 10 percent of the certificate value. There was a slight positive bias to the Seronorm results. The results demonstrate a considerable variation in the excretory behaviour of the subjects. The number of samples given during the trial varied between 21 and 38 and the total volume passed varied between 4 and 14 litres. Obviously liquid intake influences this behaviour. Subject F takes no hot drinks, and had the lowest liquid intake and hence the lowest volume output. Subject J passed the largest total volume of urine in the trial but did not drink excessive quantities relative to the other subjects. Subject B, the female, passed over 13 litres during the trial but had one of the lower liquid intakes. This may have been due to the differences between male and female metabolism. The mean sample size varied between 200 and 450 ml. This was presumably a function of the bladder capacities of the subjects.

Table 6.8 Total Arsenic in the Urine of Subject (A) Prior to Freezing

Sample	Time of Collection (hours)	Volume (ml)	Arsenic ($\mu g\ l^{-1}$)	Arsenic (μg)
A 1	-4.50	330	92 ± 14	30.4 ± 4.6
A 2	-1.00	195	188 ± 17	36.7 ± 3.3
A 3	1.30	200	40 ± 6	8.0 ± 1.1
A 4	4.30	190	697 ± 23	132.4 ± 4.3
A 5	7.20	240	340 ± 17	81.6 ± 4.0
A 6	9.20	140	602 ± 23	84.2 ± 3.2
A 7	19.10	390	283 ± 19	110.4 ± 7.4
A 8	22.35	260	56 ± 1	14.5 ± 0.1
A 9	25.25	540	77 ± 2	41.5 ± 1.1
A10	27.10	290	372 ± 11	107.0 ± 3.3
A11	31.10	790	174 ± 2	137.2 ± 1.7
A12	33.50	480	85 ± 4	40.8 ± 1.7
A13	37.14	410	86 ± 8	35.3 ± 3.4
A14	43.10	220	251 ± 5	55.2 ± 1.2
A15	48.15	310	361 ± 1	112.0 ± 0.1
A16	55.25	310	299 ± 3	92.6 ± 0.9
A17	59.20	200	65 ± 3	13.0 ± 0.7
A18	67.50	370	186 ± 7	68.8 ± 2.6
A19	73.10	260	107 ± 6	27.8 ± 1.4
A20	79.00	640	42 ± 2	27.0 ± 1.3
A21	82.22	1005	14 ± 1	14.0 ± 0.1
A22	83.20	550	17 ± 2	9.4 ± 0.9
A23	91.50	560	48 ± 7	26.9 ± 4.1
A24	96.51	295	50 ± 5	14.8 ± 1.4
A25	102.09	540	141 ± 11	76.1 ± 5.8
Totals	-	9715	-	1393

$\bar{x}$ = 389 ± 208

Table 6.9 **Total Arsenic in the Urine of Subject (B) Prior to Freezing**

Sample	Time of Collection (hours)	Volume (ml)	Arsenic ($\mu g\ l^{-1}$)	Arsenic (μg)
B 1	-4.35	465	123 ± 14	57.2 ± 6.5
B 2	-0.55	490	54 ± 22	26.6 ± 10.7
B 3	1.30	240	109 ± 18	26.2 ± 4.5
B 4	2.10	200	25 ± 10	5.0 ± 0.1
B 5	3.15	700	20 ± 3	14.0 ± 1.8
B 6	5.00	440	76 ± 1	33.4 ± 0.4
B 7	7.15	260	122 ± 3	31.8 ± 1.5
B 8	8.30	160	82 ± 2	13.1 ± 0.3
B 9	10.25	430	33 ± 2	14.2 ± 0.7
B10	11.20	295	16 ± 3	4.7 ± 1.0
B11	20.10	410	33 ± 1	13.5 ± 0.2
B12	22.25	290	105 ± 9	30.5 ± 2.5
B13	26.10	450	141 ± 4	63.5 ± 1.9
B14	28.20	410	403 ± 11	165.1 ± 4.4
B15	29.15	385	130 ± 2	50.1 ± 0.5
B16	31.20	510	145 ± 4	74.0 ± 2.0
B17	32.20	335	-	-
B18	33.10	150	174 ± 3	26.1 ± 0.5
B19	35.35	455	115 ± 3	52.3 ± 1.2
B20	43.45	370	209 ± 4	77.0 ± 1.5
B21	45.10	50	206 ± 8	10.3 ± 0.4
B22	46.45	540	53 ± 2	28.6 ± 1.0
B23	50.25	230	208 ± 19	47.9 ± 4.4
B24	54.10	125	277 ± 11	34.6 ± 1.3
B25	58.20	295	154 ± 11	45.4 ± 3.3
B26	68.15	565	113 ± 3	63.8 ± 1.8
B27	72.45	115	228 ± 10	26.2 ± 1.2
B28	75.20	130	182 ± 5	23.7 ± 0.6
B29	80.10	310	150 ± 4	46.4 ± 1.1
B30	83.40	480	64 ± 4	30.8 ± 1.9
B31	92.15	655	59 ± 2	38.6 ± 1.3
B32	93.50	80	150 ± 8	12.0 ± 0.6
B33	98.30	625	43 ± 3	26.8 ± 1.8
B34	101.10	700	24 ± 3	16.8 ± 2.0
B35	105.35	320	52 ± 4	16.5 ± 1.2
B36	105.40	100	-	-
B37	106.40	65	66 ± 6	4.3 ± 0.4
Totals	-	12830	-	1249

$\bar{x}$ = 347 ± 182

Table 6.10 Total Arsenic in the Urine of Subject (C) Prior to Freezing

Sample	Time of Collection (hours)	Volume (ml)	Arsenic ($\mu g\ l^{-1}$)	Arsenic (μg)
C 1	2.0	437	105 ± 3	45.7 ± 1.1
C 2	4.42	835	202 ± 14	168.7 ± 11.7
C 3	7.30	325	89 ± 5	28.8 ± 1.6
C 4	9.25	725	78 ± 7	56.6 ± 5.0
C 5	20.40	350	113 ± 7	39.5 ± 2.3
C 6	28.40	290	821 ± 64	238.1 ± 18.7
C 7	31.10	430	216 ± 8	92.7 ± 3.7
C 8	33.00	395	148 ± 2	58.5 ± 1.0
C 9	34.45	495	107 ± 2	53.0 ± 0.5
C10	38.00	300	149 ± 2	44.6 ± 0.7
C11	46.35	300	267 ± 4	80.1 ± 1.1
C12	54.00	435	116 ± 12	50.6 ± 0.7
C13	54.40	500	24 ± 5	12.1 ± 2.5
C14	60.00	290	90 ± 6	26.2 ± 1.6
C15	71.40	380	113 ± 3	42.9 ± 1.0
C16	78.20	335	84 ± 16	28.1 ± 5.4
C17	81.25	250	36 ± 4	9.0 ± 1.0
C18	86.45	400	49 ± 6	19.6 ± 2.5
C19	96.00	410	46 ± 2	18.9 ± 0.7
C20	99.05	460	22 ± 4	10.0 ± 1.9
C21	103.45	235	25 ± 3	5.9 ± 0.7
Totals	-	8577		1130

$\bar{x}$ = 408 ± 146

Table 6.11 Total Arsenic in the Urine of Subject (D) Prior to Freezing

Sample	Time of Collection (hours)	Volume (ml)	Arsenic ($\mu g\ l^{-1}$)	Arsenic (μg)
D 1	-3.15	250	142 ± 5	35.5 ± 1.3
D 2	1.15	310	205 ± 13	63.6 ± 4.0
D 3	5.00	355	175 ± 3	62.1 ± 1.2
D 4	7.20	350	115 ± 3	40.1 ± 1.0
D 5	10.05	410	119 ± 4	49.0 ± 1.7
D 6	16.45	270	159 ± 3	42.9 ± 0.9
D 7	23.05	460	94 ± 4	43.3 ± 2.0
D 8	25.30	220	230 ± 2	50.6 ± 0.4
D 9	27.20	180	366 ± 8	66.0 ± 1.5
D10	29.10	155	-	-
D11	31.20	375	189 ± 3	70.8 ± 1.0
D12	33.00	460	117 ± 1	54.0 ± 0.5
D13	34.20	390	176 ± 4	68.5 ± 1.7
D14	45.45	245	410 ± 7	100.4 ± 1.7
D15	51.30	480	94 ± 3	44.9 ± 1.2
D16	54.00	490	51 ± 1	24.9 ± 0.6
D17	55.30	360	174 ± 17	62.7 ± 6.0
D18	59.00	275	187 ± 2	51.4 ± 0.6
D19	68.45	270	476 ± 7	128.4 ± 1.8
D20	71.20	400	52 ± 3	20.7 ± 1.2
D21	78.50	270	144 ± 13	40.0 ± 3.5
D22	81.30	350	149 ± 8	52.2 ± 2.6
D23	86.25	490	84 ± 8	41.1 ± 3.9
D24	91.45	180	218 ± 8	39.3 ± 1.5
D25	98.40	175	251 ± 10	44.0 ± 1.7
D26	100.35	460	49 ± 5	22.7 ± 2.2
D27	103.45	600	34 ± 3	20.6 ± 1.2
Totals	-	9230	-	1340

$\bar{x}$ = 342 ± 116

Table 6.12 Total Arsenic in the Urine of Subject (E) Prior to Freezing

Sample	Time of Collection (hours)	Volume (ml)	Arsenic ($\mu g\ l^{-1}$)	Arsenic (μg)
E 1	-4.15	450	130 ± 8	58.6 ± 3.7
E 2	1.45	380	180 ± 15	68.4 ± 5.6
E 3	7.10	320	358 ± 17	114.5 ± 5.4
E 4	11.15	175	133 ± 2	23.2 ± 0.4
E 5	20.30	600	213 ± 4	127.8 ± 2.1
E 6	27.00	275	56 ± 1	15.3 ± 0.2
E 7	31.00	225	695 ± 14	156.3 ± 3.1
E 8	39.30	600	221 ± 5	132.4 ± 3.2
E 9	47.15	450	176 ± 4	79.1 ± 1.8
E10	53.18	245	215 ± 12	52.6 ± 2.9
E11	57.00	200	182 ± 4	36.5 ± 0.8
E12	58.45	360	-	-
E13	69.45	830	79 ± 3	65.4 ± 2.2
E13A	78.30	350	55 ± 6	19.4 ± 2.1
E14	79.30	600	18 ± 1	10.9 ± 0.8
E15	82.00	810	5 ± 2	3.9 ± 1.8
E16	82.30	660	7 ± 1	4.6 ± 0.7
E17	82.45	380	45 ± 4	17.1 ± 1.6
E18	94.40	675	40 ± 3	27.0 ± 2.0
E19	99.50	410	61 ± 1	25.0 ± 0.4
E20	105.00	425	47 ± 5	19.8 ± 2.3
E21	108.45	450	62 ± 4	27.9 ± 1.9
Totals	-	9870		1086

$\bar{x}$ = 449 ± 188

Table 6.13 Total Arsenic in the Urine of Subject (F) Prior to Freezing

Sample	Time of Collection (hours)	Volume (ml)	Arsenic ($\mu g\ l^{-1}$)	Arsenic (μg)
F 1	-2.30	210	32 ± 4	6.6 ± 0.9
F 2	2.20	140	36 ± 4	5.1 ± 0.5
F 3	5.00	200	333 ± 11	66.4 ± 1.7
F 4	7.10	90	333 ± 3	30.1 ± 1.0
F 5	10.30	390	77 ± 3	29.9 ± 1.0
F 6	11.00	240	27 ± 4	6.4 ± 1.0
F 7	21.15	250	125 ± 9	31.2 ± 2.2
F 8	27.20	175	741 ± 9	129.6 ± 1.5
F 9	31.00	220	879 ± 27	193.4 ± 6.0
F10	32.45	330	304 ± 6	100.2 ± 2.1
F11	45.40	325	447 ± 16	145.4 ± 5.3
F12	49.40	140	270 ± 24	37.8 ± 3.4
F13	53.15	125	249 ± 11	31.1 ± 0.2
F14	60.20	270	167 ± 2	45.0 ± 0.5
F15	68.45	155	250 ± 16	38.7 ± 2.4
F16	76.05	170	94 ± 2	16.0 ± 0.4
F17	79.45	220	115 ± 14	25.2 ± 3.0
F18	88.50	210	118 ± 13	24.8 ± 2.6
F19	96.00	110	136 ± 11	15.0 ± 1.3
F20	103.00	190	148 ± 6	28.1 ± 1.2
F21	106.45	90	105 ± 14	9.5 ± 1.3
Totals	-	4250		984

$\bar{x}$ = 202 ± 80

Table 6.14 Total Arsenic in the Urine of Subject (G) Prior to Freezing

Sample	Time of Collection (hours)	Volume (ml)	Arsenic ($\mu g\ l^{-1}$)	Arsenic (μg)
G 1	-5.00	270	146 ± 33	39.4 ± 8.8
G 2	-1.30	285	113 ± 9	32.3 ± 2.5
G 3	1.15	150	239 ± 28	35.6 ± 4.1
G 4	4.00	185	905 ± 48	167.5 ± 8.9
G 5	7.05	330	489 ± 15	161.4 ± 5.0
G 6	8.45	320	749 ± 23	240.0 ± 7.5
G 7	11.00	310	216 ± 6	67.0 ± 1.9
G 8	21.15	620	184 ± 11	114.2 ± 7.0
G 9	26.30	250	576 ± 13	144.0 ± 3.3
G10	31.00	440	179 ± 5	78.8 ± 1.8
G11	35.00	130	381 ± 12	49.6 ± 1.6
G12	43.30	310	372 ± 26	115.4 ± 8.1
G13	49.10	210	372 ± 21	78.1 ± 4.4
G14	51.30	230	187 ± 9	43.2 ± 2.0
G15	54.40	420	57 ± 3	24.1 ± 1.1
G16	58.55	310	172 ± 2	53.3 ± 0.6
G17	67.45	470	186 ± 7	87.2 ± 3.1
G18	72.10	220	89 ± 7	19.6 ± 1.4
G19	76.30	265	119 ± 1	31.5 ± 0.3
G20	83.00	370	132 ± 7	49.0 ± 2.4
G21	91.50	570	92 ± 10	52.3 ± 5.9
G22	97.00	650	56 ± 5	36.5 ± 3.2
G23	99.00	150	113 ± 2	16.9 ± 0.4
G24	105.00	190	80 ± 5	15.2 ± 1.0
Totals		7655		1753

$\bar{x}$ = 319 ± 145

Table 6.15 Total Arsenic in the Urine of Subject (H) Prior to Freezing

Sample	Time of Collection (hours)	Volume (ml)	Arsenic ($\mu g\ l^{-1}$)	Arsenic (μg)
H 1	-4.00	530	16 ± 2	8.5 ± 1.0
H 2	-0.50	100	45 ± 5	4.5 ± 0.5
H 3	3.30	235	151 ± 6	35.5 ± 1.3
H 4	7.05	230	136 ± 3	31.3 ± 0.7
H 5	7.55	170	115 ± 7	19.6 ± 1.2
H 6	9.35	175	168 ± 9	29.4 ± 1.5
H 7	10.55	460	42 ± 2	19.1 ± 1.1
H 8	11.30	550	26 ± 3	14.1 ± 1.4
H 9	20.00	360	143 ± 6	51.4 ± 2.1
H10	23.25	140	157 ± 9	22.0 ± 1.3
H11	27.30	320	505 ± 28	161.6 ± 8.9
H12	32.30	415	294 ± 20	122.0 ± 8.3
H13	33.35	290	99 ± 4	28.7 ± 1.0
H14	34.45	410	87 ± 3	35.7 ± 0.9
H15	44.10	670	75 ± 9	49.9 ± 6.0
H16	49.30	190	157 ± 3	29.9 ± 0.6
H17	53.55	310	75 ± 2	23.1 ± 0.6
H18	54.40	290	30 ± 1	8.6 ± 0.3
H19	55.10	410	45 ± 2	18.5 ± 0.9
H20	60.20	510	22 ± 2	11.1 ± 1.1
H21	67.45	600	73 ± 2	43.8 ± 1.3
H22	71.45	480	64 ± 9	30.5 ± 4.1
H23	76.00	180	64 ± 3	11.6 ± 0.5
H24	77.00	340	86 ± 4	29.1 ± 1.5
H25	80.20	250	112 ± 3	28.0 ± 0.9
H26	91.15	520	185 ± 8	96.0 ± 3.9
Totals		9235		952

$\bar{x}$ = 355 ± 158

Table 6.16 Total Arsenic in the Urine of Subject (I) Prior to Freezing

Sample	Time of Collection (hours)	Volume (ml)	Arsenic ($\mu g\ l^{-1}$)	Arsenic (μg)
I 1	-4.45	310	48 ± 1	14.9 ± 0.3
I 2	-0.35	145	32 ± 1	4.6 ± 0.1
I 3	4.00	210	83 ± 3	17.4 ± 1.0
I 4	5.15	180	74 ± 3	13.3 ± 0.5
I 5	8.05	250	72 ± 1	18.0 ± 0.3
I 6	10.45	420	45 ± 2	18.8 ± 0.7
I 7	19.10	280	187 ± 5	52.2 ± 1.5
I 8	23.15	180	83 ± 3	14.9 ± 0.5
I 9	25.30	250	73 ± 4	18.3 ± 1.0
I10	29.12	150	804 ± 25	120.5 ± 3.7
I11	31.30	100	1103 ± 167	110.3 ± 16.7
I12	34.20	290	354 ± 4	102.5 ± 1.2
I13	43.30	440	206 ± 9	90.5 ± 4.1
I14	52.50	240	373 ± 16	89.6 ± 3.9
I15	60.30	250	252 ± 13	62.9 ± 3.3
I16	69.30	250	241 ± 37	60.1 ± 9.3
I17	70.20	145	168 ± 14	24.4 ± 2.0
I18	80.10	230	135 ± 4	31.0 ± 0.9
I19	84.00	205	86 ± 1	17.5 ± 0.2
I20	92.15	180	123 ± 14	22.1 ± 2.4
I21	97.15	140	163 ± 1	22.8 ± 0.1
I22	99.15	440	31 ± 1	13.6 ± 0.6
I23	101.55	400	37 ± 2	14.6 ± 0.6
I24	105.00	210	76 ± 5	15.9 ± 1.1
Totals	-	5895	-	972

$\bar{x}$ = 246 ± 97

Table 6.17 **Total Arsenic in the Urine of Subject (J) Prior to Freezing**

Sample	Time of Collection (hours)	Volume (ml)	Arsenic ($\mu g\ l^{-1}$)	Arsenic (μg)
J 1	-4.40	430	31 ± 4	13.1 ± 1.5
J 2	-0.45	150	104 ± 18	15.6 ± 2.7
J 3	1.45	150	292 ± 26	43.8 ± 3.9
J 4	4.35	190	247 ± 18	47.0 ± 3.4
J 5	7.10	185	171 ± 18	31.5 ± 3.3
J 6	10.21	335	95 ± 2	31.7 ± 0.8
J 7	20.00	620	98 ± 4	60.8 ± 2.4
J 8	22.50	230	57 ± 3	13.0 ± 0.6
J 9	25.15	130	138 ± 10	17.9 ± 1.4
J10	26.30	220	330 ± 22	72.6 ± 4.8
J11	27.20	170	280 ± 10	47.7 ± 1.7
J12	29.15	160	529 ± 17	84.6 ± 2.7
J13	31.15	500	188 ± 2	93.9 ± 1.1
J14	32.00	420	57 ± 2	23.8 ± 1.0
J15	32.30	300	61 ± 1	18.2 ± 0.4
J16	34.20	565	59 ± 1	33.1 ± 0.3
J17	34.47	300	23 ± 2	7.0 ± 0.6
J18	35.31	450	29 ± 2	13.1 ± 0.9
J19	40.55	830	101 ± 13	83.8 ± 10.5
J20	46.10	330	123 ± 4	40.4 ± 1.3
J21	48.00	320	58 ± 4	18.6 ± 1.3
J22	52.05	170	177 ± 6	30.1 ± 1.0
J23	54.40	500	86 ± 1	43.0 ± 0.1
J24	55.20	340	7 ± 1	2.4 ± 0.3
J25	57.00	500	30 ± 2	14.7 ± 1.2
J26	58.00	700	23 ± 2	16.2 ± 1.3
J27	60.10	450	59 ± 3	26.5 ± 1.5
J28	62.00	340	33 ± 3	11.1 ± 0.9
J29	69.58	510	90 ± 3	45.9 ± 1.5
J30	72.57	180	58 ± 1	10.4 ± 0.2
J31	81.00	600	57 ± 1	34.0 ± 0.8
J32	82.10	565	15 ± 1	8.3 ± 0.2
J33	82.40	300	13 ± 2	4.0 ± 0.6
J34	83.35	460	3 ± 1	1.3 ± 0.3
J35	84.20	220	19 ± 2	4.1 ± 0.4
J36	92.30	410	77 ± 3	31.7 ± 1.4
J37	98.00	210	104 ± 1	21.7 ± 0.2
J38	103.20	330	87 ± 6	28.8 ± 2.0
Totals	-	13770		1150

$\bar{x}$ = 362 ± 173

The concentration and quantity of arsenic in the urine samples varied considerably from subject to subject and person to person. Arsenic concentrations ranged from less than 5 to over a 1000 $\mu g\ l^{-1}$, with the highest levels usually occurring 3 or 4 hours after a fish meal, and continuing for the next few samples. Higher levels were also associated with the first urine sample of the morning following overnight concentration. Normal arsenic levels in urine have been reported to lie in the range 15-120 $\mu g\ l^{-1}$ (194). A number of the subjects _e.g._ G, E, D, had levels in excess of these normal values at the commencement of the trial, _i.e._ prior to consuming any fish meals. The levels in most cases had returned to normal by the end of the trial with a few notable exceptions _e.g._ A, H. A possible explanation for this behaviour will be proposed later. The total arsenic output profile of each subject during the course of the trial is shown graphically in Figures 6.3-6.12.

The profiles are very variable in appearance, with perhaps to most remarkable being Figure 6.9 representing the subject who had the highest levels of arsenic in urine, sample G6 containing 240 μg, following the first fish meal. Most of the other subjects showed a peak following the plaice meal although subject D was anomalous in that the output was steady until 60 hours into the trial when a peak output was given. The results for subject C, Figure 6.5, are 'ideal' in that they follow the profile that might have been

