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THE DEVELOPMENT OF ON-LINE MICROWAVE DIGESTION TECHNIQUES FOR ENVIRONMENTAL MATRICES

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THE DEVELOPMENT OF ON-LINE MICROWAVE DIGESTION TECHNIQUES FOR ENVIRONMENTAL MATRICES

by

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ABSTRACT

THE DEVELOPMENT OF ON-LINE MICROWAVE DIGESTION TECHNIQUES FOR ENVIRONMENTAL MATRICES

Kathryn Judith Lamble

As a consequence of our advancing knowledge of the impact of trace elements in environmental and biological systems there is increasing need for quantitative analytical techniques to accurately, sensitively and rapidly determine a wide range of trace elements in environmental samples. Since their emergence in 1975 microwave digestion techniques have revolutionised conventional approaches to sample digestion and have rapidly gained widespread acceptance as an effective method of sample preparation. This thesis describes how a number of novel on-line microwave digestion techniques have been developed, characterised and applied to the digestion of a range of environmental matrices which have proved time consuming or troublesome to digest by conventional techniques.

A variety of batch open focused microwave digestion methods have been developed and utilised for the analysis of a range of environmental samples including tea leaves, seaweed and sediment samples. The techniques were optimised to determine a range of trace metals including Al, As, Ba, Ca, Cr, Cu, Fe, K, Mg, Mn, Pb, Ni, Ti, V and Zn and in each case were successfully validated by the analysis of suitable certified reference materials. These techniques demonstrated a number of advantages over conventional methods including less reagent usage, less contamination and substantial time savings.

Speciation studies have been a focus for this work and have included the evaluation of an acetic acid microwave extraction method for the extraction of tributyltin, dibutyltin, monobutyltin and triphenyltin from sediment samples prior to analysis by high performance liquid chromatography-inductively coupled plasma-mass spectrometry (HPLC-ICP-MS). In addition the speciation of arsenic in biological samples was addressed by the development and evaluation of an on-line microwave digestion technique. This enabled the speciation of arsenobetaine (AsBet), monomethylarsonic acid (MMA), dimethylarsinic acid (DMA) and inorganic arsenic following their separation by HPLC. Decomposition was achieved by microwave digestion and followed by pre-reduction with L-cysteine and detection by hydride generation-atomic absorption spectrometry. Simple modification of the technique also facilitated the determination of total arsenic. In addition the total reducible arsenic species (inorganic arsenic, MMA and DMA) were determined directly by on-line pre-reduction-HG-AAS. The results obtained were in good agreement with the certified values for the reference materials NRCC DORM-1 (dogfish muscle) and TORT-1 (lobster hepatopancreas).

The on-line microwave digestion approach was further developed for the determination of the total mercury content of solid environmental samples. This involved the combination of an on-line microwave digestion and a bromide/bromate oxidation reaction to facilitate complete oxidation of the organomercury species in slurried biological samples. Following this approach detection could be performed by cold vapour-atomic fluorescence spectrometry, giving a limit of detection of 13 ng l^{-1}. In addition a batch method in which samples were digested with a mixture of hydrogen peroxide, nitric acid and sulphuric acid was developed and studied using temperature profile measurements. Both methods were successfully validated by analysis of the certified reference materials NRCC DORM-2 and PACS-1 (harbour marine sediment).

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AUTHOR'S DECLARATION

At no time during the registration for the degree of Doctor of Philosophy has the author been registered for any other University award.

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Signed...Kramble.....

Date.....7/2/97.....

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LIST OF ABBREVIATIONS

AAS	Atomic absorption spectrometry
AsBet	Arsenobetaine
AFS	Atomic fluorescence spectrometry
CV-AAS	Cold vapour-atomic absorption spectrometry
CCD	Charge-coupled device
CID	Charge-injection device
CTD	Charge transfer device
DBT	Dibutyltin
DC	Direct current
DMA	Dimethylarsinic acid
ET-AAS	Electrothermal atomisation-atomic absorption spectrometry
FAAS	Flame atomic absorption spectrometry
FAES	Flame atomic emission spectrometry
FI	Flow injection
GC	Gas chromatography
HG-AAS	Hydride generation-atomic absorption spectrometry
HPLC	High performance liquid chromatography
ICP-AES	Inductively coupled plasma-atomic emission spectrometry
ICP-MS	Inductively coupled plasma-mass spectrometry
MBT	Monobutyltin
MD	Microwave Digestion
MIP-AES	Microwave induced plasma-atomic emission spectrometry
MMA	Monomethylarsonic acid