Figure 6.3 Total Arsenic in the Urine of Subject A Prior to Sample Feezing

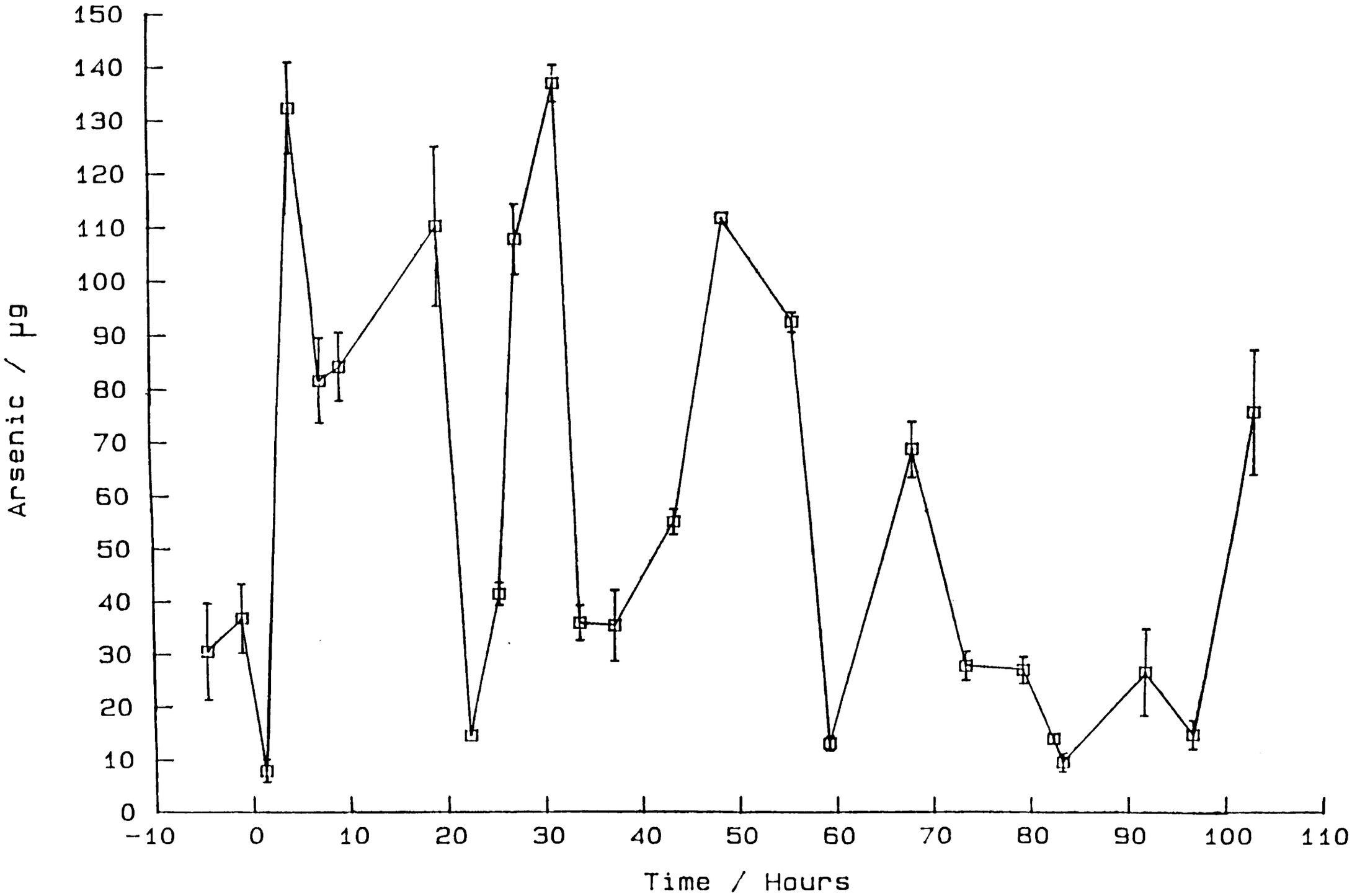


Figure 6.4 Total Arsenic in the Urine of Subject B Prior to Freezing

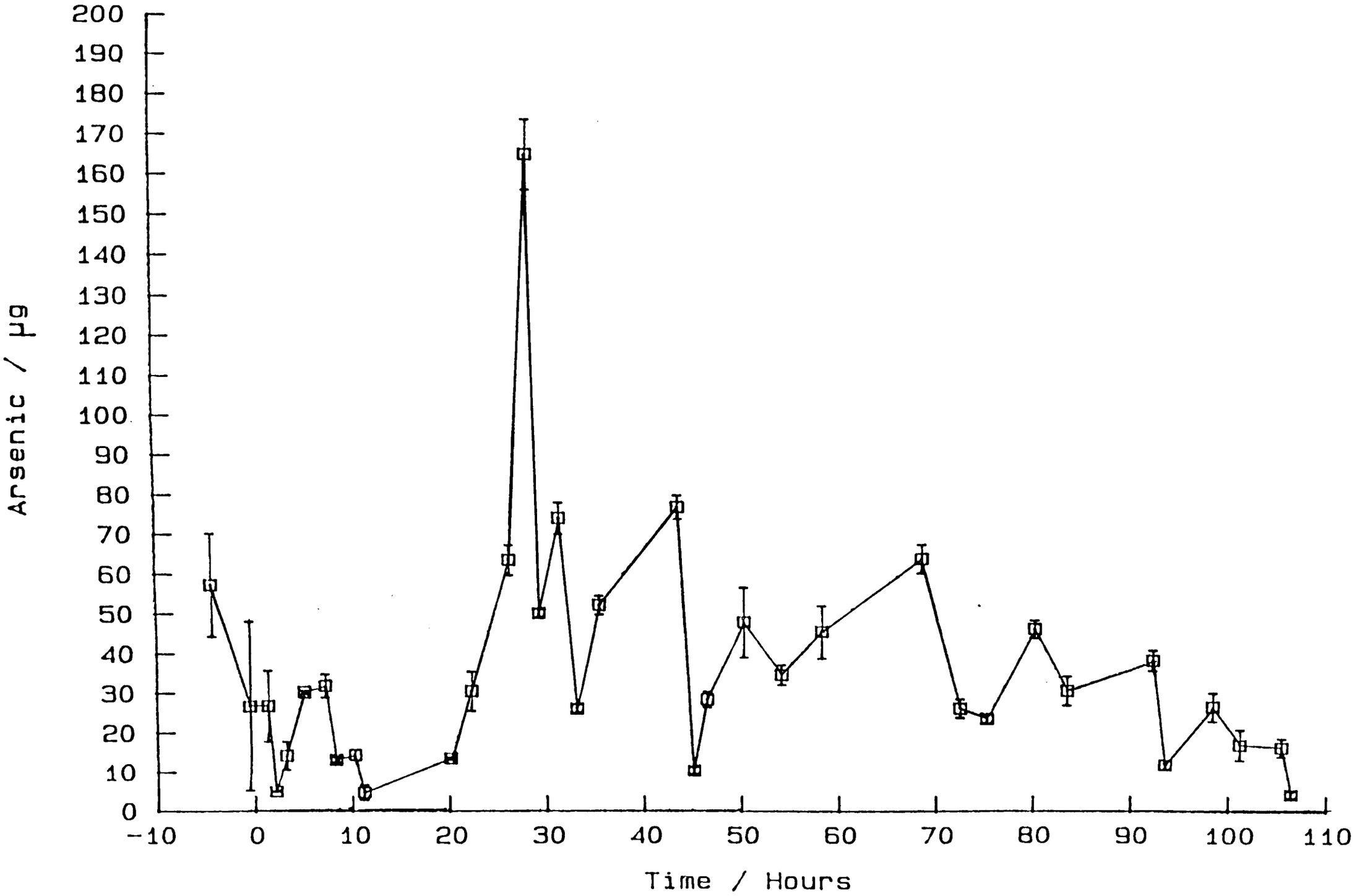


Figure 6.5 Total Arsenic in the Urine of Subject C Prior to Freezing

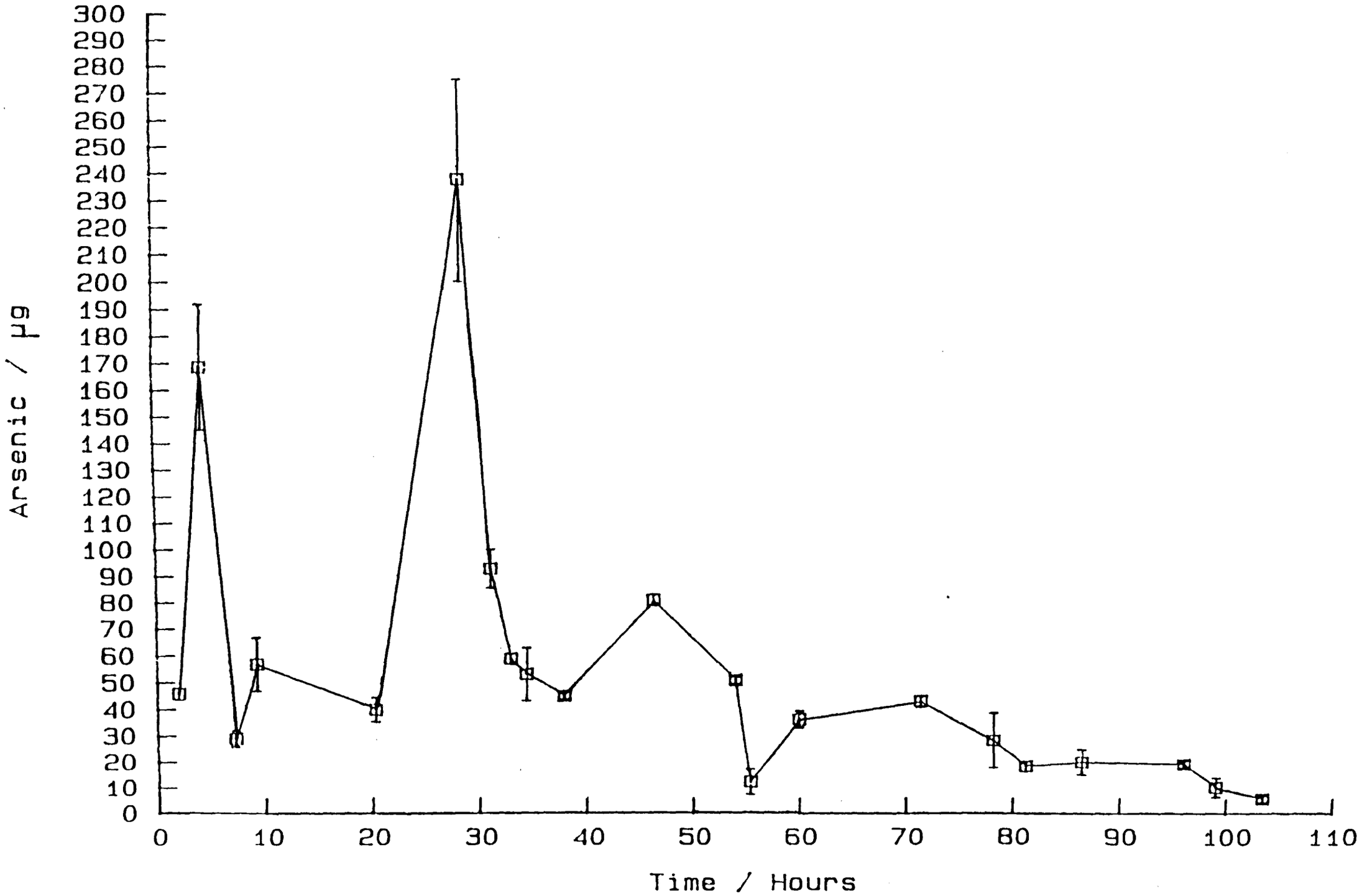


Figure 6.6 Total Arsenic in the Urine of Subject D Prior to Sample Freezing

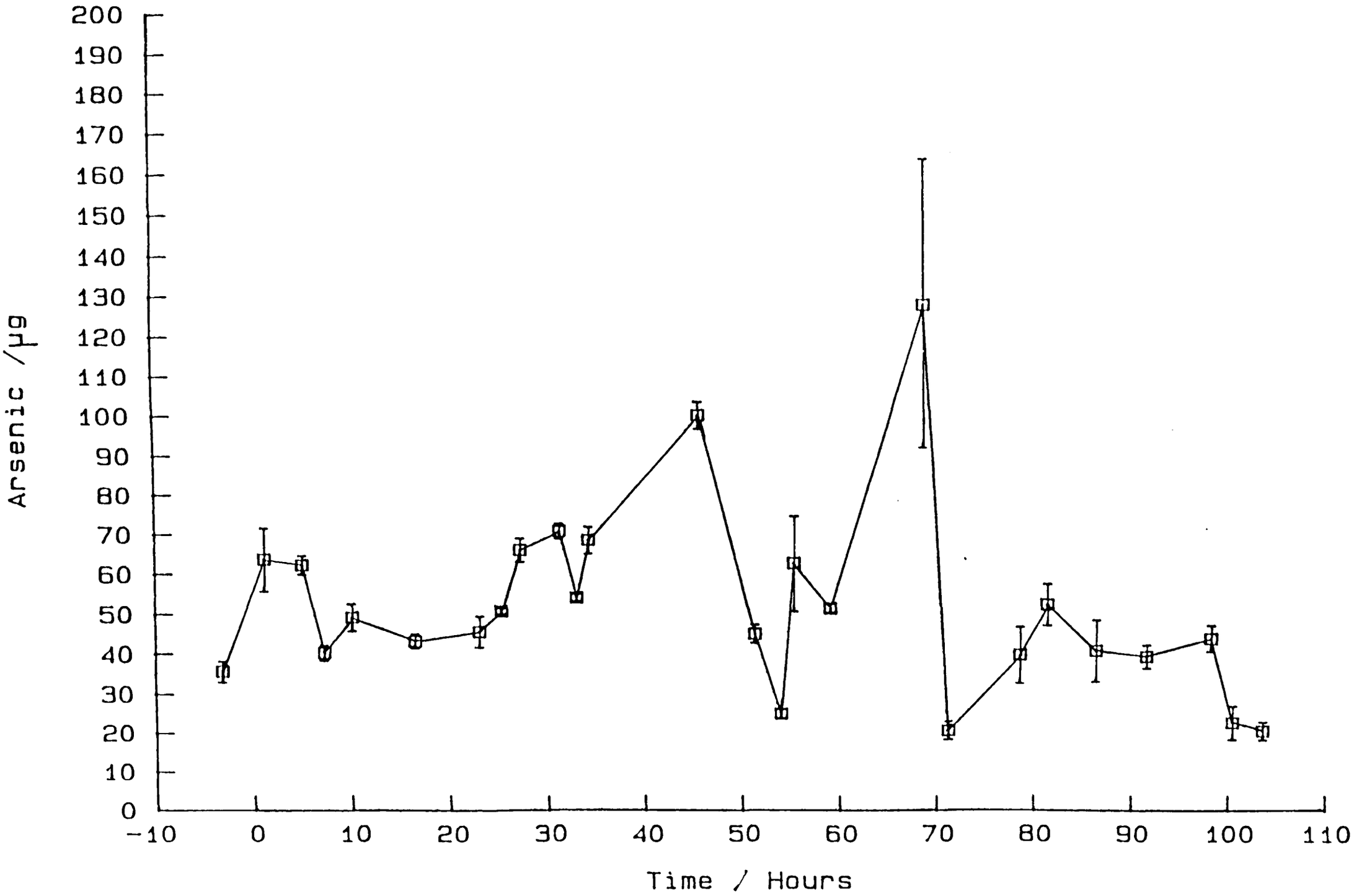


Figure 6.7 Total Arsenic in the Urine of Subject E Prior to Sample Freezing

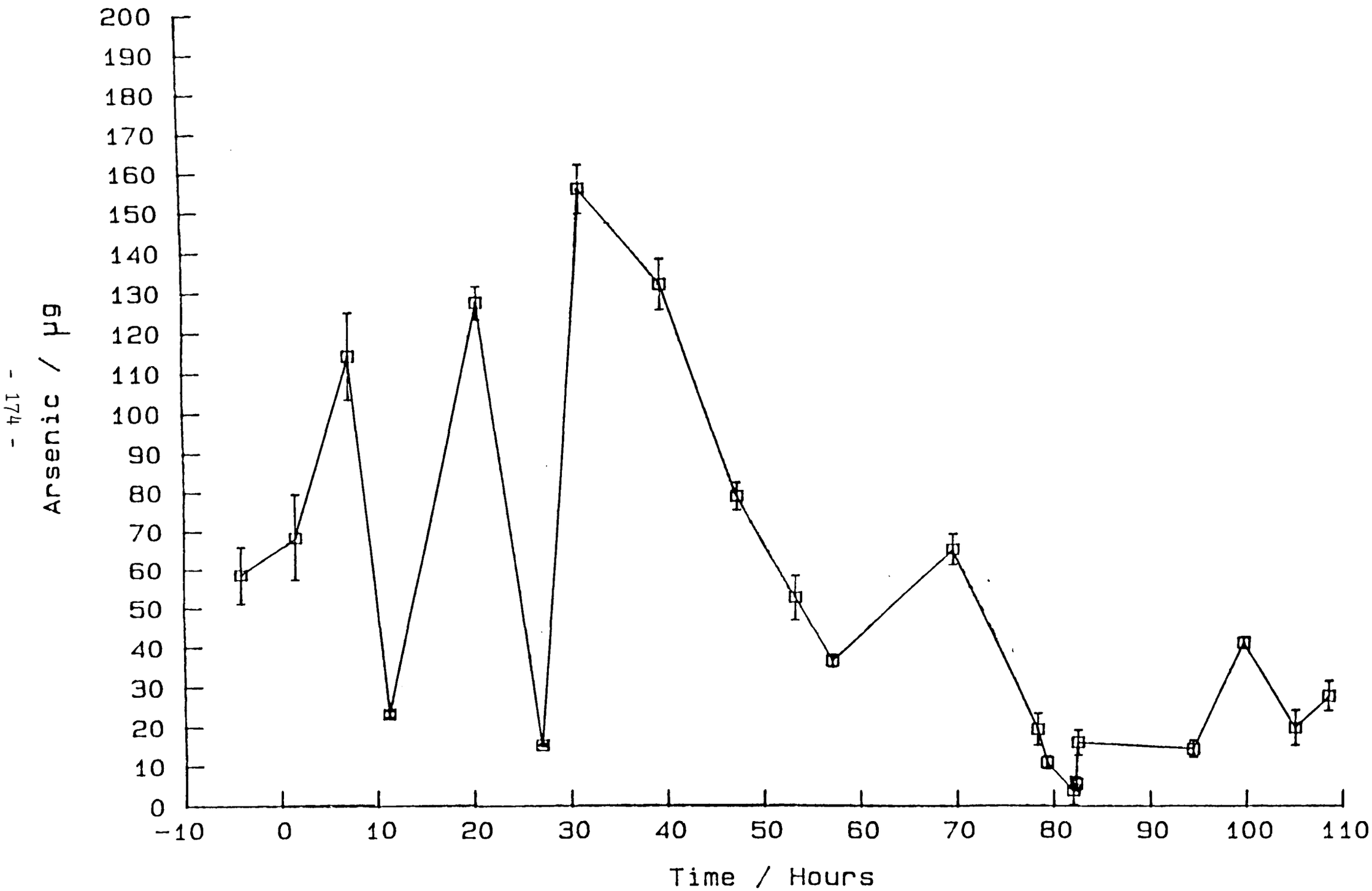


Figure 6.8 Total Arsenic in the Urine of Subject F Prior to Sample Freezing

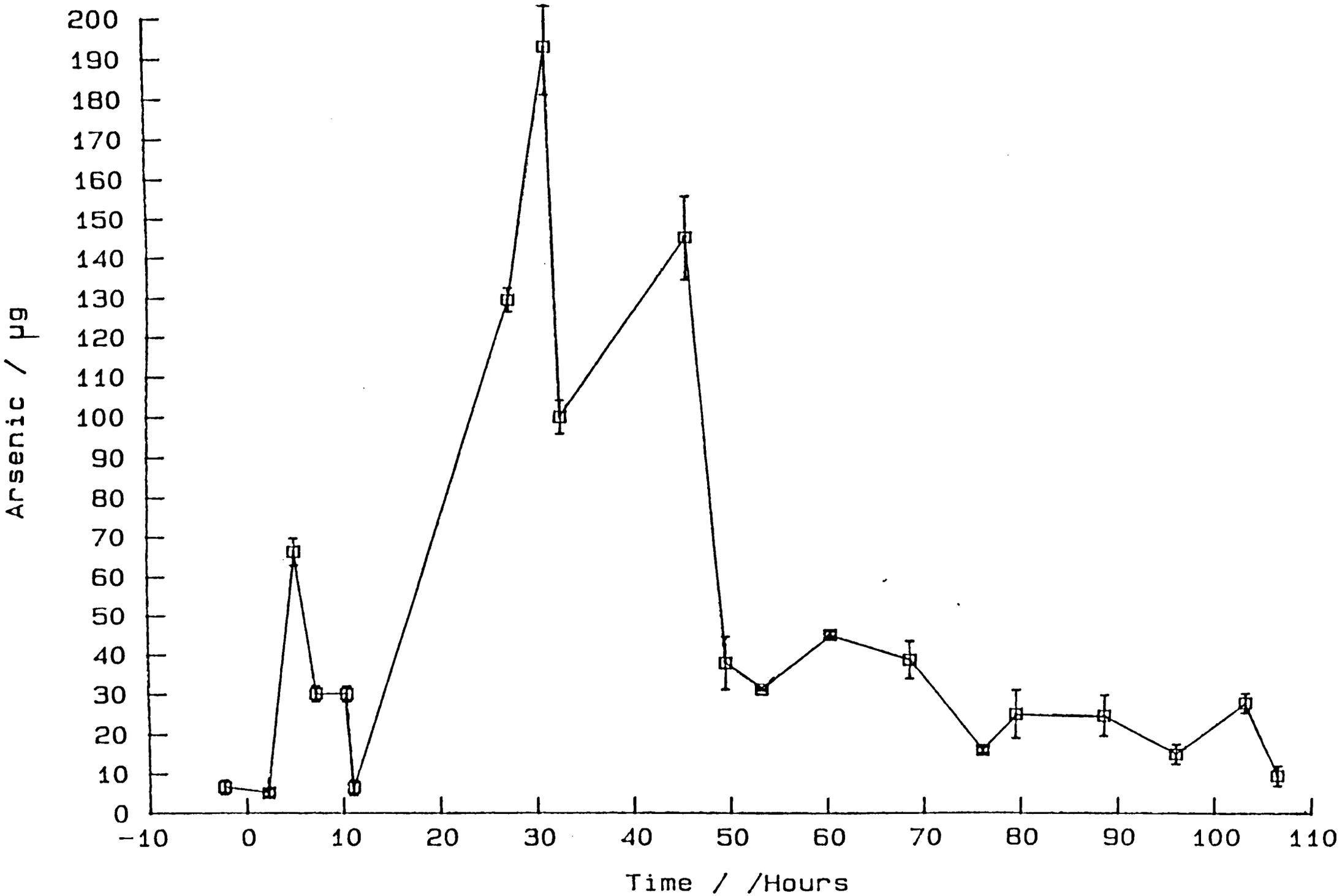


Figure 6.9 Total Arsenic in the Urine of Subject G Prior to Sample Freezing

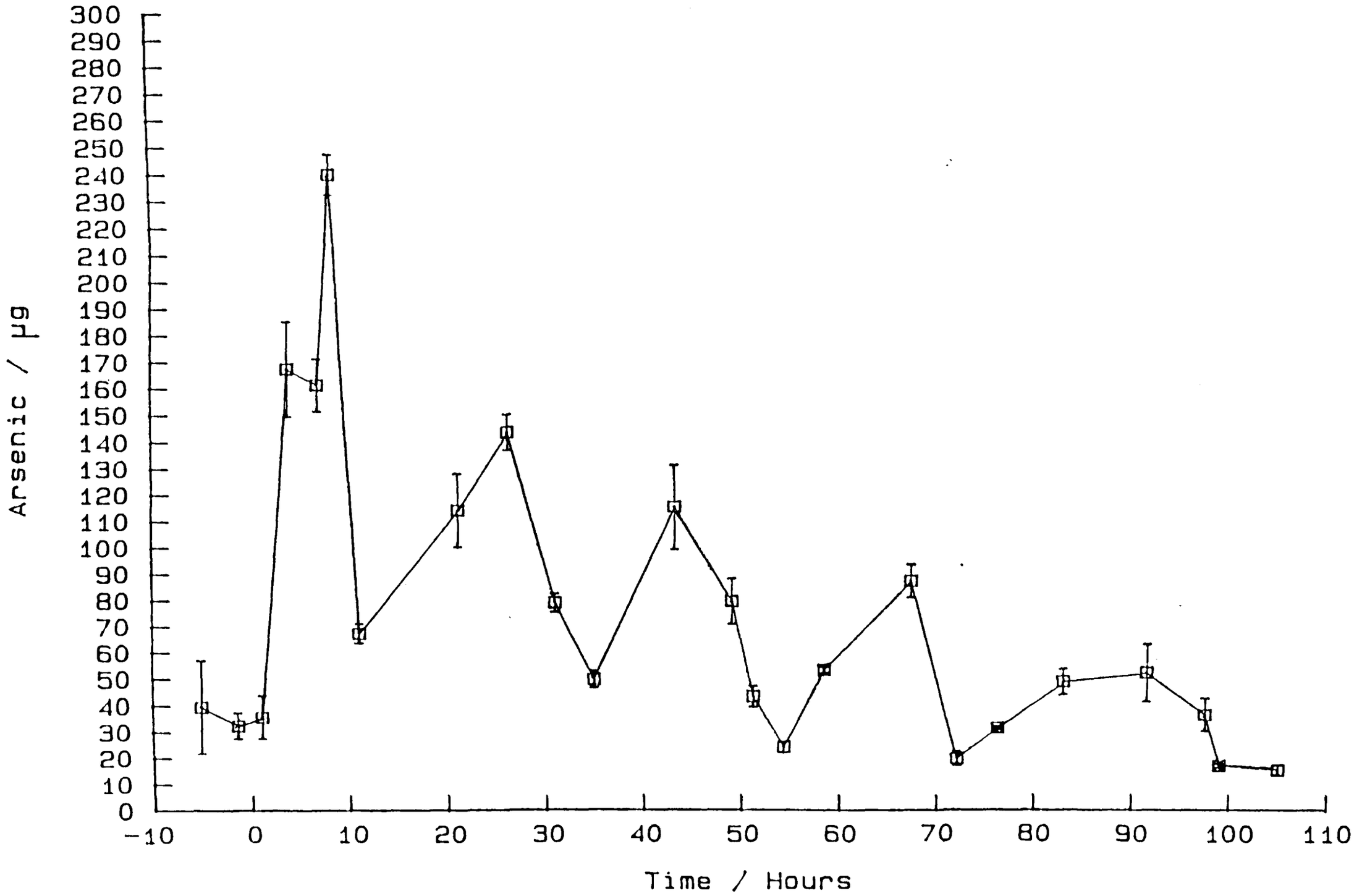


Figure 6.10 Total Arsenic in the Urine of Subject H Prior to Sample Freezing

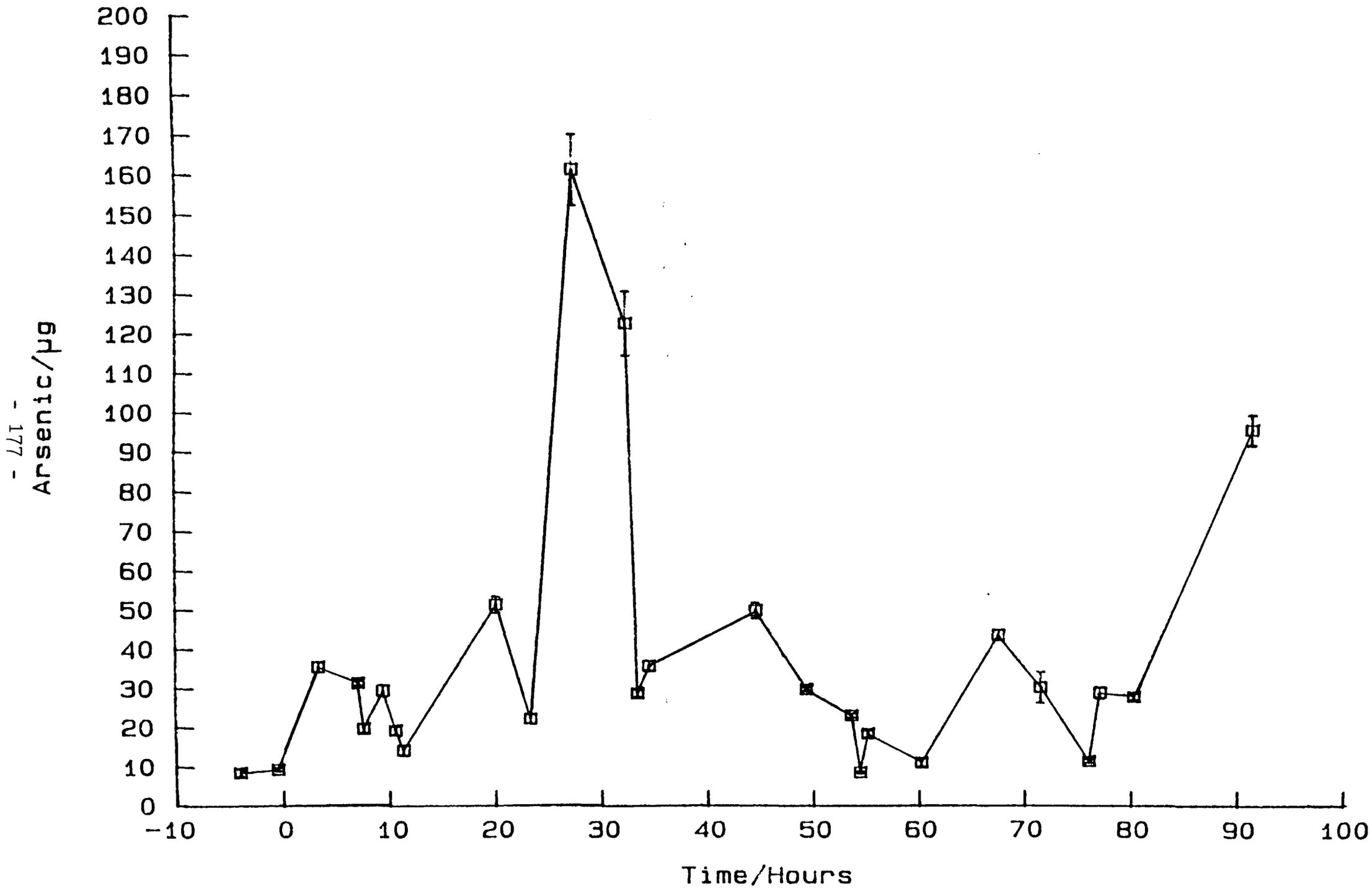


Figure 6.11 Total Arsenic in the Urine of Subject I Prior to Freezing

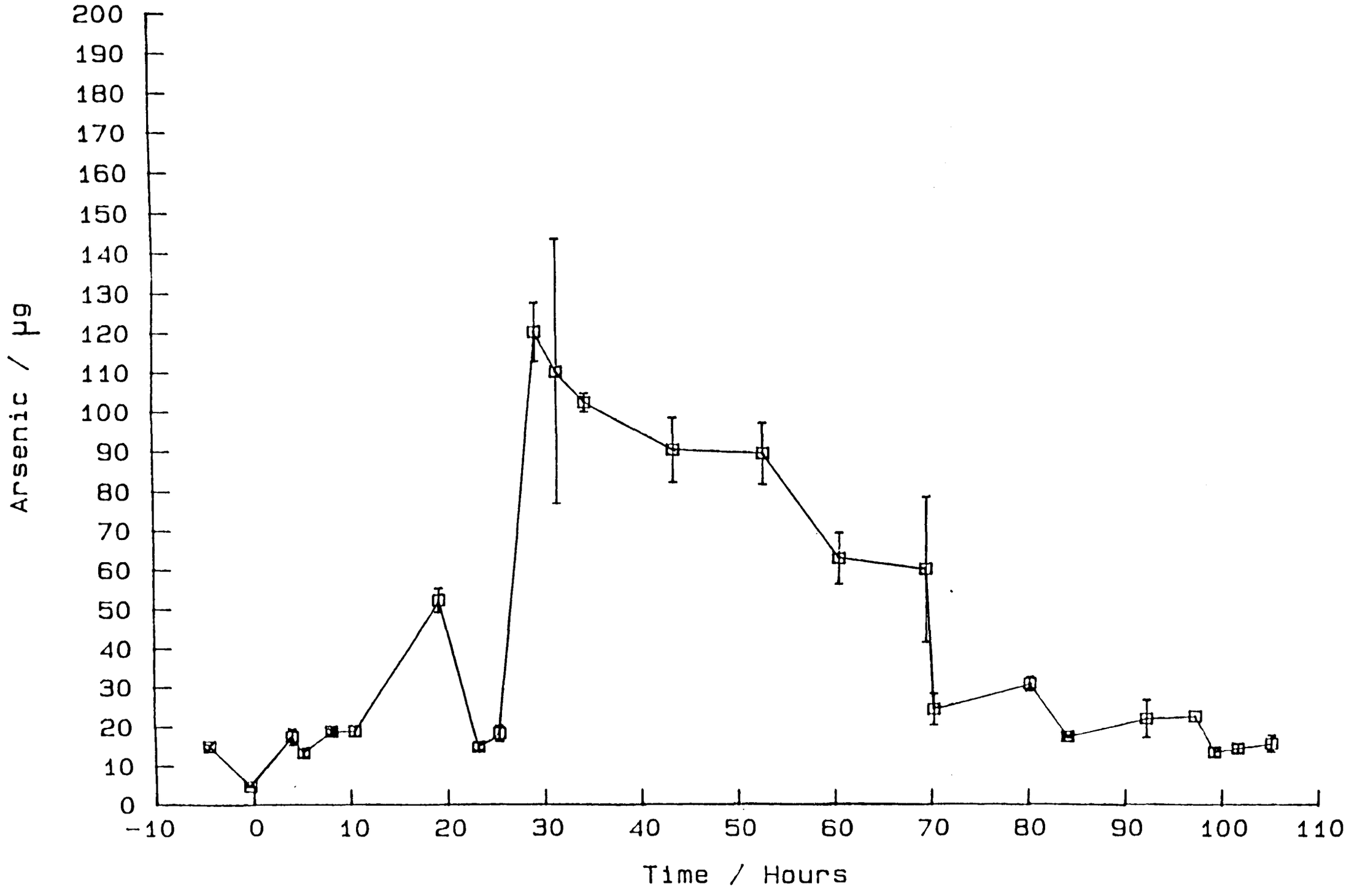
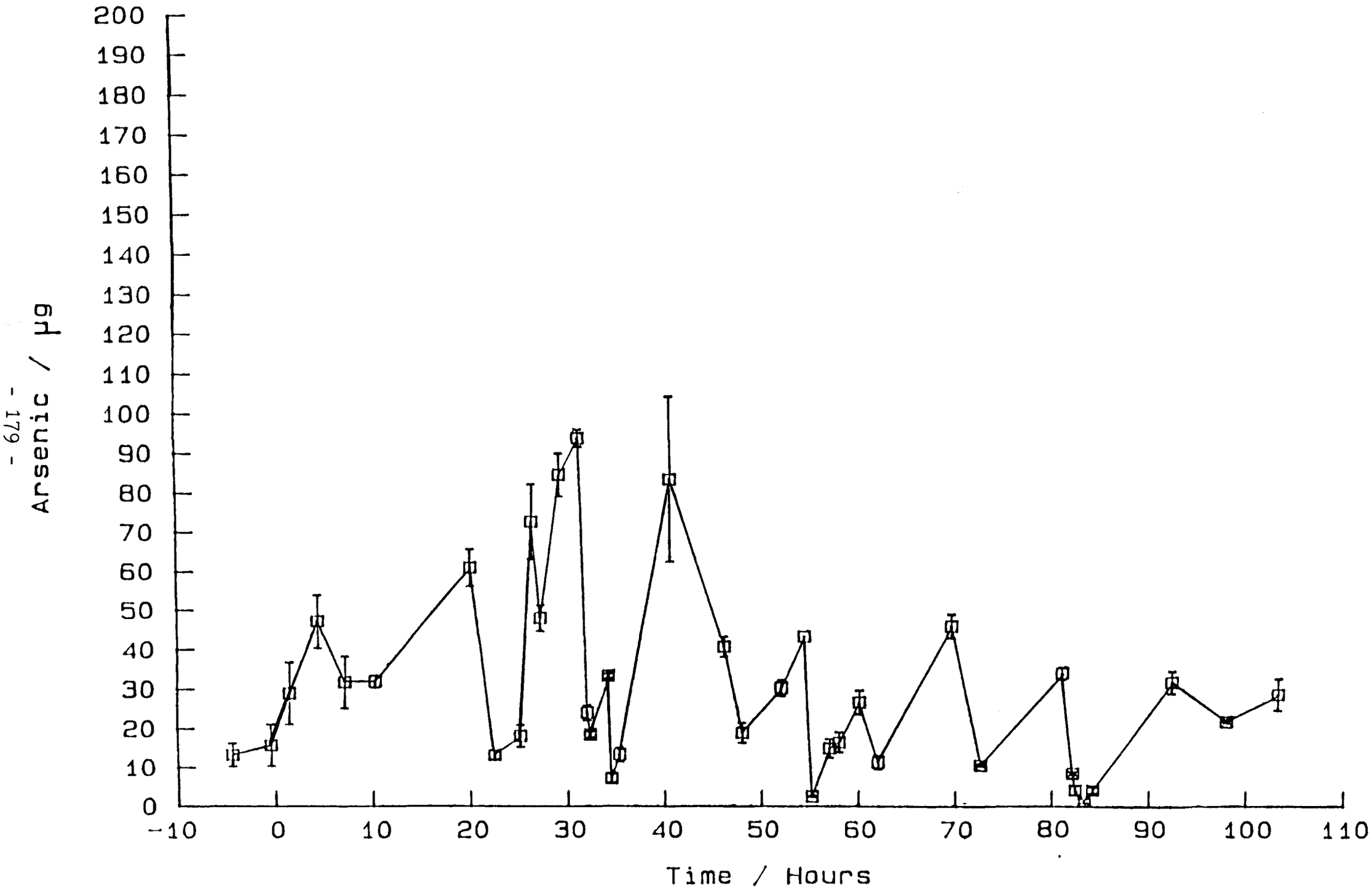


Figure 6.12 Total Arsenic in the Urine of Subject J Prior to Sample Freezing



expected at the start of the trial with initially low levels of arsenic that rise after eating cod, rapidly decay and then rise even higher following the consumption of plaice (which as previously noted usually contains higher levels of arsenic) and then degrades to basal levels. The rapid excretion of fish arsenic has been noted in the literature, 70% normally being excreted within 3 days of consumption (199). For all the subjects, except A and H, output had fallen to approximately 20 μg by the end of the trial. The variability of the profiles is principally due to the frequency at which samples were given. To eliminate this variation the total arsenic passed in twelve hour periods was plotted, the results being shown in Figures 6.13 and 6.14. These results shown much closer similarity from subject to subject, with rises associated with the fish meals followed by a rapid decline in output as the trial progressed. The outputs were lower in the trial hours 12-24, 36-48, etc. as these times corresponded to night time. The anomalous levels of arsenic in the urine of subject G are again apparent, as they are in Figures 6.15 and 6.16 in which the cumulative arsenic outputs as the trial progressed are plotted. The mean total arsenic output of the subjects, excluding G, was 1136 μg with all nine subjects lying within two standard deviations of the mean i.e. between 822-1450 μg. The relative standard deviation was 13.8% which, for biological systems, implies acceptable precision. Table 6.18 shows the cumulative arsenic total as the trial progressed. These results clearly show that if the arsenic

Figure 6.13

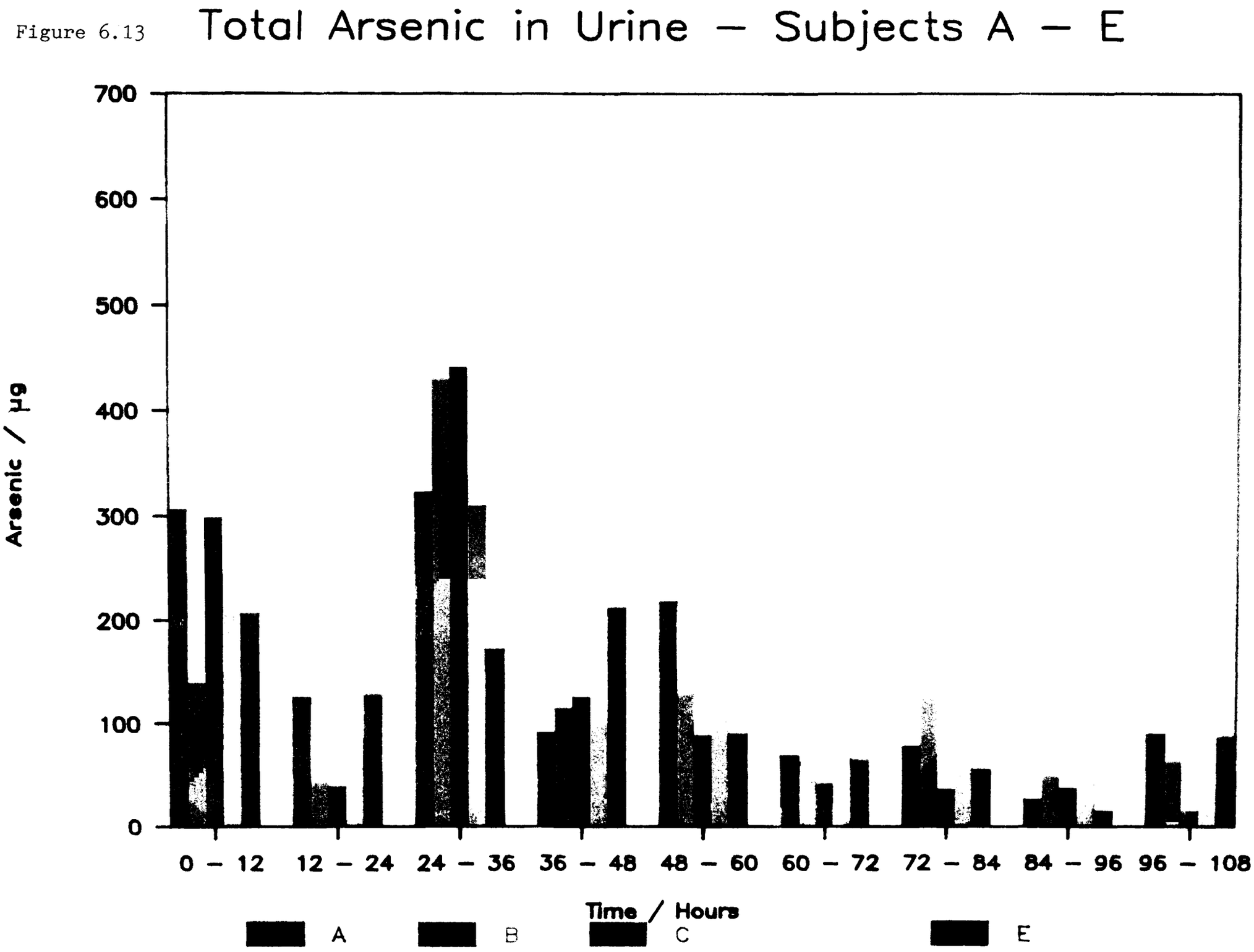

Total Arsenic in Urine — Subjects A — E
Arsenic / µg
700
600
500
400
300
200
100
0
0 — 12
12 — 24
24 — 36
36 — 48
48 — 60
60 — 72
72 — 84
84 — 96
96 — 108
Time / Hours
A
B
C
E

Figure 6.14

Total Arsenic in Urine — Subjects F — J

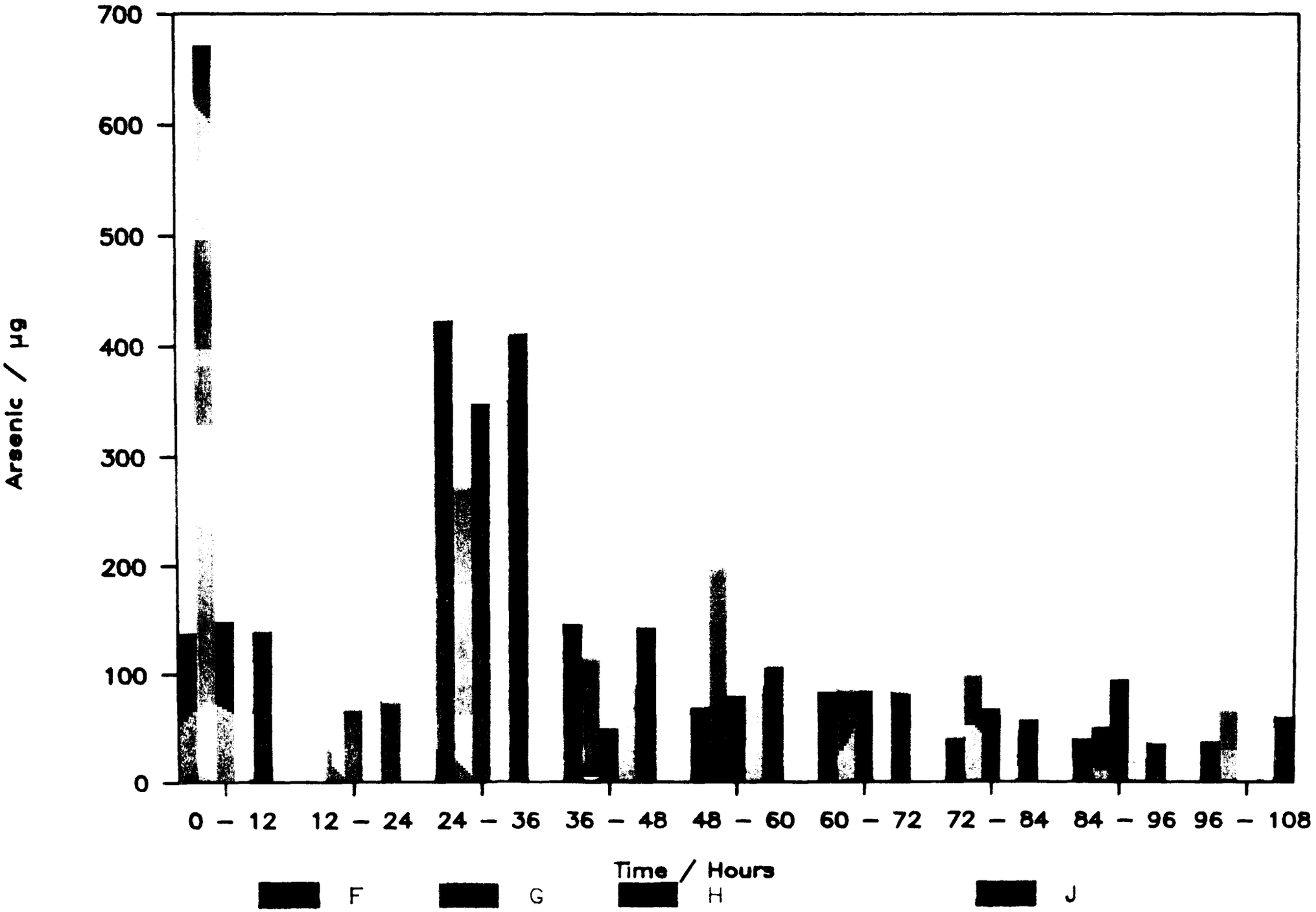


Figure 6.15 Cumulative Totals for Arsenic in Urine - Subjects A - E.

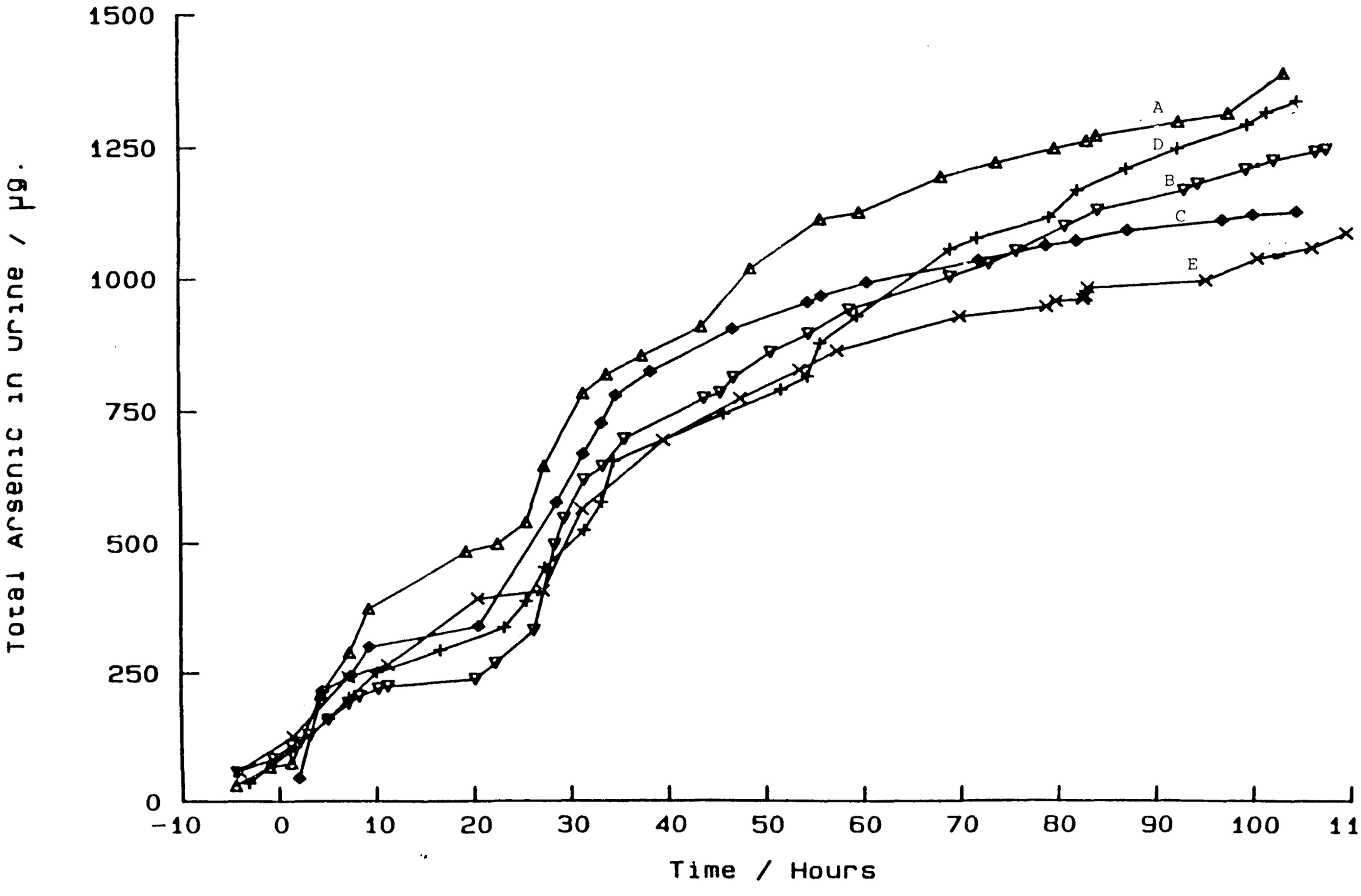


Figure 6.16 Cumulative Total Arsenic in the Urine of Subjects F - J.

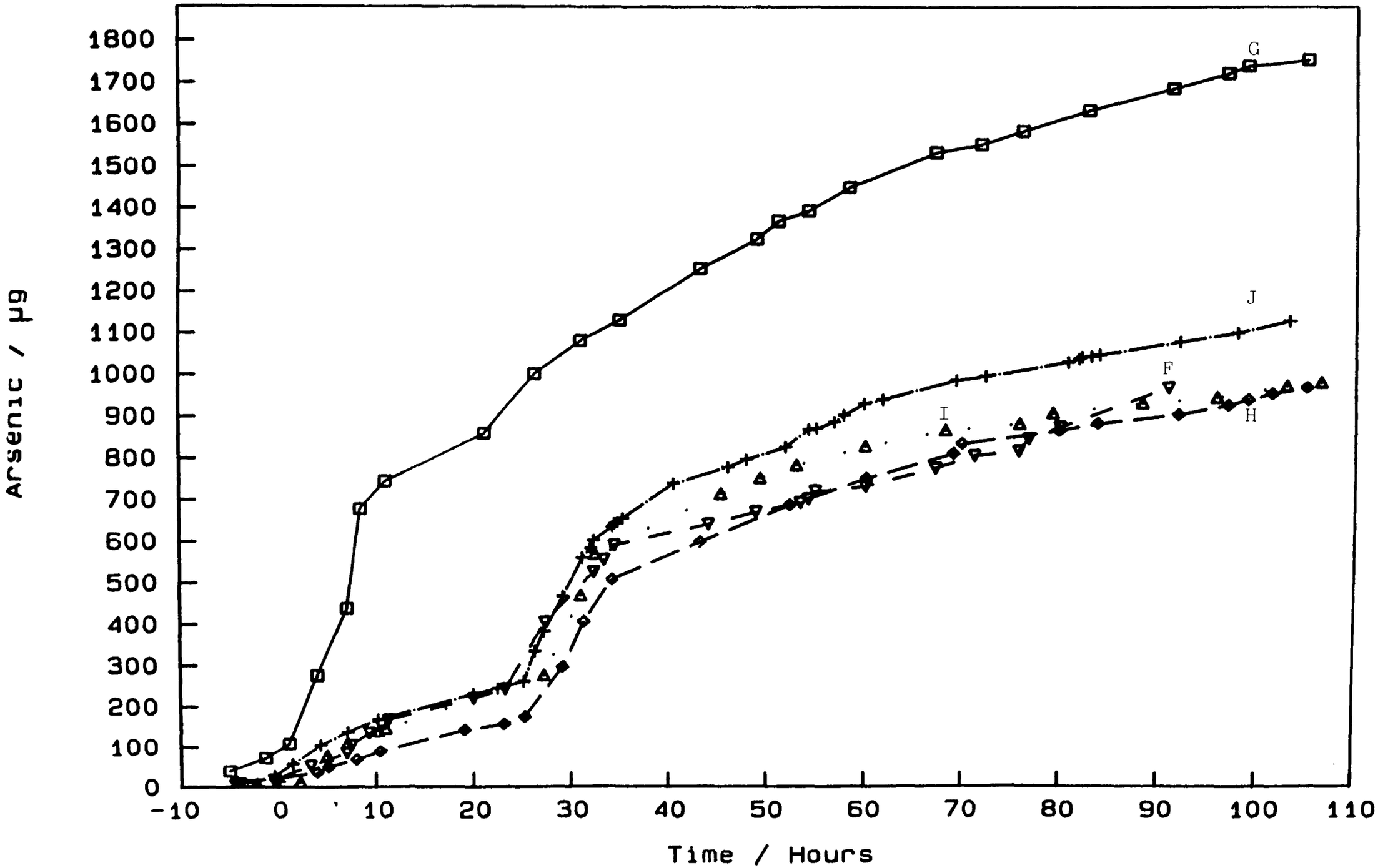


Table 6.18 Progressive Arsenic Output During the Dietary Trial. Units are μg As.