NIES	National Institute for Environmental Studies
NRCC	National Research Council Canada
PAHs	Polyaromatic hydrocarbons
PTFE	Polytetrafluoroethylene
RF	Radio frequency
SBR	Signal to background ratio
TBT	Tributyltin
TePhT	Tetraphenyltin
TPhT	Triphenyltin
UV	Ultraviolet light

1. Introduction

1 INTRODUCTION

1.1 Trace Element Analysis of Environmental Samples

The industrial activities of humans have had a major impact on the environment. We have now synthesised and characterised over five million compounds and the chemical industry produces about one hundred and fifty millions tons of synthetic chemicals annually, including inorganic, organic and organometallic compounds[1]. Much of the chemical and waste products of modern society are released into the environment either during their use, production, storage, transport or during their ultimate disposal. These emissions often result in widespread pollution and disturbance of natural systems and thus the determination of specific chemical species in a wide range of environmental samples has proved necessary to understand their behaviour in the environment. From these studies the impact, fate and mobility of such pollutants partitioned between the air, land and water interfaces can be monitored and predicted, and decisions regarding their future use can be made.

The determination of trace elements is of particular importance when studying the environment. The concentration of an element is defined as 'trace' when it is present at a level of 1 $\mu g\ g^{-1}$ or lower[2]. Although a large number of trace elements are essential to human health e.g. Cr, Cu, Fe, Mn, Zn, considerable interest has been generated in recent years concerning those metals considered toxic e.g. Al, As, Cd, Cr, Hg, Pb and Sn. The main exposure routes to humans are considered to originate from air inhalation and the ingestion of food and water. Exposure to such metals has been associated with a variety of diseases including cancer (As[3], Cr), respiratory system disorders (As[3]), reproductive damage (As[3], Hg[3], Pb[4]) and disorders of the kidneys (Cd[4]), liver (As[3], Cd[4]) and nervous system (Al, Pb[4],

Hg[3], Sn[3]). The determination of toxic metals is thus essential in the assessment of their toxicological impact to humans and other organisms.

The applications of trace element analysis are numerous and rapidly increasing. In the food industry a wide range of plant, food and beverage samples are routinely analysed to comply with governmental legislation controlling the maximum admissible concentrations of these metals in foods for human consumption. Trace elements considered essential to health are also determined to ascertain the importance of their nutritional and biological role in the body and for the labelling of foods with their mineral and nutrient contents. In agriculture the need to determine micronutrient and toxic elements in samples such as soil, plant tissue, grains, forages, animal feeds, fertilisers etc. has increased in recent years. This information is an essential requirement of soil fertility programmes, nutrient budget and cycling investigations, environmental monitoring and elemental uptake studies. Over the last decade the environmental effects of the mining industry have also come under close scrutiny. This has led to a demand for more stringent controls on emissions and effluents and to the monitoring and reclamation of disused sites, in many cases requiring detailed trace element analysis.

The vast majority of routine elemental analyses are capable of determining only the total concentration of a particular element in a sample. In some cases this is an acceptable indicator of toxicity, however toxicity is often highly dependent on the chemical form of the analyte i.e. on its speciation. The term 'speciation' can be defined as the qualitative identification and quantitative determination of the individual chemical forms that comprise the total concentration of a given trace element in a sample[5]. In recent years more emphasis has been assigned to the task of developing analytical methods, particularly coupled (hybrid) techniques, which are capable of determining the individual forms of an element in a sample.

To conclude, quantitative analytical techniques are required to accurately and rapidly determine a variety of trace metals with sufficient sensitivity for application to a range of environmental samples. This requirement has led to major technological advances in the development of analytical instrumentation, enabling the analyst to obtain vast amounts of elemental information in a shorter time and with increased sensitivity than was ever possible before. This is especially true of techniques such as inductively coupled plasma-atomic emission spectrometry (ICP-AES) and inductively coupled plasma-mass spectrometry (ICP-MS) which can obtain multielement information in a fraction of the time needed to prepare the samples. However prior to analysis by most quantitative analytical techniques there is an intrinsic requirement for the sample to be converted into a liquid form. For solid samples this can be achieved by undertaking some form of digestion procedure.

To be effective, sample digestion methods must efficiently decompose the sample matrix so that the analytes of interest are completely released, solubilised and are in a form compatible with the analytical method of choice. Effective methods of sample digestion are therefore a crucial prerequisite to accurate analytical analysis. However technological advances in this area have been somewhat slow in comparison to the developments to analytical instrumentation. Until recently sample digestion methods have been largely limited to the conventional techniques of wet digestion and dry ashing. Such techniques are often time consuming, may be the source of contamination and losses of analyte and generally require a great deal of operator attention, skill and experience in order to gain accurate and precise results. As a consequence sample preparation is often regarded as the weak link in sample analysis, and an area which provides much scope for improvement.