	Progressive Arsenic Output (μg)				
Subject	hrs 0-24	hrs 0-48	hrs 0-72	hrs 0-96	hrs 0-96+
A	431	844	1158	1263	1344
B	184	731	923	1101	1170
C	340	907	1039	1096	1131
D	301	711	1044	1216	1303
E	334	717	872	942	1031
F	138	705	858	963	1016
G	786	1174	1461	1613	1682
H	234	633	799	964	964
I	135	557	814	885	955
J	213	749	957	1051	1112

intake from the set meals was 0.8 mg as predicted then all this arsenic had been excreted within 72 hours of eating the first meal. The results also suggest that the high initial arsenic levels recorded in subject G's urine may be because he ate the second fillet from the cod containing 18.9 mg kg^{-1} arsenic. If this was the case then G would have consumed 1.43 mg of arsenic from the set meals. Again, this had been excreted within 72 hours. On the basis of these results it seems likely that none of the arsenic taken in by the body from fish based meals is assimilated, but passes out in the urine within 48-72 hours of consumption.

Following the screening of all the urine samples for total arsenic, selected samples were defrosted, filtered and total arsenic again determined by N_2 addition-ICP-MS. Arsenobetaine (AB) was determined by HPLC-ICP-MS and DMAA, MMAA and inorganic arsenic (As_i) by hydride generation - cryogenic trapping-AAS (HG-CT-AAS). The results are shown in Tables 6.19-6.28, and graphically in Figures 6.17-6.26. The urine samples from B, D and H were not analysed by HG-CT-AAS due to time constraints but the results by HPLC-ICP-MS indicate that AB was the dominant arsenic compound in the samples. Several points arise from the results:

(1) A significant fraction of the arsenic was lost following freezing. This was because when the samples were defrosted there was precipitation of solids. These were removed by filtration which probably also

Table 6.19 Arsenic Species in the Urine of Subject A. Units are μg As

Sample	Time (hours)	Total Before Freezing	Total After Freezing	A-B	DMAA	MMAA	As_i
A 1	-4.5	30.4 ± 4.6	12.8 ± 3.3	7.5 ± 2.0	<0.2	<0.3	<0.3
A 2	-1.0	36.7 ± 3.3	2.3 ± 0.8	5.9 ± 0.2	<0.1	<0.2	1.2
A 5	7.2	81.6 ± 4.0	29.2 ± 5.3	44.6 ± 0.8	0.1	<0.2	0.2
A 6	9.2	84.2 ± 3.2	17.8 ± 3.5	18.9 ± 2.6	0.4	<0.1	0.1
A 7	19.1	110.4 ± 7.4	21.7 ± 2.1	17.2 ± 2.0	0.2	<0.4	<0.4
A 8	22.4	14.5 ± 0.1	7.8 ± 1.4	21.3 ± 3.1	<0.1	<0.2	0.3
A10	27.1	108.0 ± 3.3	64.2 ± 8.6	72.2 ± 2.0	<0.2	<0.3	<0.3
A11	31.1	137.2 ± 1.7	81.8 ± 4.0	88.5 ± 5.0	<0.4	<0.8	<0.8
A12	33.5	35.8 ± 1.7	18.3 ± 0.8	26.4 ± 3.4	<0.3	<0.5	4.7
A14	43.1	55.2 ± 1.2	31.7 ± 2.0	30.1 ± 1.3	NP	NP	NP
A15	48.2	112.0 ± 0.1	37.0 ± 5.3	37.0 ± 4.3	<0.1	<0.2	<0.2
A16	55.3	92.6 ± 0.9	39.6 ± 3.7	44.6 ± 1.2	0.7	<0.3	1.1
A18	67.5	68.8 ± 2.6	27.6 ± 1.8	21.3 ± 1.7	<0.2	<0.4	3.0
A23	91.5	27.7 ± 4.1	25.0 ± 3.6	31.9 ± 1.7	<0.3	<0.6	5.0

NP = Not Performed

Table 6.20 **Arsenic Species in the Urine of Subject B.**
Units are μg As.

Sample	Time (hours)	Total As Before Freezing	Total As After Freezing	A-B
B 1	-4.35	57.2 ± 6.5	12.1 ± 2.3	<9.1
B 3	1.30	26.7 ± 4.5	3.9 ± 0.2	19.6 ± 0.7
B 7	7.15	31.8 ± 1.5	40.3 ± 3.0	31.2 ± 3.4
B 8	8.30	13.1 ± 0.3	18.4 ± 0.6	13.5 ± 1.8
B13	26.10	63.6 ± 1.9	15.3 ± 1.8	12.2 ± 1.8
B14	28.20	165.1 ± 4.4	131.6 ± 1.2	114.3 ± 2.8
B16	31.20	74.2 ± 2.0	67.3 ± 5.1	26.5 ± 2.0
B20	43.45	77.0 ± 1.5	71.4 ± 2.6	67.3 ± 2.1
B24	54.10	34.6 ± 1.3	23.9 ± 0.8	27.3 ± 1.5
B29	80.10	46.4 ± 1.1	34.4 ± 0.6	45.9 ± 3.7
B37	106.40	4.3 ± 0.4	4.4 ± 0.3	2.3 ± 0.3

Table 6.21 **Arsenic Species in the Urine of Subject C.** Units are μg As

Sample	Time (hours)	Total Before Freezing	Total After Freezing	A-B	DMAA	MMAA	As_i
C 1	2.0	45.7 ± 1.1	34.2 ± 1.4	24.8 ± 2.1	<1.1	<2.2	2.2
C 2	4.42	168.7 ± 11.7	86.0 ± 4.2	52.3 ± 9.4	<1.1	<2.1	4.4
C 3	7.30	28.8 ± 1.6	32.8 ± 3.3	19.7 ± 0.7	<0.4	<0.7	2.0
C 5	20.40	34.5 ± 2.3	39.4 ± 2.1	33.4 ± 2.9	0.6	<0.7	1.5
C 6	28.40	238.1 ± 18.7	187.8 ± 3.2	229.4 ± 5.7	0.6	<0.6	1.5
C 7	31.10	92.7 ± 3.7	85.0 ± 4.8	88.0 ± 4.1	3.2	<1.1	2.0
C 8	33.00	58.5 ± 1.0	52.5 ± 4.1	42.1 ± 6.9	<0.4	<0.8	1.5
C11	46.35	80.7 ± 1.1	58.5 ± 3.9	56.8 ± 1.0	<0.4	<0.8	1.3
C12	54.00	50.6 ± 0.7	34.8 ± 5.5	35.5 ± 2.4	<0.5	<0.9	1.2
C15	71.40	42.9 ± 1.0	38.9 ± 1.1	33.9 ± 2.4	<0.5	<1.0	1.0
C17	81.25	9.0 ± 1.0	4.7 ± 2.0	4.4 ± 1.2	1.3	0.3	0.8
C21	103.45	5.6 ± 0.7	4.4 ± 2.8	2.8 ± 0.9	2.0	1.6	0.5

Table 6.22 **Arsenic Species in the Urine of Subject D.**
Units are μg As

Sample	Time (hours)	Total As Before Freezing	Total As After Freezing	A-B
D 3	5.0	62.1 ± 1.2	36.2 ± 2.1	45.1 ± 2.8
D 5	10.05	49.0 ± 1.7	43.5 ± 4.9	32.6 ± 1.0
D 6	16.45	42.9 ± 0.9	43.7 ± 2.2	46.5 ± 0.3
D 8	25.30	50.6 ± 0.4	46.0 ± 1.5	46.5 ± 0.3
D 9	27.20	66.0 ± 1.5	60.3 ± 4.5	62.1 ± 0.4
D11	31.20	70.8 ± 1.0	53.6 ± 1.9	43.9 ± 6.3
D12	33.00	54.0 ± 0.5	49.2 ± 1.4	41.4 ± 2.9
D22	81.30	52.2 ± 2.6	45.5 ± 1.8	34.3 ± 0.1
D25	98.00	44.0 ± 1.7	34.0 ± 1.8	30.0 ± 0.8
D26	100.35	22.7 ± 2.2	28.5 ± 1.8	37.3 ± 4.1

Table 6.23 **Arsenic Species in the Urine of Subject E.** Units are μg As

Sample	Time (hours)	Total Before Freezing	Total After Freezing	A-B	DMAA	MMAA	As_i
E 1	-4.15	58.6 ± 3.7	20.1 ± 5.0	11.0	<1.8	<0.9	<3.6
E 2	1.45	68.4 ± 5.6	20.1 ± 3.8	13.8	<1.5	<0.8	<3.0
E 4	11.15	23.2 ± 0.4	41.5 ± 2.8	28.5	<0.4	<0.2	<0.7
E 5	20.30	127.8 ± 2.1	42.6 ± 6.0	34.0	<1.2	<0.6	<2.4
E 6	27.00	15.3 ± 0.2	88.0 ± 1.9	8.9	1.2	<0.6	<2.2
E 7	31.00	156.3 ± 3.1	110.7 ± 4.1	101.3	2.3	<0.5	<1.8
E 8	39.30	132.4 ± 3.2	88.8 ± 9.6	64.7	3.0	<0.6	<2.4
E 9	47.15	79.1 ± 1.8	50.4 ± 5.9	33.5	1.9	<0.5	<1.8
E10	53.18	52.6 ± 2.9	27.2 ± 3.4	27.5	<1.0	<0.3	<1.0
E11	57.00	36.5 ± 0.8	21.2 ± 3.4	15.6	<0.4	<0.2	<0.8
E13A	78.30	19.4 ± 2.1	12.4 ± 1.8	12.4	0.8	<0.4	<1.4
E15	82.00	3.9 ± 1.8	2.4 ± 0.5	<20.0	NP	NP	NP
E21	108.45	27.9 ± 1.9	9.5 ± 0.3	NP	<1.8	<0.9	<3.6

NP = Not Performed

Table 6.24 Arsenic Species in the Urine of Subject F. Units are μg As

Sample	Time (hours)	Total Before Freezing	Total After Freezing	A-B	DMAA	MMAA	As_i
F 1	-2.30	6.6 ± 0.9	10.2 ± 1.3	8.6 ± 2.2	<0.4	<0.4	<0.2
F 2	2.20	5.1 ± 0.5	7.3 ± 0.9	4.4 ± 0.4	<0.3	<0.3	<0.2
F 3	5.00	66.4 ± 1.7	85.7 ± 5.3	72.8 ± 0.7	<0.4	<0.4	<0.2
F 4	7.10	30.0 ± 1.0	35.6 ± 1.0	31.1 ± 1.1	<0.2	<0.2	0.3
F 5	10.30	29.9 ± 1.0	31.0 ± 0.9	31.7 ± 3.7	1.2	<0.8	<0.4
F 6	11.00	6.4 ± 1.0	2.8 ± 0.6	<4.8	<0.5	<0.5	<0.2
F 7	21.15	31.2 ± 2.2	29.7 ± 2.0	24.3 ± 2.8	NP(1)	NP	NP
F 9	31.00	193.4 ± 6.0	168.0 ± 6.6	142.3 ± 10.3	<0.4	<0.4	<0.2
F11	45.40	145.4 ± 5.3	97.4 ± 7.2	87.4 ± 9.8	<0.7	<0.7	1.8
F13	53.20	31.1 ± 0.2	21.8 ± 1.0	17.7 ± 1.7	0.4	<0.3	<0.1
F16	76.10	16.0 ± 0.4	17.1 ± 0.2	13.8 ± 2.0	0.3	<0.3	<0.2
F18	88.50	24.8 ± 2.6	22.9 ± 0.4	20.5 ± 2.3	0.6	<0.4	<0.4
F20	103.00	28.1 ± 1.2	18.4 ± 0.7	22.4 ± 0.6	0.8	2.1	2.3

NP = Not Performed

(1) Sample F7 could not be analysed by HG-CT-AAS due to foaming and the formation of a precipitate in the gas liquid separator.

Table 6.25 **Arsenic Species in the Urine of Subject G.** Units are μg As

Sample	Time (hours)	Total As Before Freezing	Total As After Freezing	A-B	DMAA	MMAA	As_i
G 1	-5.00	39.4 ± 8.8	24.8 ± 2.2	21.1 ± 1.8	<0.3	<0.5	<0.5
G 2	-1.30	32.3 ± 2.5	14.1 ± 5.1	17.2 ± 0.9	<0.3	<0.6	<0.6
G 3	1.20	35.6 ± 4.1	15.2 ± 2.1	15.8 ± 5.4	<0.2	<0.3	1.7
G 4	4.00	167.5 ± 8.9	165.6 ± 6.3	157.1 ± 7.4	1.8	<0.4	<0.4
G 5	7.05	161.4 ± 5.0	138.8 ± 13.9	167.0 ± 7.6	0.3	<0.7	<0.7
G 6	8.45	240.0 ± 7.5	63.0 ± 3.5	97.4 ± 9.3	<0.3	<0.6	<0.6
G 7	11.00	67.0 ± 1.9	51.5 ± 7.8	50.7 ± 1.7	<0.3	<0.6	<0.6
G 8	21.15	114.2 ± 7.0	99.8 ± 3.7	72.8 ± 8.1	<0.6	<1.2	3.6
G10	31.00	78.8 ± 1.8	105.6 ± 2.2	76.3 ± 4.7	<0.4	<0.9	1.0
G11	35.00	49.6 ± 1.6	51.1 ± 3.4	37.4 ± 3.3	0.3	0.3	0.4
G12	43.30	115.4 ± 8.1	112.2 ± 10.5	111.0 ± 5.1	0.3	1.5	0.7
G15	54.40	24.1 ± 1.1	22.9 ± 0.5	21.6 ± 0.8	<0.4	<0.8	1.5
G17	67.45	87.2 ± 3.1	79.9 ± 2.8	71.9 ± 2.9	<0.5	<0.9	<0.9
G23	99.00	16.9 ± 0.4	16.4 ± 0.8	13.6 ± 1.0	<0.2	<0.3	<0.3

Table 6.26 **Arsenic Species in the Urine of Subject H.**
Units are μg As

Sample	Time (hours)	Total As Before Freezing	Total As After Freezing	A-B
H 1	-4.00	8.5 ± 1.0	14.3 ± 0.5	6.9 ± 1.4
H 3	3.30	35.5 ± 1.3	48.4 ± 1.2	48.0 ± 7.3
H 6	9.35	29.4 ± 1.5	25.6 ± 1.4	20.4 ± 0.4
H 9	20.00	51.4 ± 2.1	38.5 ± 2.2	36.2 ± 1.3
H10	23.25	22.0 ± 1.3	21.6 ± 1.4	20.0 ± 1.9
H11	27.30	161.6 ± 8.9	119.7 ± 2.6	111.0 ± 5.0
H12	32.30	122.9 ± 8.3	77.2 ± 5.8	84.2 ± 3.3
H19	55.10	18.5 ± 0.9	19.4 ± 1.5	14.3 ± 0.5
H21	67.45	43.8 ± 1.3	41.4 ± 2.4	33.0 ± 1.6
H26	91.15	96.0 ± 3.9	97.8 ± 4.7	100.7 ± 3.6

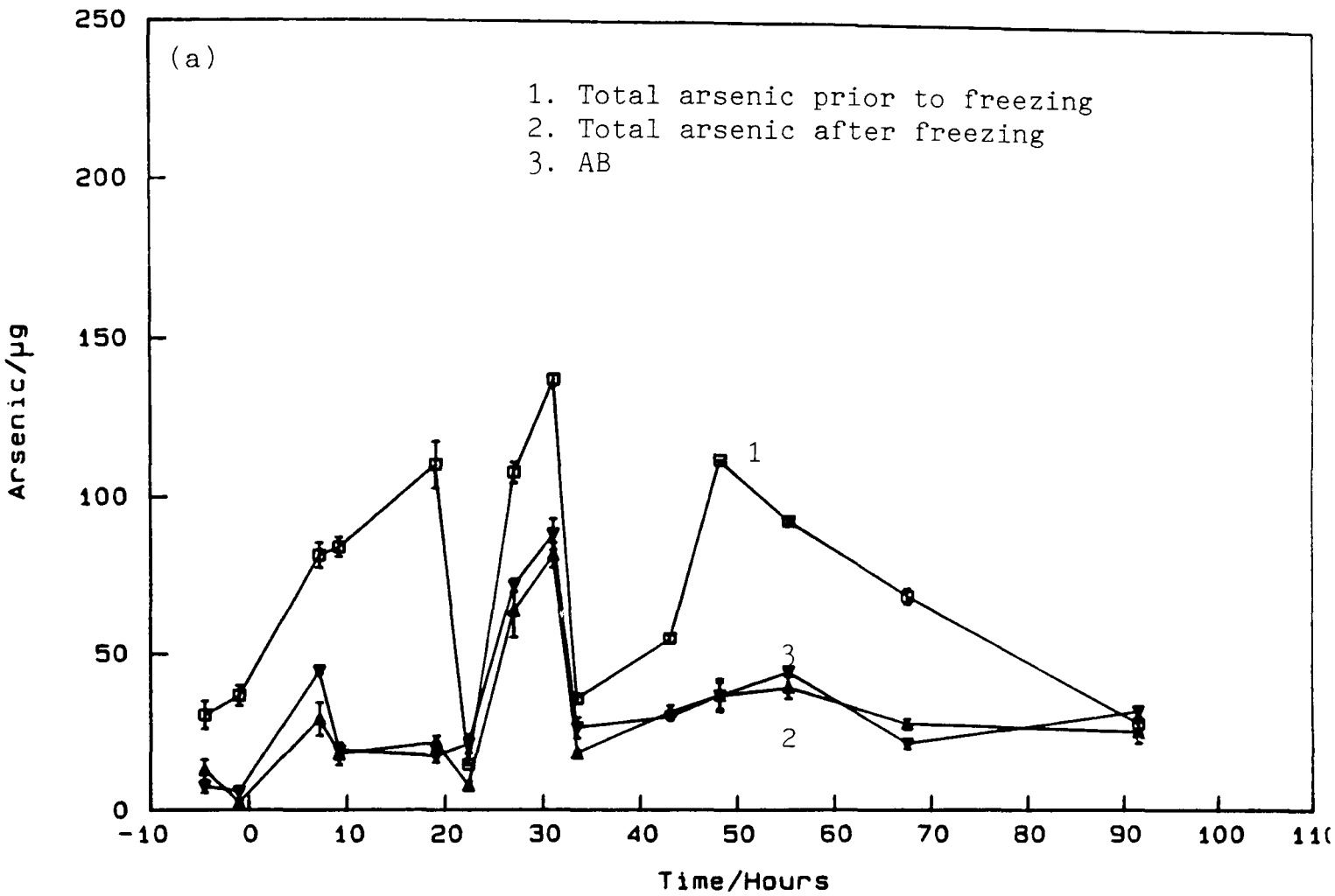
Table 6.27 Arsenic Species in the Urine of Subject I. Units are μg As

Sample	Time (hours)	Total As Before Freezing	Total As After Freezing	A-B	DMAA	MMAA	As_i
I 1	-4.45	14.9 ± 0.3	37.2 ± 3.7	13.8 ± 4.5	5.3	<0.6	<0.4
I 2	-0.35	4.6 ± 0.1	57.1 ± 0.4	61.7 ± 1.8	0.7	<0.6	0.2
I 3	4.00	17.4 ± 1.0	74.8 ± 5.0	60.7 ± 0.5	<0.4	<0.8	<0.2
I 4	5.15	13.3 ± 0.5	19.6 ± 8.5	35.6 ± 3.6	1.8	<1.0	<0.2
I 5	8.05	18.8 ± 0.3	28.3 ± 1.5	41.5 ± 1.3	<0.5	<1.0	<0.3
I 7	19.10	52.2 ± 1.5	33.6 ± 1.4	59.0 ± 4.0	1.3	<1.1	<0.3
I 8	23.15	14.9 ± 0.5	11.7 ± 3.6	14.5 ± 2.1	2.0	<0.7	<0.2
I 9	25.30	18.3 ± 1.0	95.5 ± 7.3	49.4 ± 9.3	1.5	<1.0	1.2
I10	29.12	120.5 ± 3.7	102.5 ± 3.2	106.0 ± 4.7	<0.8	<0.6	<0.3
I12	34.20	102.5 ± 1.2	71.3 ± 4.4	103.9 ± 10.4	2.9	<1.2	<0.6
I13	43.30	90.5 ± 4.1	83.2 ± 2.6	77.1 ± 2.6	7.2	<1.7	<0.9
I16	69.30	60.2 ± 9.3	44.0 ± 4.3	58.2 ± 6.6	4.1	<1.0	<0.5
I19	84.00	17.5 ± 0.2	11.9 ± 1.0	10.3 ± 0.3	1.6	<0.8	<0.4
I21	97.15	22.8 ± 0.1	14.2 ± 1.6	14.1 ± 2.3	2.2	<0.6	<0.3
I22	99.15	13.6 ± 0.6	8.2 ± 2.2	1.8 ± 0.9	2.1	<1.8	<0.9

Table 6.28 Arsenic Species in the Urine of Subject J. Units are μg As

Sample	Time (hours)	Total As Before Freezing	Total As After Freezing	A-B	DMAA	MMAA	As_i
J 1	-4.4	13.1 ± 1.5	9.0 ± 2.0	25.8 ± 7.5	<0.2	<0.4	6.1
J 2	-0.45	15.6 ± 2.7	2.9 ± 1.0	3.0 ± 1.0	<0.2	<0.3	1.5
J 3	1.45	28.8 ± 3.9	7.5 ± 0.3	12.1 ± 2.9	<0.2	<0.3	2.1
J 4	4.35	47.0 ± 3.4	36.7 ± 1.5	41.1 ± 2.0	<0.2	<0.5	1.6
J 6	10.21	31.7 ± 0.8	28.0 ± 12.0	43.0 ± 0.1	<0.3	<0.7	1.5
J 8	22.50	13.0 ± 0.6	6.9 ± 0.9	19.0 ± 3.2	1.5	<0.5	1.2
J10	26.30	72.6 ± 4.8	44.4 ± 1.5	46.7 ± 1.8	<0.2	<0.6	0.6
J11	27.20	47.7 ± 1.7	37.9 ± 1.2	39.2 ± 5.1	<0.2	<0.4	<0.4
J12	29.20	84.6 ± 2.7	54.7 ± 4.5	85.8 ± 8.5	<0.2	<0.3	<0.3
J15	32.30	18.2 ± 0.4	8.7 ± 2.7	<7.5	2.0	<0.6	<0.6
J19	40.55	83.4 ± 10.5	32.4 ± 0.8	<21.0	1.3	<1.7	<1.7
J22	52.10	30.1 ± 1.0	17.0 ± 1.2	15.2 ± 1.9	<0.2	<0.3	<0.3
J27	60.10	26.5 ± 1.5	2.3 ± 0.9	<11.3	1.3	<0.9	<0.9
J30	73.00	10.4 ± 0.2	9.5 ± 1.6	14.5 ± 2.0	<0.2	<0.4	<0.4
J31	81.00	34.0 ± 0.8	21.6 ± 2.4	22.8 ± 2.4	<1.5	<3.0	<3.0
J38	103.20	28.8 ± 2.0	17.5 ± 1.0	24.1 ± 5.1	<0.3	<0.7	<0.7

Figure 6.17(a) and 6.17(b) Arsenic Species in the Urine of Subject A



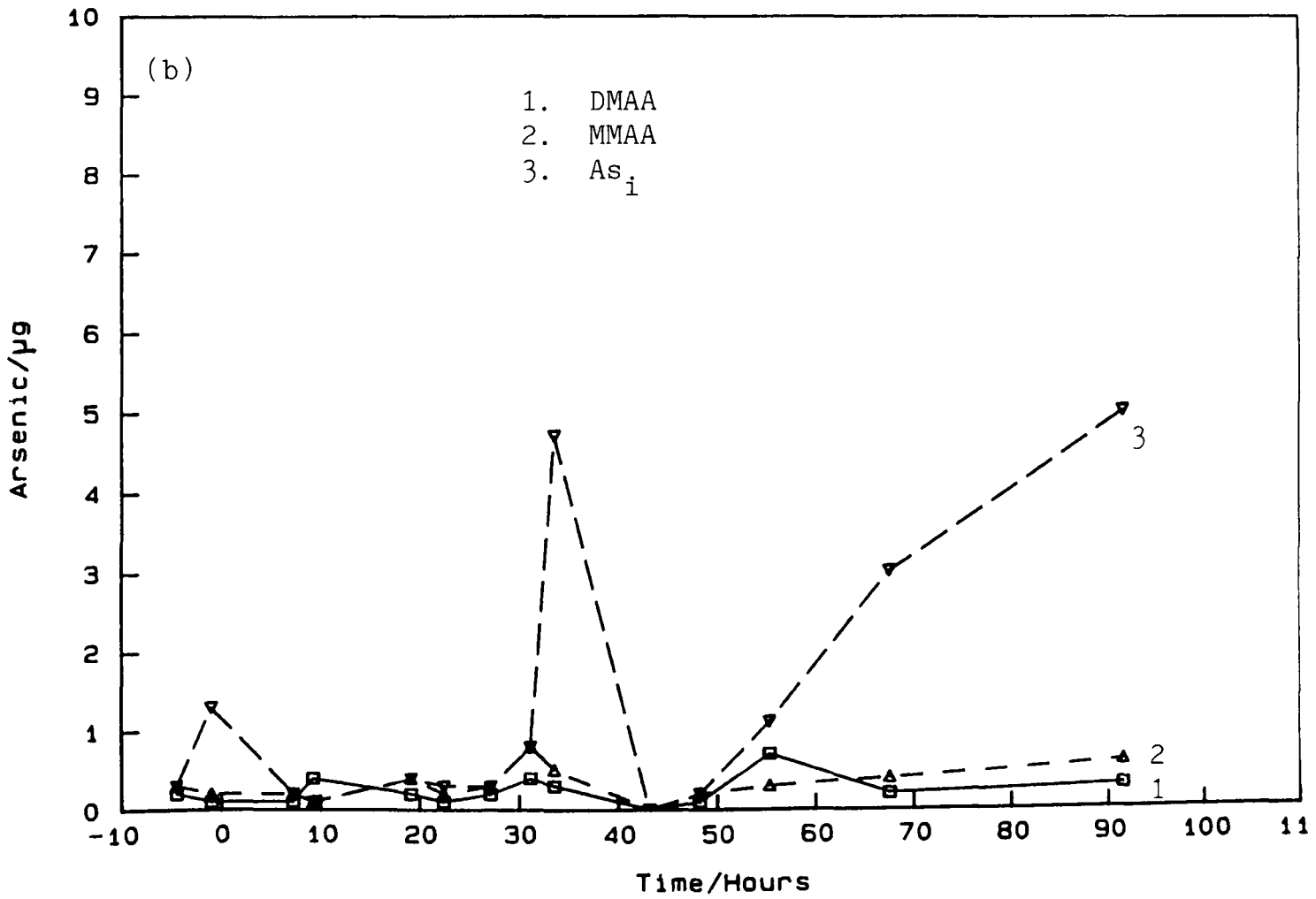


Figure 6.18 Arsenic Species in the Urine of Subject B

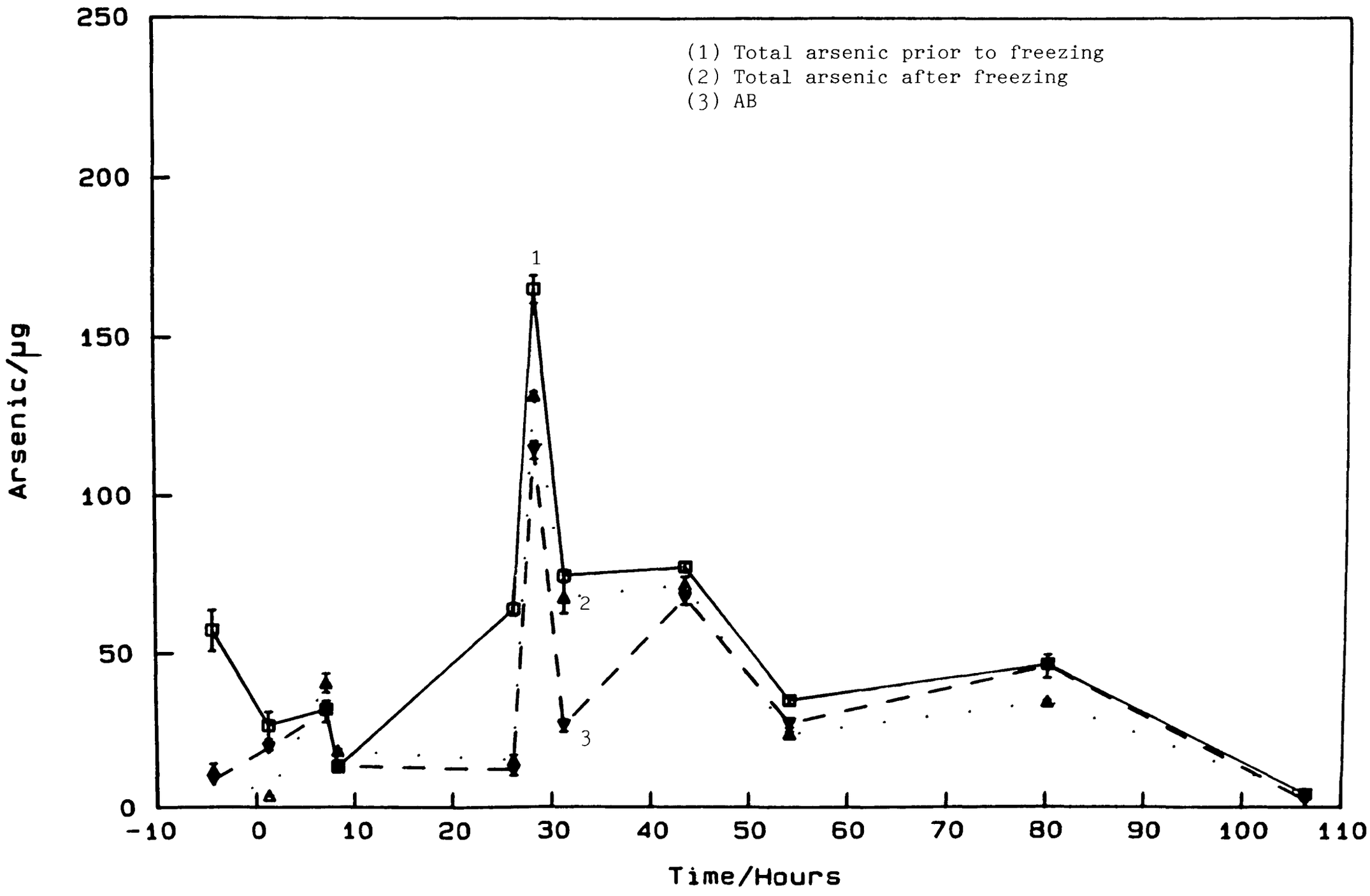
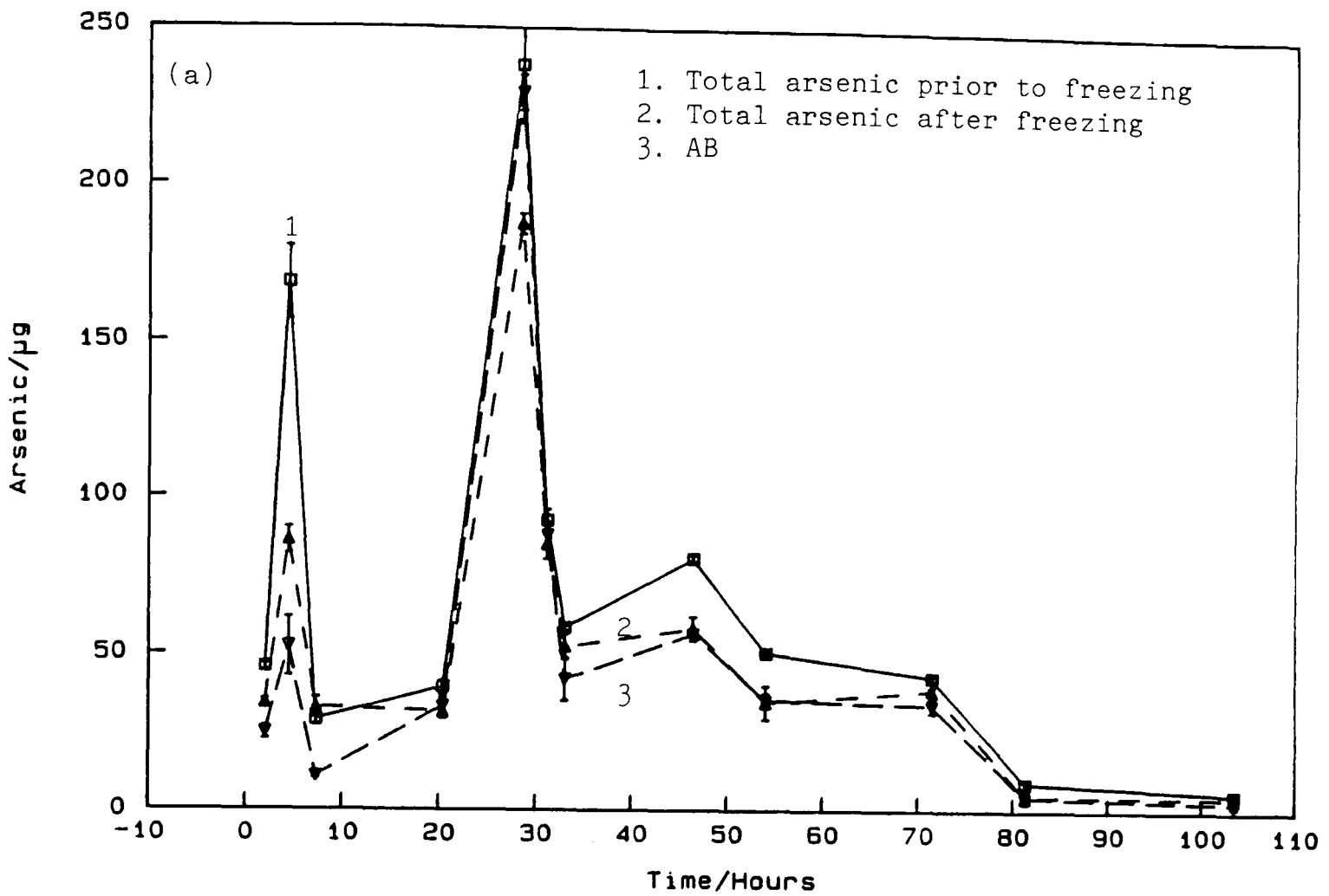


Figure 6.19(a) and 6.19(b) Arsenic Species in the Urine of Subject C



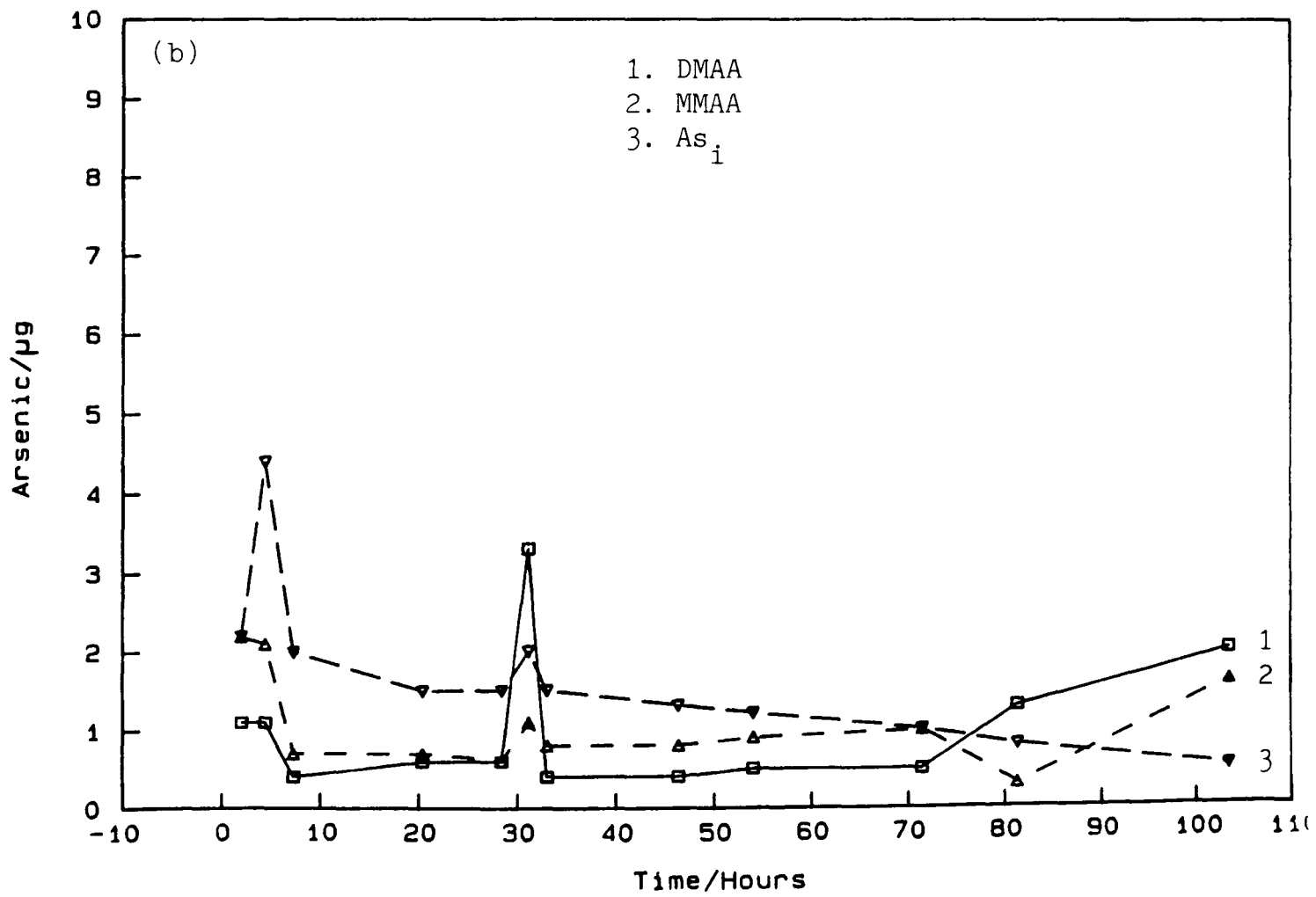


Figure 6.20 Arsenic Species in the Urine of Subject D

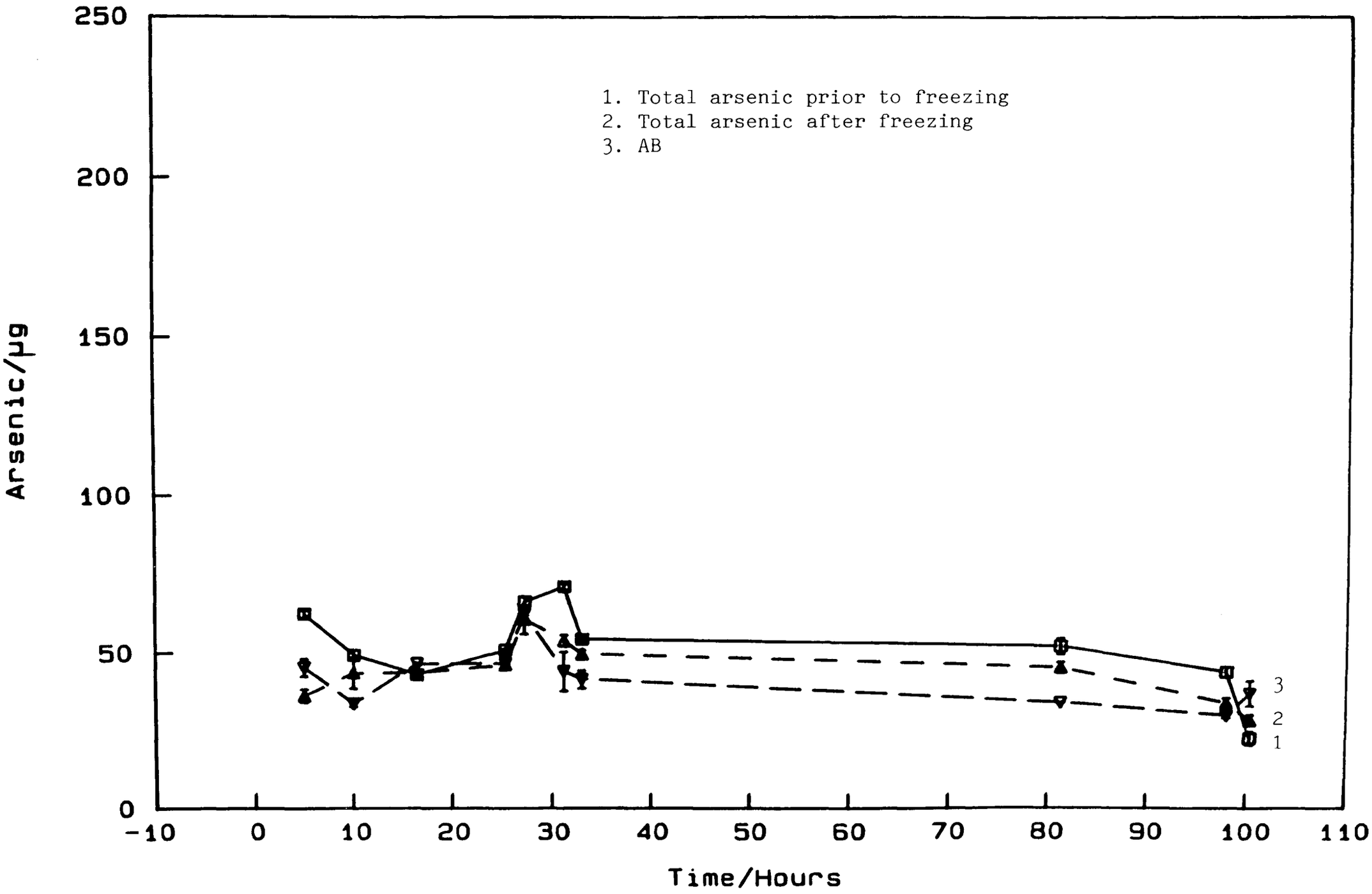


Figure 6.21(a) and 6.21(b) Arsenic Species in the Urine of Subject E

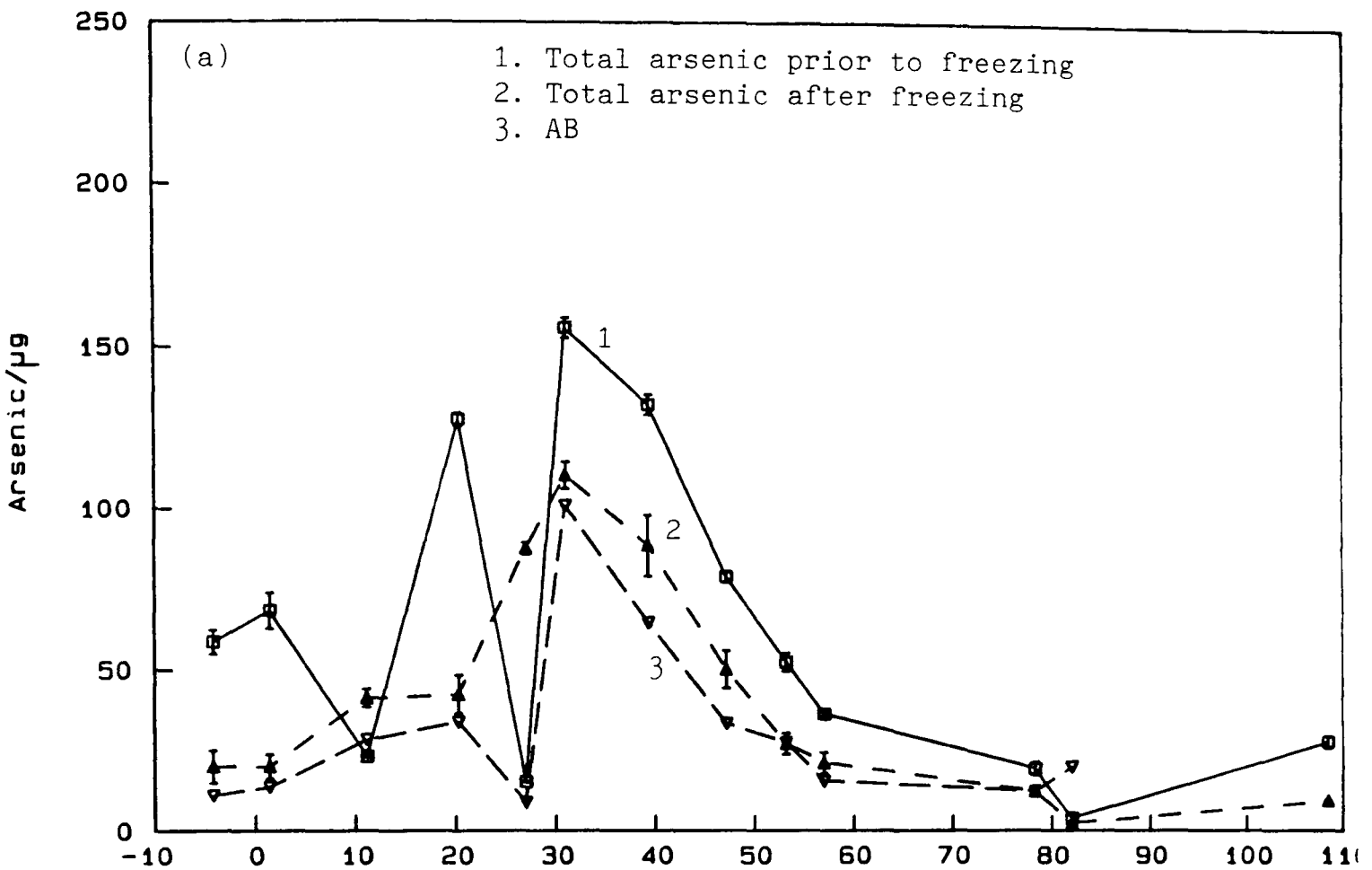
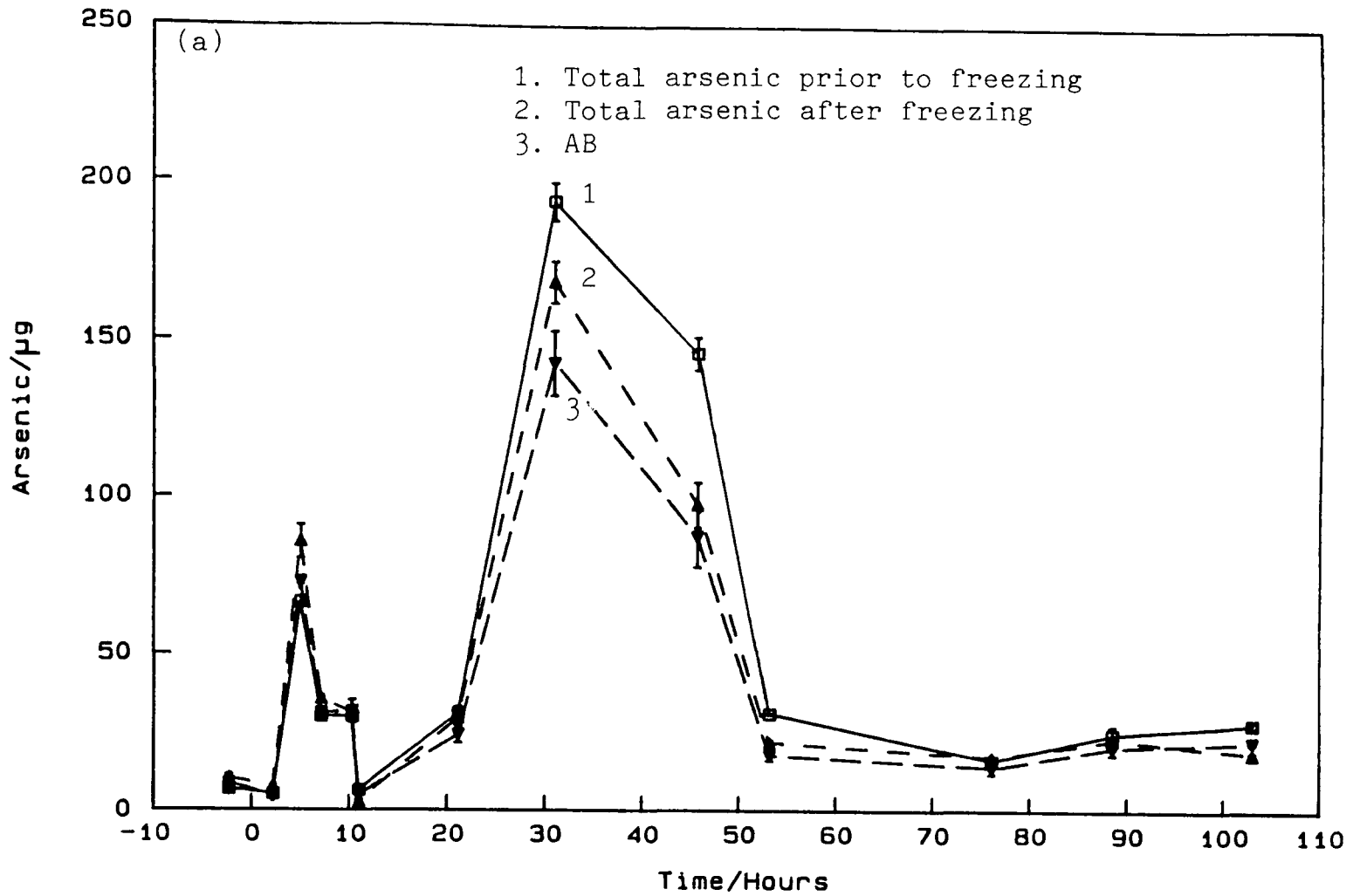


Figure 6.22(a) and 6.22(b) Arsenic Species in the Urine of Subject F



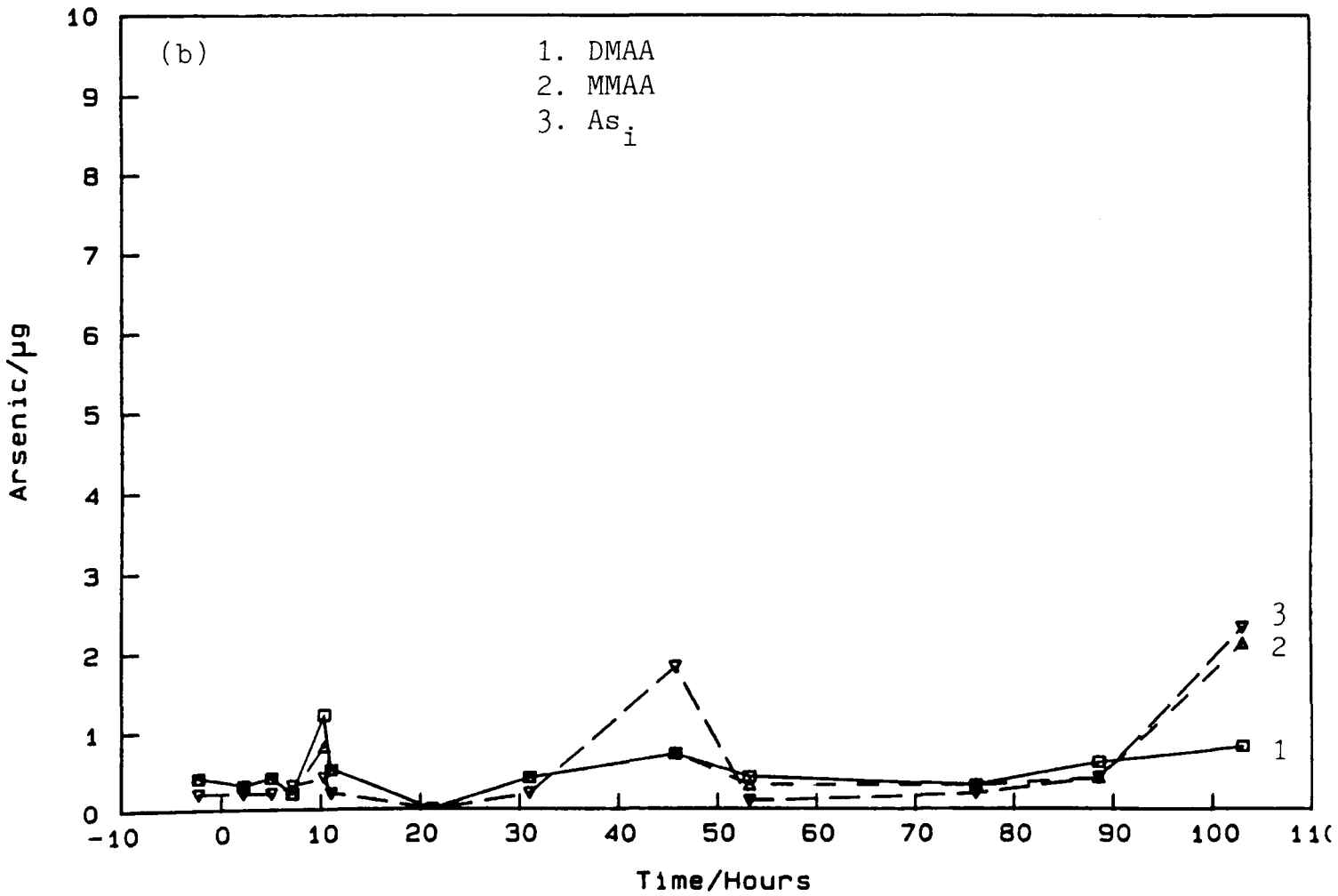


Figure 6.23(a) and (b) Arsenic Species in the Urine of Subject G

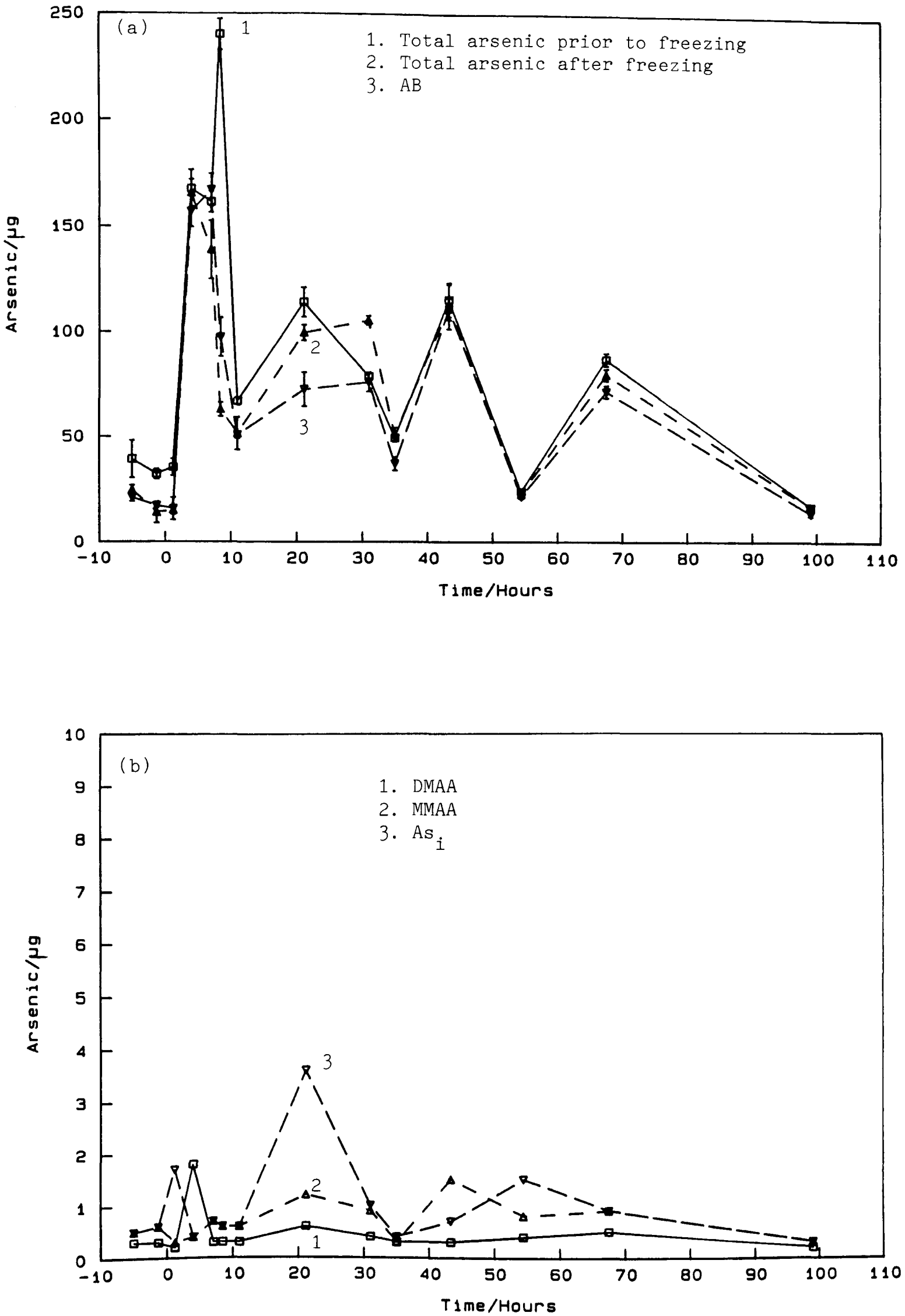


Figure 6.24 Arsenic Species in the Urine of Subject H

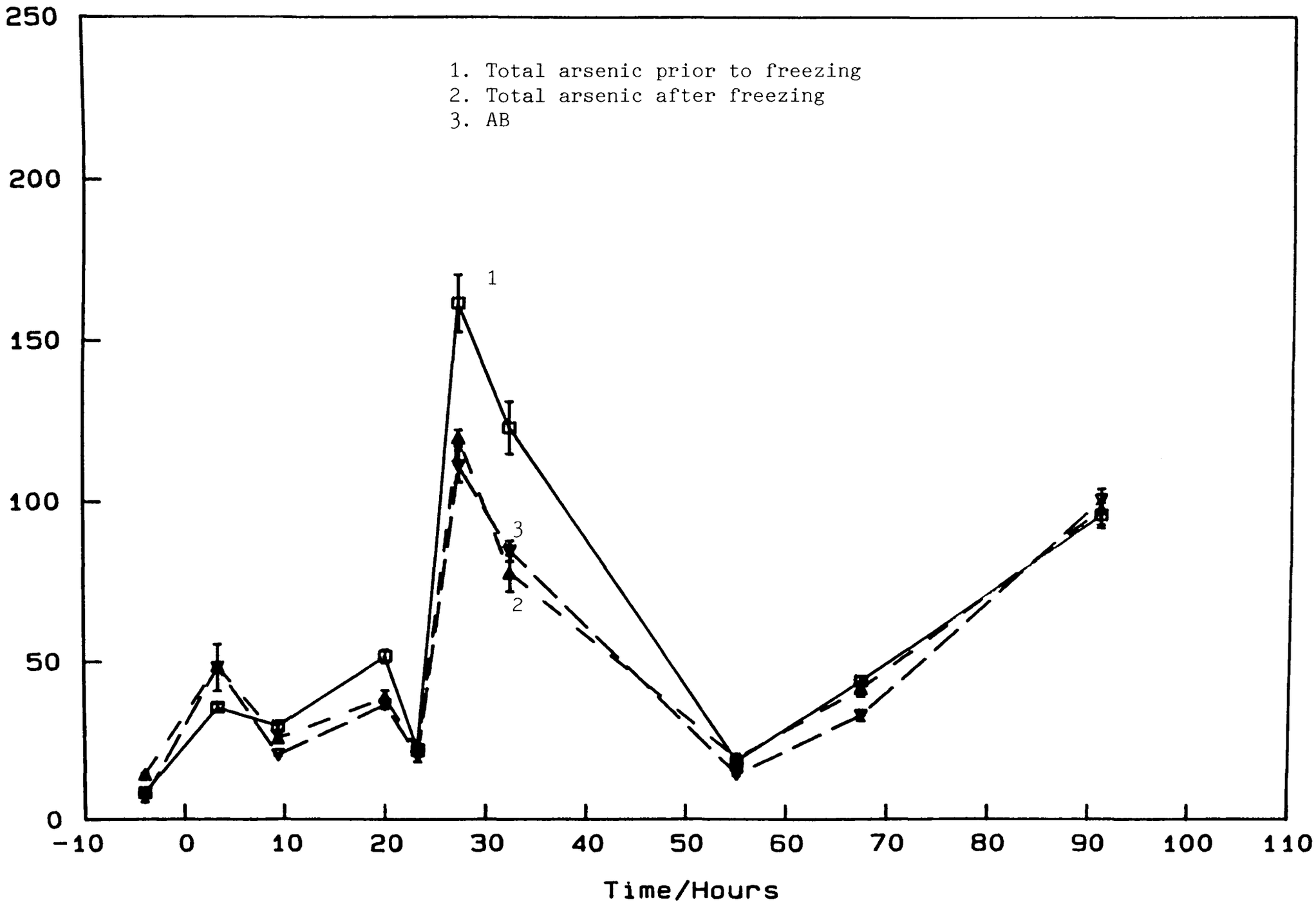
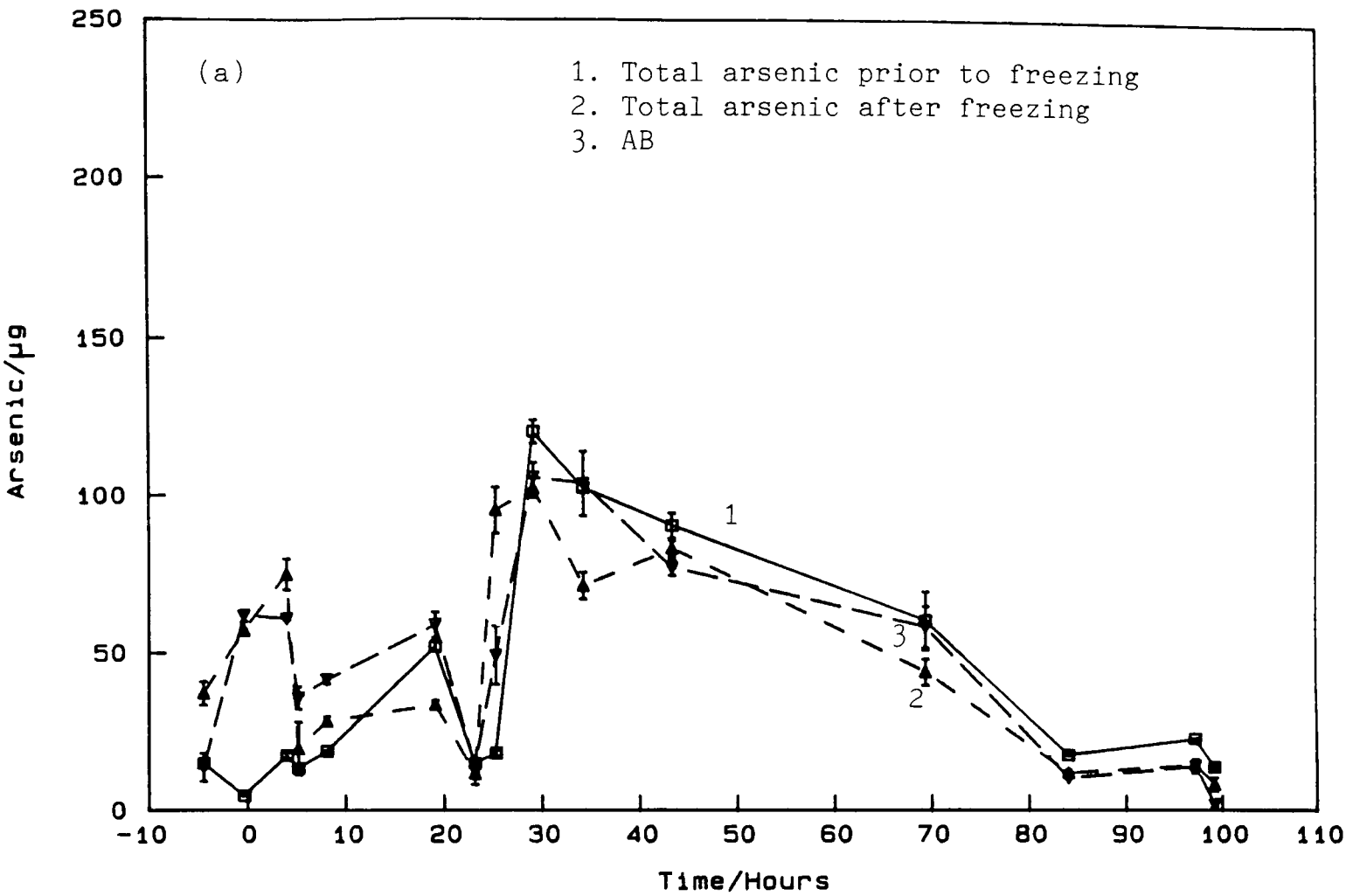


Figure 25(a) and (b) Arsenic Species in the Urine of Subject I



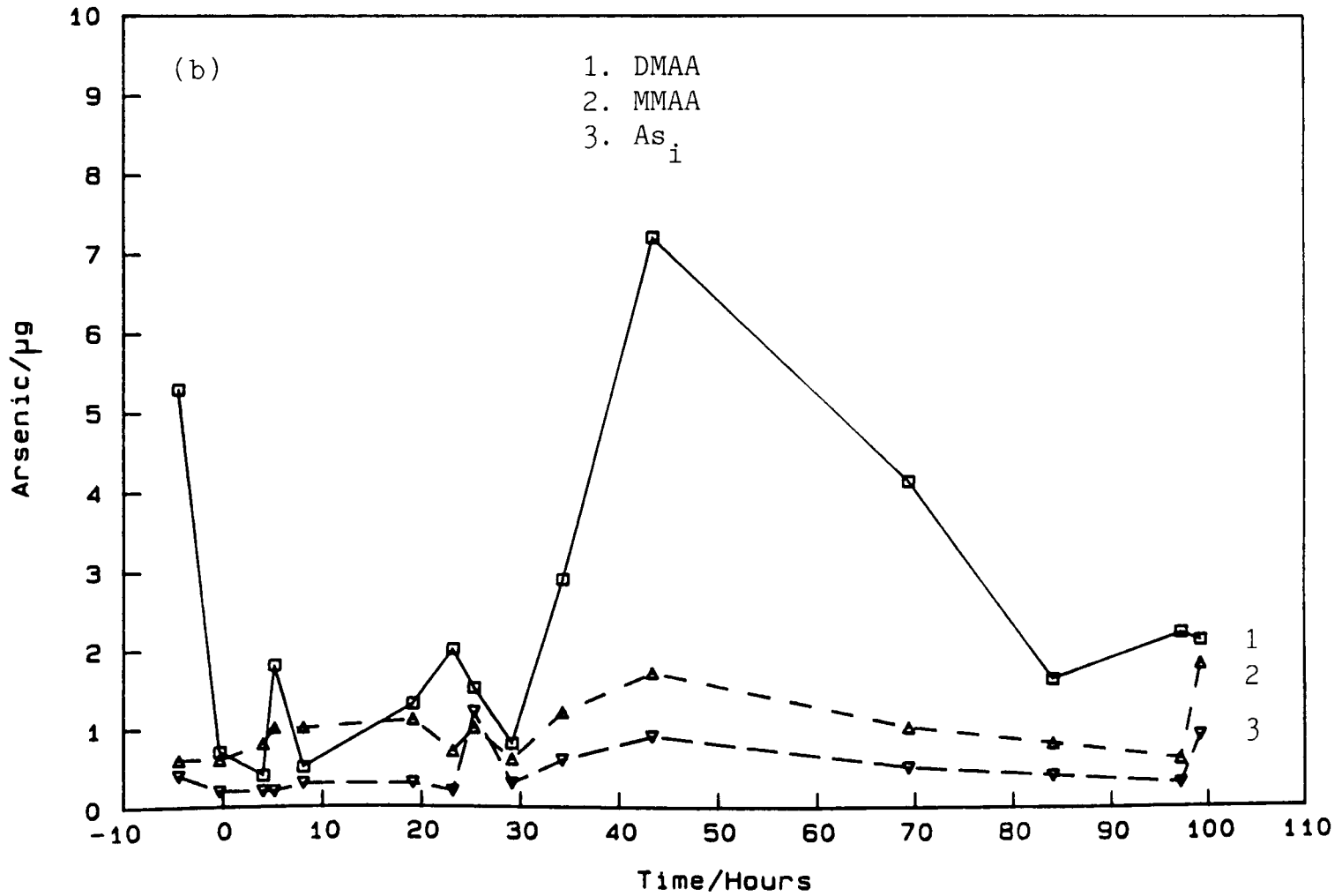
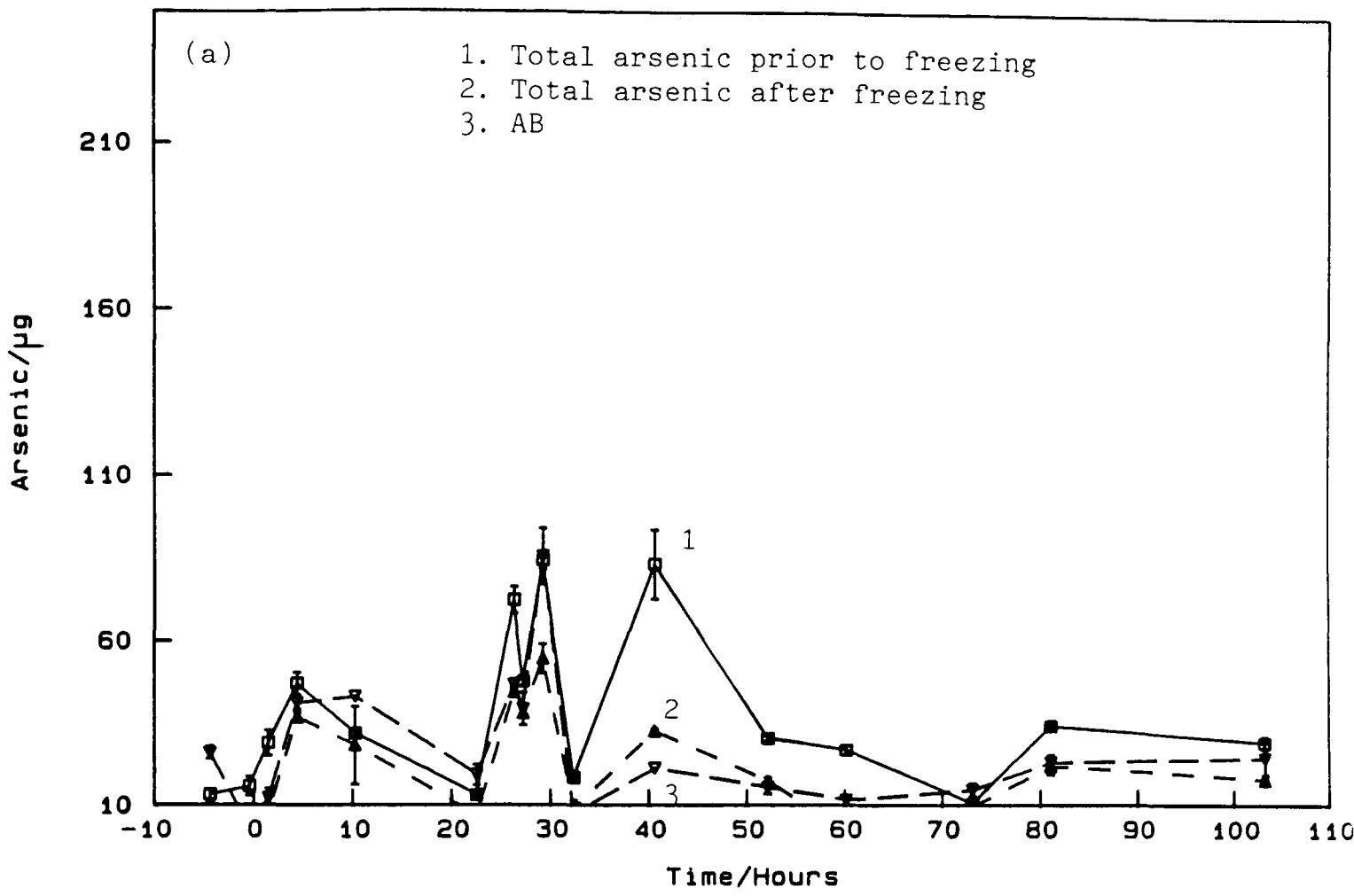
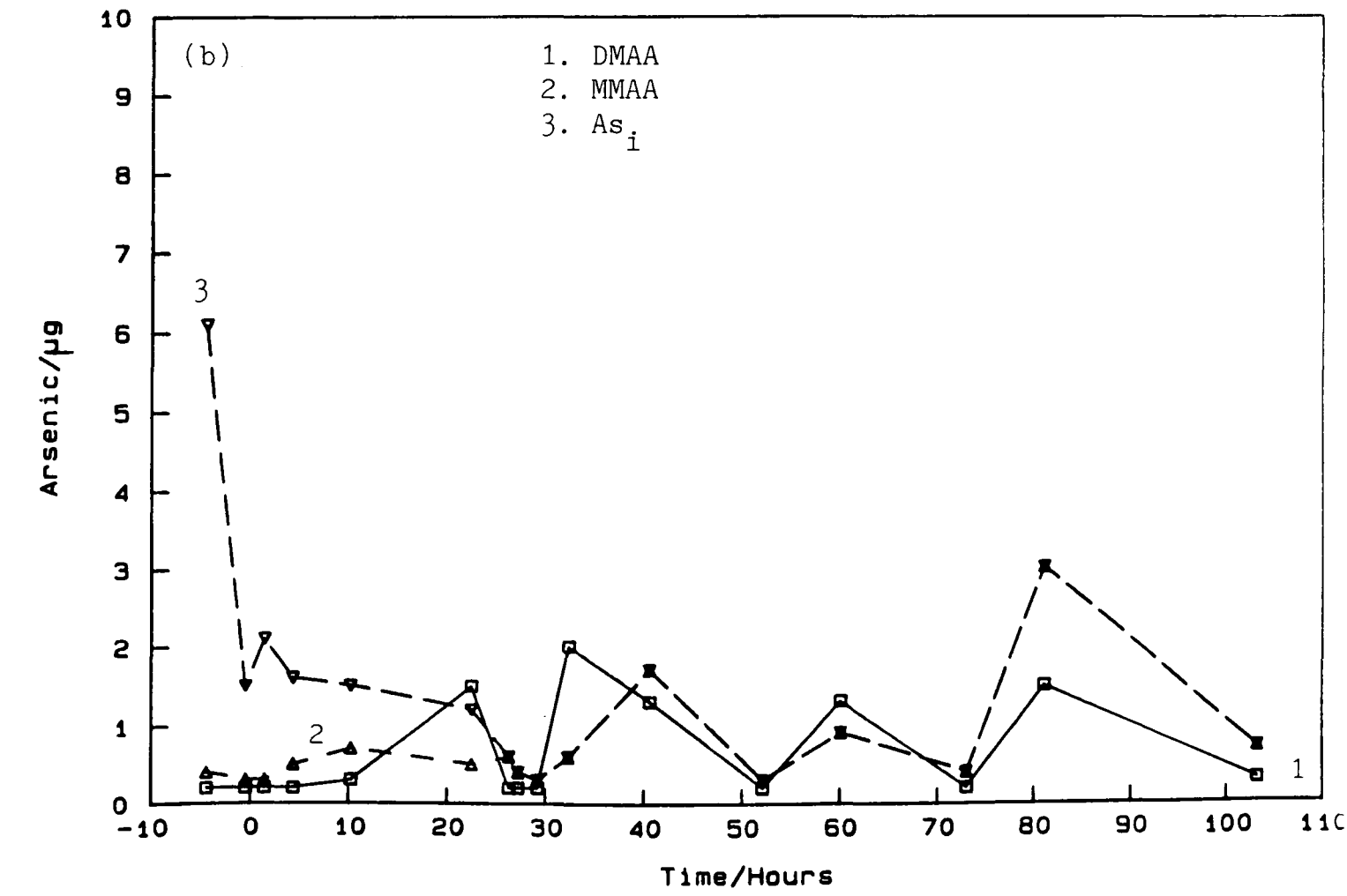


Figure 6.26(a) and (b) Arsenic Species in the Urine of Subject J





removed arsenic. To confirm this hypothesis the solids from 30 ml of sample A18 were collected and allowed to dry. They were found to constitute 0.5% m/v of the sample and, after N_2 addition-ICP-MS analysis, to contain 2.1 mg kg^{-1} arsenic (dry weight). It is worthy of note that arsenic was lost with the solids when the body arsenic load was highest. As the trial progressed the arsenic before and after freezing remained relatively unchanged.

(2) Arsenobetaine was the predominant arsenic species in the urine before consuming any fish meals. This is a very surprising finding. Earlier studies (5; 98; 103) failed to identify arsenobetaine in the urine of subjects who had not eaten fish. This was probably because the methods were based around hydride generation which would not detect arsenobetaine as it is non-reducible. Both Chana and Smith (103) and Foa et al. (105) observed that the sum of DMAA, MMAA and As_i was considerably less than total arsenic determined following sample ashing. In a reference population of 148 subjects Foa et al. (105) found DMAA, MMAA and As_i only accounted for 10 percent of the total arsenic.

Arsenobetaine in urine may arise from the practice of feeding poultry, sheep and pigs fish protein. It was particularly interesting to note that the rise in urinary arsenic excretion for subjects A and H followed consumption of a 'Christmas dinner'. It is common practice for farmers

to fatten turkeys prior to Christmas with fish protein (200). To confirm that poultry were a dietary source of arsenobetaine chicken breasts and poultry food were trypsin extracted and analysed by HPLC-ICP-MS. The breasts were found to contain 80-160 μg As kg^{-1} dry weight as arsenobetaine. Preliminary studies of the poultry feed suggest arsenobetaine is the primary arsenic species present. These studies are continuing. These results are interesting in that they point to the accumulation of arsenobetaine in the flesh of these animals. Previous studies on a number of animals (100; 201) have indicated arsenobetaine is rapidly excreted in the urine, although traces may persist for over a week.

Overall the results show, with a few exceptions, that arsenobetaine is the predominant species in urine. The arsenobetaine profile closely follows, and is virtually superimposed on, that of the total arsenic after freezing, throughout the duration of the study for most of the subjects. The early results for I, I1-17 are rather poor. The urine reference material run with these samples was one of the few not to give close agreement with the certified value and this may indicate the real sample results are in error. However the results have not been excluded because other solutions run with I1-I7 as quality control checks gave good agreement with expected values.

The results attest to a rapid excretion of arsenobetaine in the urine, the rate of removal being dependent on the subject, e.g. subjects E and I took approximately 60 hours to clear the arsenobetaine ingested from the fish whereas subject C took approximately 30 hours. These results are in accordance with the findings of other studies (97; 105; 199).

The results for HG-CT-AAS of the samples demonstrated that there was no metabolic degradation of arsenobetaine to give enhanced levels of the toxic species DMAA, MMAA and As_i. Indeed the levels of these species never exceeded 20 $\mu g\ l^{-1}$, the highest result being 6.1 μg of As_i in sample J1. As the volume of J1 was 430 ml this corresponds to a level of 14 $\mu g\ l^{-1}$ and is no cause for concern. MMAA was found in a few samples, at low levels. The fluctuations shown in the Figures is generally a result of multiplying values at the detection limit by large sample volumes e.g. sample C1. MMAA levels at 2.5 $\mu g\ l^{-1}$ or less have been reported by other authors (5; 104). DMAA levels up to 7.2 μg were recorded in the urine of the subjects. DMAA levels are usually found in excess of MMAA levels (5; 103; 104) probably as a result of in vivo methylation (105; 106). In this study the elevated levels of DMAA observed in some subjects, e.g. C, E, I, J, were probably associated with ingestion of the leek soup 24 hours into the trial. Certainly in the subjects mentioned DMAA peaks were observed within the 24 hours following the meal. Subject I was

unique in that most of his urine contained DMAA throughout the trial. Why this was so is unclear, but may be a function of individual metabolic differences. Subjects E, F, G and I also showed small DMAA peaks that corresponded to arsenobetaine peaks following the cod meals. This might indicate (a) the cod meal contained traces of DMAA or, (b) arsenobetaine was degraded to DMAA. This is rather unlikely as it would have had to occur very rapidly. Even if this were the case, the levels are too low to be of concern.

The literature predicts inorganic arsenic to be the minor arsenic constituent of urine (5; 103; 105; 199) as a result of methylation to MMAA and DMAA (106; 199). The results of this study confirm the results detailed in past papers, and where As_i was identified the amount was below 10 μg.

To confirm the integrity of the HG-CT-AAS results the individual arsenic species were spiked at 5 $\mu g\ l^{-1}$ in selected urine samples. Recoveries lay between 75-85%. Some of the urine samples were also surveyed by ICP-MS for the presence of interfering transition metals such as iron and copper. The levels were found to be below 1.0 $mg\ l^{-1}$, which can be regarded as insignificant.

6.5 Conclusions

In a number of set meals, fish was identified as the

predominant source of arsenic in the diet. Arsenobetaine constituted the major arsenic burden in these fish. Apparently all the arsenic ingested from the set meals was excreted in the urine within 72 hours of the commencement of the trial. The arsenic passed unchanged as arsenobetaine into the urine. There was no evidence of any degradation of arsenobetaine. The body receives arsenobetaine from another dietary source, possibly as a result of the practice of feeding chickens, swine etc. fish protein. Arsenobetaine was found at 80-160 μg As kg^{-1} in chicken breasts.

MMAA, DMAA and inorganic arsenic were determined in the volunteers urine, all at below 10 μg per sample. DMAA was the predominant form, possibly because it was ingested in one of the meals.

Finally, an ICP-MS method involving low bleeds, typically 0.03 $lmin^{-1}$, of nitrogen into the nebuliser gas was developed to overcome the polyatomic ion $^{40}Ar^{35}Cl^{+}$ which normally prevents the determination of arsenic in samples containing high levels of chloride. Using this method it was possible to determine arsenic in urine, a certified reference material containing in excess of 5 percent chloride and synthetic sodium chloride solutions. The results were in good agreement with expected values.

7. CONCLUSIONS AND SUGGESTED FURTHER WORK

Following the successful development of the HPLC-ICP-MS methodology this study has concentrated on three areas:

1. Arsenic and arsenic species in plants.

   Soils in the former arsenic mining regions of the Tamar Valley were found to contain, in some instances, in excess of 1.0 percent w\w arsenic. A range of crops were planted on soils containing 800 and 14000 mg As kg^{-1} dry weight. The results indicated that the higher level arsenical soil was too toxic to support growth, with only strawberries and some small potatoes growing. The lower arsenical soil supported the growth of more crops but most were severely stunted and would be difficult to retail. The uptake of arsenic from the soil was low, with maximum arsenic levels of 60-70 mg kg^{-1} dry weight being found in unpeeled potatoes from the high arsenic soil. The source of the arsenic was probably soil which had not been washed from the skin. Scanning electron microscopy with energy dispersive X-ray spectrometry could be used in the future for obtaining profiles through the plants and assessing whether the arsenic is in or on the peel.

   Speciation of the plants was made difficult by the poor efficiencies, approximately 10 percent, of the extraction procedures applied. Nevertheless it was

possible to characterise MMAA, DMAA, arsenite and arsenate in the plants, all at levels below 5 mg As kg^{-1} dry weight. The highest levels recorded were in the unpeeled potatoes.

The results for this stage of the study were the least satisfactory. There is a pressing need for an efficient extraction procedure for plants, possibly based on the action of enzymes such as amylases and cellulases which would release cell contents. This procedure could then be used in tandem with HPLC-ICP-MS to broaden the range of plants speciated and possibly determine varietal differences in uptake.

Total arsenic results varied depending on the method used, i.e. HGAAS, GFAAS, ICP-MS, but as none of the results demonstrated high arsenic levels in the plants it can be concluded that, provided they are cleaned adequately prior to consumption, there is no dietary risk associated with crops grown in arsenical soils. The N_2 addition-ICP-MS method described in Section 6.2 should allow more accurate determinations of arsenic in plants in the future.

2. Arsenic and arsenic species in fish.

Arsenobetaine has been clearly demonstrated to be the predominant arsenic species in cod, dab, haddock, lemon sole, mackerel, plaice and whiting. The study should

now be extended to include shellfish and other commonly eaten fish e.g. herring.

The majority of the results of the trypsin extraction showed that the human digestive system does not degrade arsenobetaine to more toxic species. However one of the plaice samples showed degradation to DMAA and one of the dab extracts contained an unidentified arsenic species. These studies should be duplicated and the nature of the unidentified species elucidated. The development of a digestion procedure that mimicked the enzymatic processes from the buccal cavity through to the gut, although complicated, would give valuable detail on whether arsenobetaine is degraded to toxic forms of arsenic. This digestion procedure could be applied to a wide range of foods and would increase understanding of arsenic transformations occurring in the body.

Research effort should also be directed towards ascertaining if there is lipid bound arsenic in mackerel and, if so, what the nature of the compound is and what the metabolic products are following human digestion.

3. The dietary trial.

The trial commenced with the development of a

successful methodology for determining arsenic in urine. N_2 addition-ICP-MS was found to overcome the polyatomic ion, $^{40}Ar^{35}Cl^+$, which interferes with arsenic determinations by ICP-MS. The mode of action of nitrogen and the feasibility of using nitrogen to overcome other polyatomic ion interferences such as $^{40}Ar^{16}O^+$ on $^{56}Fe^+$ remain to be elucidated. Nitrogen ablates the nickel sampler cone so the possibility of using resistant cones, possibly platinum tipped, should be investigated.

The dietary trial was worthy of a three year study in itself and this would have been advantageous as it would allow more subjects to be studied, a higher proportion of females and possibly age differences in excretory behaviour to be investigated. Future trials should be run over a longer period so the normal excretion patterns prior to eating the set meals can be assessed. More importantly samples of every meal eaten by the subjects should be collected. If this had been done the sources of the arsenobetaine in the urine of subject A and H at the end of the trial could have been identified. Assessing whether or not arsenobetaine, and possibly other arsenic species, accumulates in the flesh of poultry, swine and sheep is an urgent research requirement. This could be significantly adding to the human arsenic body burden.

As conducted the study trial demonstrated that, as expected, the bulk of the arsenic in the diet came from fish and was present as arsenobetaine. All of the arsenic consumed from the set meals was excreted within 72 hours of consumption. Arsenobetaine from the fish meals appeared in the urine within 4-6 hours of consumption and passed out over a period of 72 hours. Arsenobetaine occurred in the urine of the subjects even when they had not consumed fish.

DMAA, inorganic arsenic and traces of MMAA were also identified in the urine, up to a maximum of 7 μg of DMAA in one sample. A more prolonged study would have allowed all of the urine samples to be speciated and any patterns in MMAA, DMAA and As_i excretion characterised. However it was still possible to observe that there was no significant degradation of arsenobetaine into more toxic arsenic species.

Finally, the results of this study allow the conclusion that arsenic in normal foodstuffs poses no significant dietary risk. Fish are the major source of arsenic in the diet and the endogenous arsenic is in a non-toxic form. Fruit and vegetables grown on arsenical soils pose no risk providing they are carefully cleaned prior to consumption.

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LECTURES AND ASSOCIATED STUDIES

i. Departmental Lecture, 16th July 1987, Plymouth Polytechnic.
Dr I. S. Krull, "Metal Speciation via High Performance Liquid Chromatography-Element Specific Detection".

ii. Plymouth Polytechnic Chemistry Society, 6th November 1987, Plymouth Polytechnic.
Dr S. Haswell, "Gas Sensors".

iii. RSC Lecture, 13th November 1987, Plymouth Polytechnic.
Dr A. Howard, "Speciation".

iv. RSC Lecture, 29th January 1988, Plymouth Polytechnic.
Prof. A. Townsend, "Flow Injection Analysis - The First Decade".

v. AGM of the Peninsula Section of the RSC, 13th May 1988, Plymouth Polytechnic.
Dr C. Fisher, "Heavy Metals in Food. Is There a Problem?"

vi. RSC Lecture, 5th July 1988, Plymouth Polytechnic.
Prof. D. Bryce-Smith, "Environmental Influences on the Way We Think and Act".

vii. Research Visit, 18th October 1988, ICI, Brixham.

viii. RSC Lecture, 20th January 1989, Plymouth Polytechnic.
Dr P. Worsfold, "Flow Injection - Hands-Off Analysis".

ix. RSC Lecture, 17 February 1989, Plymouth Polytechnic.
Prof M. Barber, "Modern Mass Spectrometry".

x. RSC Lecture, 20th October 1989, Polytechnic South West.
Dr P. Dewick, "Poisonous Plants - Medical Agents".

xi. Weekly Departmental Research Seminars, 1987-90, Plymouth Polytechnic/Polytechnic South West.

MEETINGS ATTENDED

i. Atomic Spectrometry Updates/Atomic Spectroscopy Group, meeting on "Trace Metal Speciation", 8th April 1987, London.

ii. Analytical Quality Assurance meeting on "Heavy Metals in Food", 10th June 1987, London.

iii. Department of the Environment Second Organotin Seminar, 7th July 1987, London.

iv. Analytical Division of the RSC, meeting on "Research and Development Topics in Analytical Chemistry" 8th and 9th July 1987, Glasgow.

v. Atomic Spectroscopy Group and Western Region jointly with the Peninsula Section of the RSC, meeting on "New Perspectives in Atomic Spectroscopy", 3rd and 4th September 1987, Plymouth.

vi. RSC meeting on "The Biological Alkylation of Heavy Elements", 17th and 18th September 1987, London.

vii. North East Region Atomic Spectroscopy and Molecular Spectroscopy Groups jointly with the UV Spectrometry Group and Atomic Spectrometry Updates of the RSC, meeting on "Recent Advances in Atomic and Molecular Spectroscopy", 19th and 20th March 1988, Hull.

viii. Atomic Spectroscopy Group of the RSC jointly with the Spectroscopy Group of the Institute of Physics, "Fourth Biennial National Atomic Spectroscopy Symposium", 29th June-1st July 1988, York.

ix. Analytical Division of the RSC, meeting on the "25th Anniversary of Research and Development Topics in Analytical Chemistry", 18th and 19th July 1988, Plymouth.

x. The Food Chemistry Group of the RSC, the Working Party on Food Chemistry of the Federation of European Chemical Societies and the Federation of European Chemical Societies joint meeting "Bioavailability '88", 21-24th August 1988, Norwich.

xi. The University of Durham and VG Elemental "The First International Conference on Plasma Source Mass Spectrometry", 12-16th September 1988, Durham.

xii. Analytical Division of the RSC, meeting on "Research and Development Topics in Analytical Chemistry", 21st and 22nd March 1989, Dublin.

xiii. Bulgarian Academy of Sciences, Bulgarian Institute of Physics, University of Sofia, The Bulgarian Commission on Spectroscopy and others, "XXVI Colloquium Spectroscopicum Internationale", 2-9th July 1989, Sofia.

xiv. The University of Surrey and the British Geological Surrey joint meeting "The Third Surrey Conference on Plasma Source Mass Spectrometry", 17-19th July 1989, Guildford.

xv. Atomic Spectroscopy Group\Atomic Spectrometry Updates joint meeting on "Lasers in Atomic Spectrometry", 30th March 1990, Guildford.

xvi. Analytical Division of the RSC meeting on "Research and Development Topics in Analytical Chemistry", 16th and 17th July 1990, Runcorn.

xvii. Atomic Spectroscopy Group of the RSC jointly with the Spectroscopy Group of the Institute of Physics," Fifth Biennial National Atomic Spectroscopy Symposium", 17-19th July 1990, Loughborough.

PRESENTATIONS AND PUBLICATIONS

Posters

i. "Novel Methods for the Determination of Methylated Metal Species", S. Branch, L. Ebdon and S. Hill. New Perspectives in Atomic Spectrometry, 3rd and 4th September 1987, Plymouth.

ii. "Novel Methods for the Determination of Methylated Species and their Application to Estuarine Samples", S. Branch, L. Ebdon, S. Hill and G. Millward. The Biological Alkylation of Heavy Elements, 17 and 18th September 1987, London.

iii. "The Quantitative Determination of Arsenic Species by Coupled High Performance Liquid Chromatography-Atomic Spectrometry", S. Branch, K.C.C. Bancroft, L. Ebdon and P. O'Neill. Fourth Biennial National Atomic Spectroscopy Symposium, 29th June-1st July 1988, York.

iv. "The Role of Plasma Source Mass Spectrometry in Arsenic Speciation", S. Branch, K.C.C. Bancroft, L. Ebdon and P. O'Neill. 25th Anniversary Research and Development Topics Meeting, 18th and 19th July 1988, Plymouth.

v. "Liquid Chromatography and the Inductively Coupled Plasma-Mass Spectrometer". S. Branch, K.C.C. Bancroft, L. Ebdon and P. O'Neill. Research and Development Topics Meeting, 21st-23rd March 1989, Dublin, Eire.

vi. "The Determination of Arsenic by Inductively Coupled Plasma-Mass Spectrometry", S. Branch, L. Ebdon and P. O'Neill. XXVI Colloquium Spectroscipum Internationale, 2nd-9th July 1989, Sofia, Bulgaria.

vii. "A Comparison of Extraction Procedures for Speciating Arsenic in Marine Food Produce", S. Branch, L. Ebdon and P. O'Neill. Research and Development Topics Meeting, 16th and 17th July 1990, Runcorn.

Oral Presentations

i. "Coupled Techniques for the Determination of Tributyltin", S. Branch, L. Ebdon and S. Hill. Second Organotin Seminar, 7th July 1987, London.

ii. "Coupled Chromatography-Atomic Spectrometry for Trace Metal Speciation", S. Branch and L. Ebdon. Bioavailability 88, 21st-24th August 1988, Norwich.

iii. "Arsenic Speciation by Coupled High Performance Liquid Chromatography-Inductively Coupled Plasma-Mass Spectrometry", S. Branch, K.C.C. Bancroft, L. Ebdon and P. O'Neill. First International Conference on Plasma Source Mass Spectrometry, 12th-16th September 1988, Durham.

iv. "Advances in Computerised Tomography Applied to Analytical Plasmas", S. Branch, L. Ebdon, B. Fairman and S. Hill. XXVI Colloquium Spectroscipum Internationale, 2nd-9th July 1989, Sofia, Bulgaria.

v. "Arsenic and the Inductively Coupled Plasma-Mass Spectrometer. Are they Compatible?" S. Branch, W. Corns, L. Ebdon and P. O'Neill. 3rd Surrey Conference on Plasma Source Mass Spectrometry, 16th-19th July 1989, Guildford.

vi. "The Determination of Arsenic in Human Urine by Inductively Coupled Plasma-Mass Spectrometry", S. Branch, L. Ebdon and P. O'Neill. 5th Biennial National Atomic Spectroscopy Symposium, 18th-20th July 1990, Loughborough.

Papers

i. "The Quantitative Determination of Arsenic Species by Coupled High Performance Liquid Chromatography-Atomic Spectrometry", S. Branch, K.C.C. Bancroft, L. Ebdon and P. O'Neill. Anal. Proc., 1989, 26, 73.

ii. "Liquid Chromatography and the Inductively Coupled Plasma-Mass Spectrometer. A Marriage Made in Heaven?" S. Branch, L. Ebdon and S. Hill. Anal. Proc., 1989, 26, 401.

iii. "The Determination of Arsenic by Hydride Generation-Inductively Coupled Plasma-Mass Spectrometry Using a Novel Tubular Membrane Gas Liquid Separator", S. Branch, W. Corns, L. Ebdon, S. Hill and P. O'Neill. J. Anal. At. Spectrom., 1990, in the press.

iv. "The Determination of Arsenic in Samples with High Chloride Content by Inductively Coupled Plasma-Mass Spectrometry", S. Branch, L. Ebdon, M. Ford, M. E. Foulkes and P. O'Neill, submitted to the J. Anal. At. Spectrom.

performed with high purity indium as a standard (m.p. 156.6 °C). Force and sample deformation calibration were also performed according to usual procedures.

TMA curves of compression (height, %) against temperature were recorded for the various paint samples (*e.g*, Fig. 1) and the following parameters were calculated: softening T (°C) or T_g, % compression (changes in the Y axis calculated over the temperature range 20–60 °C), and the linear compression coefficient (α) (where $\alpha = L_2-L_1/T_2-T_1$) which was also calculated over the temperature range 20 to 60 °C.

The results obtained are given in Tables 1–4.

Table 3. Effect of moisture on Young's modulus (E) for the prussian blue - linseed oil (41%) and safflower oil (3.2%) paint film (Winsor and Newton)

	Softening T/°C	Compression, %	E/mN m^{-2}
$CaCl_2$ (5% RH)	41	1.1	1242
$MgCl_2$ (33% RH)	39	1.6	853
NaCl (74% RH)	36	1.7	804
Water (100% RH) (6 days in water)	28	4.2	325

E = Stress (F/A)/strain (δL/L).
δL was evaluated in the temperature region 20–60 °C.

Table 4. Effect of moisture on samples from the 19th century

	Softening T/°C	Compression, %
Ground layer (Fig. 1)—		
Ambient (50–60%)	39	7.2
After soaking in water (17 h)	34.5	18.0
Blue paint layer—		
Ambient (50–60%)	48	3.1
After soaking in water (17 h)	34.6	22.1

For the ground layer the linear compression coefficients (α) (over the temperature range 20–60 °C) of the ambient and water-soaked samples were also calculated:

	α/mm m^{-1} K^{-1}
Ground (ambient)	13.2×10^{-4}
Ground (wet)	38.8×10^{-4}
Lead white - linseed oil (amb.)	10.0×10^{-4}
Lead white - linseed oil (3 days in water)	14.6×10^{-4}
Lead white - linseed oil (9 days in water)	36.2×10^{-4}

Conclusions

Results indicate that TMA is a technique which can measure the changes which take place in paint films when they are exposed to various levels of relative humidity and temperature. Measurements on a 10-year old lead white - linseed oil paint film reveal the following.

(*a*) T_g values vary with relative humidity (Fig. 2).
(*b*) % Compression values vary with relative humidity (Fig. 3).
(*c*) Young's modulus varies with relative humidity (for the system prussian blue - linseed oil) (Fig. 4).

Results also demonstrate that pigment type affects the T_g values (Fig. 5).

Further work is in progress to quantify the behaviour of paint films with variations in temperature and relative humidity.

We would like to thank Perkin Elmer Ltd. (Beaconsfield) and in particular Dr. T. Lever for lending us the TMA7 for the purpose of this study.

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Liquid Chromatography - Inductively Coupled Plasma - Mass Spectrometry for Monitoring Tributyltin in Waters

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The role of individual chemical species in determining the environmental behaviour of an element is now well established. One prime example is the marine antifouling agent tributyltin (TBT), which is known to affect seed oysters and dog whelks at environmental concentrations below 100 ng l^{-1}. Inorganic tin species do not act in this manner. In the United Kingdom a target water quality level for TBT has been set at 20 ng l^{-1}. In order to monitor this level an analytical method of high sensitivity and selectivity is required. Methods which have been suggested include coupled high-performance liquid chromatography (HPLC) - atomic absorption spectrometry,[1] hydride generation - atomic absorption spectrometry,[2,3] gas chromatography - flame photometry[3] and HPLC with fluorimetric detection.[4] Various methods using selective extraction followed by graphite furnace atomic absorption spectrometry[5] have been reported. In this paper we consider the coupling of HPLC with inductively coupled plasma mass spectrometry (ICP-MS) utilising two different types of plasma torch and assess implications for the analysis of tin species.

Experimental

Apparatus

The HPLC system consisted of a mobile phase of 80 + 20 methanol - water, 0.1 M with respect to ammonium acetate, delivered by an HPLC pump (Waters Associates Inc., MA, USA) to a 25 × 0.4 cm column packed with Partisil 10 μm SCX (Whatman, Maidstone, Kent). A 5 × 0.4 cm column packed with the same material was placed in line to protect the analytical column. The column outlet was connected via a short length of silicone rubber tubing to either an atomic absorption spectrometer (SP9, Philips Scientific, York Street, Cambridge) fitted with a slotted tube atom trap, or an inductively coupled plasma mass spectrometer (Plasmaquad 2, VG Elemental, Winsford, Cheshire). The ICP-MS instrument was used either with a standard plasma torch or a reduced argon flow plasma torch. Samples and standards were introduced via a Rheodyne 7125 injection valve (Rheodyne Inc., Cotati, CF, USA).

Reagents and Standards

All reagents were of AnalaR or Aristar grade (BDH Chemicals, Poole, Dorset). Organotin standards were provided by Aldrich Chemical Co., Gillingham, Dorset. De-ionised, doubly distilled water was used throughout.

Results and Discussion

The HPLC - AAS system used has been described previously.[1]

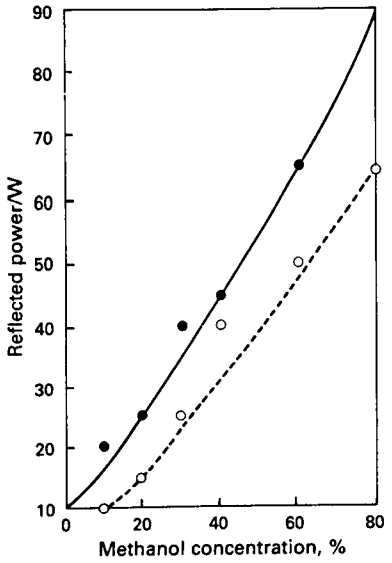

Fig. 1. Effect of methanol concentration on reflected power. ●, 1.4 kW; ○, 1.8 kW

The limit of detection for TBT using this arrangement is 200 ng (as tin). Thus, large preconcentration factors are required in order to measure the levels in environmental samples. It is also advantageous, with this system, to use large sample injections, typically 1.5 ml. This practice, however, reduces column lifetime owing to the build-up on the column of preconcentrated impurities. It was considered that ICP-MS, with its superior powers of detection, would ameliorate these problems. The principal practical difficulty with the use of ICP-MS was the requirement to introduce a mobile phase containing a high concentration of organic solvent. This led to elevated reflected powers (Fig. 1), even at high forward powers, resulting from the increased organic solvent loading of the plasma. Prolonged reflected powers of these magnitudes lead to generator cut-out. Furthermore, soot deposited on the faces of the sampler and skimmer cones within the ICP-MS interface region resulted in elevated noise and decreased signals. These problems can be overcome by introducing oxygen into the nebuliser gas but this may lead to reflected powers of greater than 100 W.

Table 1. Instrumental operating conditions

HPLC system—		
Mobile phase	80 + 20 methanol - water, 0.1 M with respect to ammonium acetate	
Stationary phase	Partisil 10 μm SCX packed into a 25 × 0.4 cm column	
Flow-rate	1.5 ml min^{-1}	
Injection volume	175 μl (ICP-MS) or 1.5 ml (FAAS)	
ICP-MS—	Standard torch	Low flow torch
Forward power	1.8 kW	1.0 kW
Reflected power	40 W	10 W
Nebuliser flow	0.8 l min^{-1}	0.65 l min^{-1}
Coolant gas	15 l min^{-1}	9 l min^{-1}
Intermediate gas	0.9 l min^{-1}	0.9 l min^{-1}
Cool bath	−10 °C	−10 °C
Data acquisition mode	Survey scan	Local mass set to 120.2 u
FAAS—		
Wavelength	286.3 nm	
Lamp current	6 mA	
Band pass	1 nm	
Hydrogen	2.6 l min^{-1}	
Air	4.0 l min^{-1}	

Another approach is to replace the standard torch with a low argon flow torch. The torch used in this study was of a Fassel design but had 1.3 mm jets in the outer and intermediate gas flow inlets and a configuration ratio of 0.82; this contrasts with gas inlets of 6 mm and a configuration ratio of 0.78 in the standard Fassel torch supplied with the instrument. The torch operated at a total argon flow of 10.55 l min^{-1} and a reflected power of typically less than 25 W. No soot deposition was observed on the cones but the reason for this was not clear. The operating conditions used for both torches and AAS are shown in Table 1.

The low flow torch was found to perform adequately with a linear calibration from 0.5–10 μg ml^{-1} of TBT, but gave a poorer limit of detection (500 ng ml^{-1}) when compared with the standard torch (25 ng ml^{-1}). This was attributed to the methanol content of the mobile phase. From Fig. 2 it can be seen that as the methanol concentration increased the tin signal degraded. The prudent maximum forward power of the low flow torch was only 1 kW because of the low coolant flow.

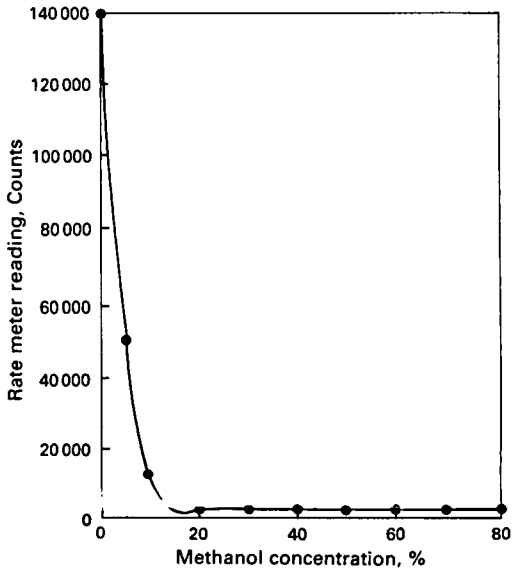

Fig. 2. Effect of methanol on tin signal using low flow torch

This generated a much smaller plasma than the standard torch, which operated at 1.8 kW, and also led to lower sensitivities. The standard torch gave linear calibrations from 0.025–1 μg ml^{-1} of tributyltin, with a limit of detection of 0.025 μg ml^{-1}, which is equivalent to 1.6 ng of tin in the injection. This is an improvement of several orders of magnitude on the HPLC - FAAS coupling.[1]

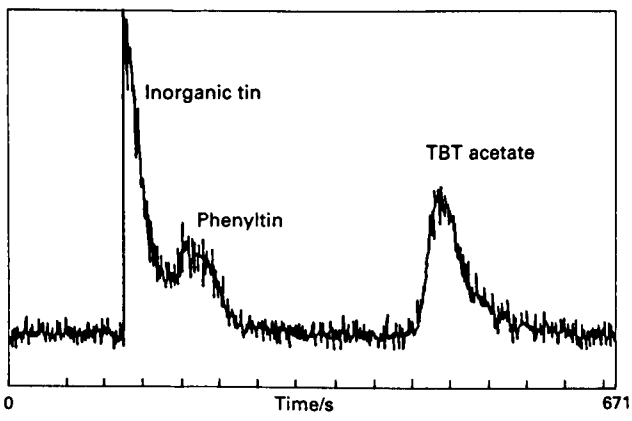

Fig. 3. Separation of organotins

Analysis of Spiked Water Samples

Four water samples containing TBT and a range of other species were prepared. The samples were diluted 100 a 10 000-fold and TBT determined in a blind trial using HPL

FAAS and HPLC - ICP-MS, respectively, following solvent extraction and pre-concentration. The results are shown in Table 2 and a sample chromatogram in Fig. 3. Excellent results

Table 2. Results of "Blind Trial" analysis of TBT spiked water samples

Solution	Spike level/ μg ml^{-1}	Results by HPLC - FAAS for 100× dilution/μg ml^{-1}	Results by HPLC - ICP-MS for 10 000× dilution/μg ml^{-1}
A	3.0	2.87 ± 0.15	3.06 ± 0.40
B	3.0	2.99 ± 0.16	3.37 ± 0.22
C	3.0	3.56 ± 0.25	2.05 ± 0.42
D	3.0	3.09 ± 0.07	3.48 ± 0.30

were obtained for all of the HPLC - FAAS determinations and resonable results for the HPLC - ICP-MS analyses. If the 10000× dilution had been analysed by HPLC - FAAS large volumes would have been required for preconcentration.

The HPLC - ICP-MS results were calculated by manual measurement of peak height; as the peaks were skewed this inevitably led to a slight degradation in accuracy and precision. New software (time resolved acquisition, version 3.1, VG Elemental) has recently been received which allows the integration of peak areas. This should further enhance performance.

Conclusion

Coupled HPLC - ICP-MS offers a sensitive and accurate method for the determination of tributyltin ions at normal environmental levels.

We are indebted to Mr. W. Corns for practical assistance.

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Two-beam Thermal Lens Spectrometer for Ultra-trace Analysis

Jun Shen and Richard D. Snook
DIAS, UMIST, P.O. Box 88, Manchester M60 1QD

Thermal lens spectroscopy is a member of a family of photoacoustic and thermo-optical techniques including photoacoustic spectrometry,[1] thermo-optical interferometric measurements,[2] thermal lens measurement,[3] photothermal deflection,[4] thermal diffraction,[5] photothermal refraction,[6] etc., which measure absorbance via the deposition of heat in a sample by non-radiative decay processes following light absorption. Because of its high sensitivity and versatility, the thermal lens technique has been employed successfully for absorbance measurements on gas,[7] solid phase[8] and solution[3] samples. By using the thermal lens effect, absolute fluorescence quantum efficiencies of fluorescent substances can also be determined precisely.[9]

The mathematical expression of the focal length of a thermal lens and the effect of a thermal lens on a laser beam through it have been derived. A sample cell is placed in a TM_{00} Gaussian profile laser beam. The sample is heated by the absorbed laser power, and a temperature gradient is established. Because the refractive index of the sample is a function of temperature a refractive index gradient is produced, thus creating an optical element. Based on an approximation in which the optical element has a parabolic refractive index distribution so that it could be treated as an optical lens, Gordon *et al.*[10] gave the focal length $F(t)$ of the thermal lens as:

$$F(t) = \frac{\pi k w^2}{P_{th}\,(dn/dT)}\,(1 + t_c/2t) \quad \ldots \quad \ldots \quad (1)$$

Here, k is the thermal conductivity (W cm^{-1}); w is the "1/e^2" radius of the laser beam in the sample (cm); P_{th}, the thermal power, is the absorbed laser power (W) which is converted to heat [if the whole absorbed power is converted into heat, P_{th} = 2.303 PA, where P is laser power (W), A is absorbance; dn/dT is the refractive index change with temperature (K^{-1}); $t_c = w^2 \rho C_p/4K$ is the critical time (s) of the sample within which, under laser illumination, the temperature reaches an equilibrium value when the rate of heat input from the laser is just balanced by the rate of heat conduction out of the sample, where ρ = density (g cm^{-3}) and C_p = specific heat (J g^{-1} k^{-1}). The thermal lens can be detected by its effect on the propagation of the pump laser (in a single-beam experiment) or another TM_{00} Gaussian profile laser probe beam passing through the thermal lens (dual-beam experiment). As most liquids have a positive coefficient of thermal expansion, dn/dT is negative and the thermal [illegible] magnitude of the thermal lens signal can be determined by monitoring the change in the far field spot size of the laser beam passing through the thermal lens. By putting a pinhole detector assembly in the far field and measuring the laser intensity at the beam centre, the far field spot size, which is inversely proportional to the laser intensity, can be determined. If the sample cell is positioned at the confocal position of the pump beam (for a single-beam experiment) or both pump and probe beam (for dual-beam, mode-matched experiment), the maximum thermal lens effect will be exhibited. The expression relating the laser intensity $I(t)$ at the beam centre in the far field as a function of time is

$$I(t) = I_{(0)}\left[1 - \theta \Big/ \left(1 + \frac{t_c}{2t}\right) + \tfrac{1}{2}\theta^2 \Big/ \left(1 + \frac{t_c}{2t}\right)\right]^{-1} \quad (2)$$

$$\theta = \frac{P_{th}\,(dn/dT)}{\lambda k} \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad (3)$$

and thus the thermal power P_{th} can be determined by measuring the initial intensity $I_{(0)}$ and the intensity after the steady state has been established $I_{(\infty)}$.
That is

$$\begin{cases} \theta = 1 - (1 + 2I)^{\frac{1}{2}} \\ I = [I_{(0)} - I_{(\infty)}]/I_{(\infty)} \end{cases} \quad \ldots \quad \ldots \quad \ldots \quad (4)$$

Taking account of the aberrant nature of the thermal lens, Sheldon *et al.*[12] put forward an aberrant lens model. According to this model, the sample should be placed at $3^{\frac{1}{2}}$ confocal position, which maximises the thermal lens effect.

Two kinds of dual-beam arrangement have been developed. One is the conventional, mode-matched alignment configuration in which the sample cell is located at the confocal position of both the pump beam and probe beam. This arrangement

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Two kinds of dual-beam arrangement have been developed. One is the conventional, mode-matched alignment configuration in which the sample cell is located at the confocal position of both the pump beam and probe beam. This arrangement

stability of some has been questioned. As an alternative the introduction of a chirally selective additive to the mobile phase in conjunction with a reversed-phase packing material can give considerable flexibility together with a reduction in costs.[7]

In this instance PGC was employed as stationary phase with the mobile phase additive, β-cyclodextrin, added (3 mM) to coat the carbon phase dynamically. In this system chiral selectivity is achieved by inclusion complexation into the cyclodextrin cavity with the possibility of additional hydrogen bonding with the external secondary hydroxyls on the cavity rim.[8]

The enantiomers of a number of beta-adrenoceptor antagonist drugs have been resolved by using this method, of which metoprolol is a typical example (Fig. 3).

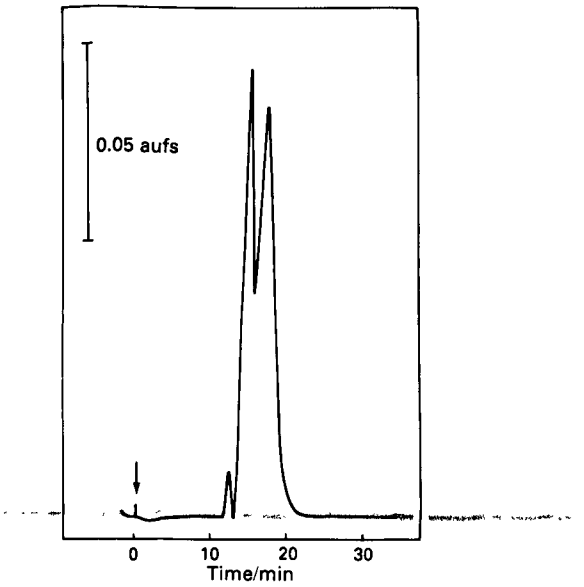

Fig. 3. Resolution of the enantiomers of metoprolol (76 μg ml^{-1}) on PGC with β-cyclodextrin (3 mM) as mobile phase additive; detection wavelength, 260 nm. For chromatographic conditions, see text

In carrying out these separations it is important to control the operating parameters, such as temperature, pH and concentration of the chiral mobile phase additive, which can significantly affect the resolution of drug enantiomers. Here again the robustness of the PGC column to differing pH values can be utilised, as illustrated with nomifensine, where lowering the pH to 2.5 yields considerable improvement in resolution of the enantiomers [Fig. 4(*a*), (*b*)].

Conclusions

The wide pH range over which PGC can be used is a major potential advantage in practical HPLC and offers it as a supplementary column packing to the existing silica-based materials. Although the retention characteristics of carbon are essentially similar to ODS silica, selectivity has been found to be different in some instances.

One area of drug analysis which should profit by the use of PGC is the separation of basic drugs that currently exhibit a number of problems in chromatography, including tailing.

The additional capability to resolve the enantiomers of chiral drugs, shown recently for the first time[9] and illustrated in the present work, indicates the wide scope of the porous graphitised carbon material.

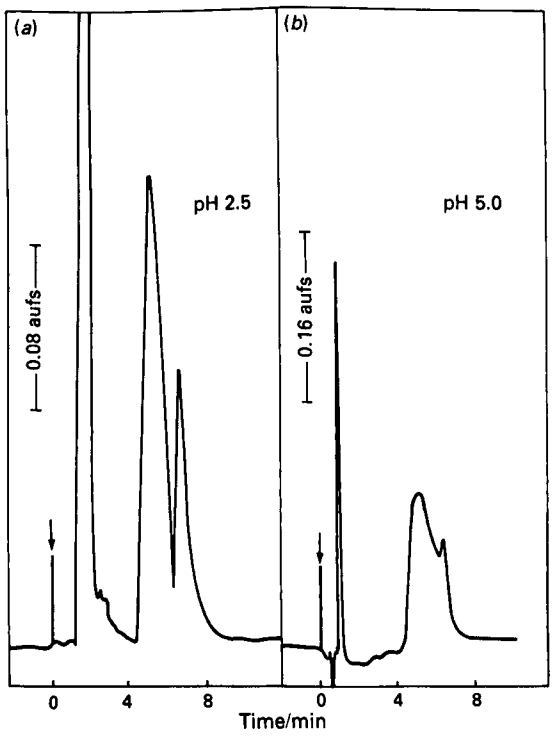

Fig. 4. Effect of mobile phase pH on the resolution of the enantiomers of nomifensine maleate (150 μg ml^{-1}). Separation on PGC with β-cyclodextrin as mobile phase additive: (*a*), pH 2.5; and (*b*), pH 5.0. Detection wavelength, 240 nm. For chromatographic conditions, see text

The contribution of Professor J. H. Knox and Dr. B. Kaur in supplying column material and advice is gratefully acknowledged.

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The Determination of Arsenic Species by Coupled High-performance Liquid Chromatography - Atomic Spectrometry

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It is now widely recognised that the physico-chemical form, or species, of an element markedly affects properties such as toxicity, biological uptake and environmental fate. Arsenic, a potentially toxic element, shows a remarkable graduation in its toxicity.[1] For example, different forms of arsenic vary in toxicity greatly, from arsenite, the most toxic, to arseno-

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betaine, in the order[1] shown below:

Arsenite > Arsenate > Monomethylarsonic Acid > Dimethylarsinic Acid > Arsenobetaine

Over the past 10–15 years a considerable volume of work has been published relating to arsenic in the marine environment, principally due to the high levels of arsenic in marine organisms as compared with terrestrial organisms.[2] In 1977 the non-toxic organo-arsenic compound arsenobetaine was isolated.[3] This compound has now been identified in a wide variety of marine biota.[4]

Much less study has been made of the terrestrial environment. For example, little is known of the speciation of arsenic in fruit and vegetables grown in the market gardens of South West England, a region of historic arsenic mining. Although the mines are now disused the soils around them still contain arsenic concentrations in excess of 1000 μg g^{-1} dry mass. Despite these high levels the soil will still support plant growth. A method was required which could be used for quantifying and speciating arsenic in plants and also be applicable to marine organisms. While such schemes have been published in the past, they have generally failed to speciate simultaneously the principal organic and inorganic arsenic species.[5–7]

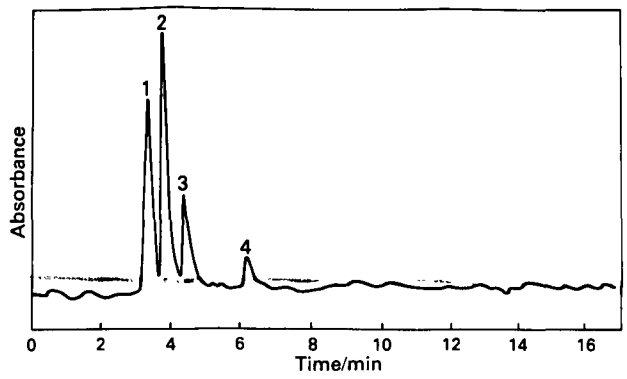

Fig. 1. Separation of a 250-μl mixture of five species. Mixture concentration was 50 μg ml^{-1} of both species. Mobile phase, 0.04 mM $NH_4H_2PO_4$, pH 6.0, flow-rate 2.0 ml min^{-1}. Atomic absorption monitored at 193.7 nm. No background correction. Peaks are: 1, arsenite; 2, monomethylarsonic acid; 3, dimethylarsinic acid; 4, arsenobetaine. Arsenate totally retained

Experimental

The analytical methodology involved coupling high-performance liquid chromatography (HPLC) to either an atomic absorption spectrometer or an inductively coupled plasma - mass spectrometer (ICP-MS).

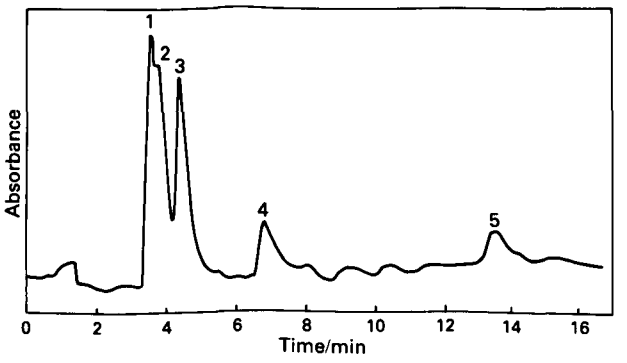

Fig. 2. Separation of a 250-μl mixture of five species. Mixture concentration was 50 μg ml^{-1} of each species. Mobile phase, 20 mM $NH_4H_2PO_4$, pH 6.0, flow-rate 2.0 ml min^{-1}. Atomic absorption monitored at 193.7 nm. No background correction. Peaks are: 1, arsenite; 2, monomethylarsonic acid; 3, dimethylarsinic acid; 4, arsenobetaine; 5, arsenate

Chromatographic Methods

Two HPLC systems were used. The first consisted of a 25 × 0.4 cm 5 μm ODS column connected in series to a 25 × 0.4 cm Partisil 5 μm SAX column (Whatman, Maidstone, Kent). The second system consisted of a 10 × 0.4 cm column packed with Benson 7–10 μm anion exchange resin (Benson, Reno, NV, USA). Mobile phase was delivered by an HPLC pump (PU4010, Philips Scientific, York Street, Cambridge). Samples were introduced via an injection valve (Rheodyne 7125, Rheodyne Inc., Cotati, California, USA).

Detectors

An atomic absorption spectrometer (SP9, Philips Scientific, York Street, Cambridge) fitted with a slotted tube atom trap was used for the initial optimisation. It was interfaced to the HPLC system as previously described.[8] For quantitative analysis an ICP - MS instrument (Plasmaquad 2, VG Elemental, Winsford, Cheshire) was used, interfaced to the HPLC system by a short length of plastic capillary tubing.

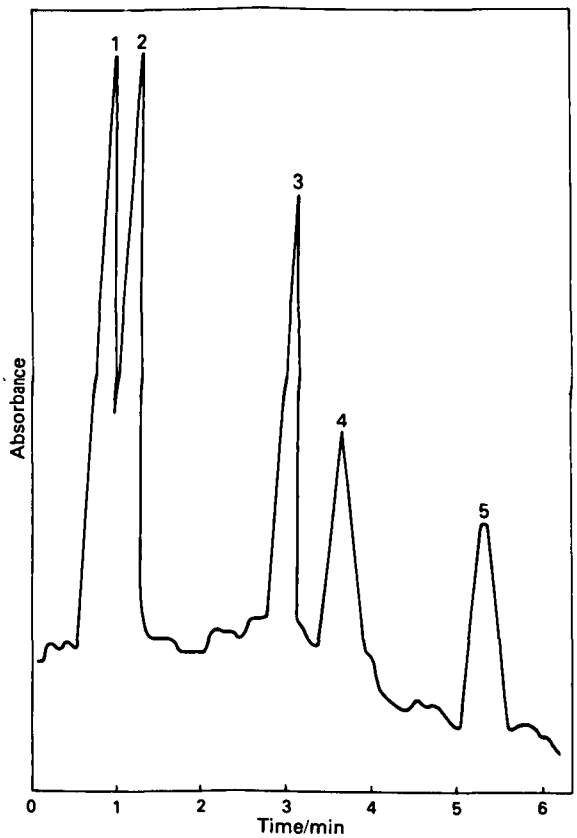

Fig. 3. Separation of a 175-μl mixture of five species, each at 100 μg ml^{-1}. Column equilibrated with 1 mM K_2SO_4, pH 10.5, and switched on injection to 50 mM K_2SO_4, pH 10.5. Flow-rate, 1.5 ml min^{-1}. Atomic absorption monitored at 193.7 nm. No background correction. Peaks are: 1, arsenobetaine; 2, dimethylarsinic acid; 3, arsenite; 4, monomethylarsonic acid; 5, arsenate

Reagents and Standards

All chemicals were of AnalaR or Aristar grade (BDH, Poole, Dorset), with the exception of the arsenate and dimethylarsinic acid (DMAA) (Sigma Chemical Co., Poole, Dorset). The monomethylarsonic acid (MMAA) was a gift from Dr. A. Howard of Southampton University, and the arsenobetaine a gift from Dr. K. Irgolic (Texas A and M University, USA). De-ionised doubly distilled water was used throughout.

Results and Discussion

A variety of mobile phases was used with the twin-column systems. The best results were obtained using a phosphate buffer system (Figs. 1 and 2). It is apparent from these results

that isocratic elution is not optimal. Low concentrations of phosphate were required to separate arsenite, MMAA, DMAA and arsenobetaine. At concentrations above 0.1 mM the resolution between arsenite and MMAA was lost. At concentrations below 0.01 mM the MMAA was retained on the column because the ionic strength of the mobile phase was too low to elute this species. Concentrations of ammonium dihydrogen phosphate above 10 mM were required to elute the strongly retained arsenate species. Thus, a step gradient was required from low concentration, to separate the arsenite, MMAA, DMAA and arsenobetaine, to a high concentration to elute the arsenate. A number of such gradients were investigated and a step gradient from 0.08 mM $NH_4H_2PO_4$, pH 6.0, to 20 mM $NH_4H_2PO_4$, pH 6.0, was found to produce an acceptable separation over a time scale of 30 min. However, this avenue of approach was not fully investigated owing to difficulties with the Partisil SAX packing. The poor stability of silica-based anion-exchange columns has been previously reported[9] and it was found that after several weeks efficiency rapidly deteriorated and all reproducibility was lost. Hence, the study was switched to the resin-based exchanger.

Table 1. Reproducibility of peak height measurements and retention times. Each figure is the mean of 8 injections

Species	Mean peak height/mm	Relative standard deviation, %	Mean retention time/min	Relative standard deviation, %
Arsenite	27.6	11.1	2.25	5.7
Arsenate	12.4	4.9	5.41	1.6
MMAA	11.2	15.3	3.28	4.8
DMAA	36.9	5.3	0.79	4.8
Arsenobetaine	36.6	3.1	0.49	5.5

The results using the Benson packing are shown in Fig. 3. Sulphate was chosen as the mobile phase with a view to using ICP-MS for detection, where phosphate might affect the sampler and skimmer cones. Acid sulphate mobile phases were avoided as these might also be deleterious. At a pH of 10.5 arsenite, arsenate, MMAA and DMAA are all ionised. This aids separation as at lower pH values arsenite (pK_{a_1} = 9.2) remains undissociated and elutes with arsenobetaine on the solvent front. The strong acid, arsenic acid (pK_{a_1} = 2.2), is strongly retained on the column so a step to high concentration sulphate was required. The analytical protocol was to equilibrate the column for 15 min with 1 M potassium sulphate solution, pH 10.5, and then to switch, on injection, to 50 mM potassium sulphate solution, pH 10.5. The lower concentration mobile phase remaining in the connecting tubing enabled separation of the first four species; the high concentration sulphate then eluted the arsenate. This method gave an efficient, highly reproducible and rapid separation, taking less than 6 min. Reproducibility data are given in Table 1. The high relative standard deviation for MMAA results from manual measurement of the peak heights.

The Benson-packed column has now been coupled to the ICP-MS with encouraging results; instrumental settings are given in Table 2. Similar separations are produced but with greatly enhanced detection limits, these being of the order of 5–10 ng ml^{-1} for each species. The sulphate has not been detrimental to the cones and the instrument has remained stable for analytical runs of up to 4 h. The HPLC - ICP-MS coupling is now being applied to determining arsenic species in fruit and vegetables.

Table 2. Instrumental settings for HPLC - ICP-MS coupling

HPLC parameters	
Mobile phase	Column equilibrated on 1 mM K_2SO_4, pH 10.5. Switched on injection to 50 mM K_2SO_4, pH 10.5
Flow-rate	1.5 ml min^{-1}
Injection volume	175 µl
ICP-MS parameters	
Nebuliser gas flow	0.72 l min^{-1}
Coolant gas flow	13 l min^{-1}
Forward power	1.35 kW
Reflected power	6 W
Data acquisition mode	Survey scan. Local mass set to 75

Conclusion

An efficient, reproducible and rapid HPLC separation of five environmentally important arsenic species has been developed. The choice of mobile phase enables a simple coupling to the ICP-MS, which offers the potential of highly sensitive determinations.

We would like to thank the Ministry of Agriculture, Fisheries and Food for a grant which has made this work possible.

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Obituary

It is with great regret that we announce the death of Dr. J. Aggett of the University of Auckland, New Zealand, the Regional Advisory Editor for New Zealand of *The Analyst*.

ERRATA

Pg 124 Column 8, line 6: '1.1' should read '10.1'.

Pg 132 Figure 5.1(B): 'Methanol/Chloroform Extract' should read 'Trypsin Extract'.

Pg 155 Line 25: '$MeOH/CH_3Cl$' should read '$MeOH/CHCl_3$'.

Pg 209 Line 16: 'is' should read 'was'.