1.2 Conventional Digestion Techniques

1.2.1 Dry Ashing Techniques

Decomposition of the sample matrix can be achieved by high temperature heating (400-1000°C) with a suitable alkali fusion reagent such as lithium borate or sodium carbonate for several hours in a muffle furnace. This is followed by dissolution of the residue in a suitable acid such as nitric or hydrochloric acid.

Although the dry ashing technique is rarely used for the decomposition of biological samples[6] it has found wide application for geological samples. However even following treatment with a suitable fusion reagent a number of important minerals such as bastnaesite, bunsenite, cassiterite, chalcocite, chalcopyrite, chromite, galena, ilmenite, monazite, pyrite, sphalerite, wolframite and zircon may not be totally soluble[7]. The major disadvantage of all fusion procedures however originates from the high levels of fusion reagents required to achieve decomposition of the sample. In addition to potential contamination of the sample, this leads to a high dissolved solid content in the final solution, which necessitates large dilution factors prior to analysis with obvious loss in sensitivity. The use of fusion reagents consisting of easily ionisable elements, particularly Ca, K and Na, can also cause severe matrix effects during analysis[6].

In contrast to most open wet digestions employing hydrofluoric acid, the determination of silicon is possible using the dry ashing technique. However a number of volatile elements such as As, Hg and Se may be lost due to the elevated temperatures reached in the ashing step. Losses of analyte may also occur due to retention on the walls of the ashing vessel or onto the constituents of the ash itself or by the formation of refractory phases which are subsequently difficult to dissolve. These problems can be minimised to some extent by the

addition of ashing aids such as calcium, magnesium and aluminium nitrates which assist the oxidation process, help to retain volatile species and transform the analytes into readily soluble nitrates. However their addition further increases blank levels and significantly increases the total dissolved solids content of the final solution, both of which are highly undesirable.

1.2.2 Wet Digestion Techniques

Conventional wet digestion procedures have been in use for more than one hundred years and involve heating of the solid sample in acid media in either a closed[6,8] or more commonly an open vessel[9,10]. Suitable heating devices for open wet digestions include flames, hotplates and aluminium heating blocks. For the determination of volatile or refractory elements closed digestions are generally more effective. The development of the technique began in 1860 when Carius described the digestion of samples with concentrated nitric acid in sealed, strong-walled glass vials (Carius tubes)[10]. However despite advances in vessel design the technique remains time consuming, inefficient and potentially dangerous. Modern conventional closed digestions involve the heating of samples in a chemically inert vessel such as a PTFE (polytetrafluoroethylene) vial surrounded by an outer stainless steel jacket. After the addition of digestion reagents the vessel is sealed and heated in a conventional oven for periods of one to several hours. Considerable care must be taken to limit the pressure build up and thus avoid the potential rupture of the vessel.

During conventional wet digestion procedures heating is generally inefficient, relying on the processes of conduction, convection and radiation to transfer heat from outside of the vessel to the centre of the sample. The vessels used are usually poor conductors and thus heat is transferred very slowly and inefficiently to the sample core. Vaporisation at the surface of the

acid reagents also occurs and as a result a thermal gradient is produced by convection currents. As a consequence of these factors only a small percentage of the sample actually reaches temperatures matching that of the heat supplied. In addition hotplates are prone to thermal gradients across their surface, thus the energy supply at different regions of the hotplate can vary greatly, leading to both poor inter and intra-batch reproducibility.

1.3 Microwave Digestion Techniques

Significant improvements in sample preparation techniques have however been made since 1975, when Abu-Samra[11] first reported the use of microwaves as a heat source for wet digestion methods. Since then the microwave digestion technique has gradually gained widespread acceptance as an effective method of sample preparation. Using this technique not only have digestion times been dramatically reduced (estimated to be of a factor of 2 to 5 times) but other benefits such as a reduction in contamination, less reagent and sample usage, a reduction in the loss of volatile species and improved safety have been reported[12].

The mechanism of microwave heating, in contrast to traditional heating methods, is based on the direct absorption of energy by polar molecules and ions. Microwaves are not only absorbed by the digestion medium but also by the sample molecules. This increases the kinetic energy of the matrix and causes internal heating and differential polarisation, which expand, agitate and rupture surface layers of the solid material, thereby exposing fresh surfaces to acid attack[13]. These effects may be highly significant, producing rates of dissolution which are far greater than those predicted by the temperature of the acids alone. Due to the fact that the sample/reagents directly absorb microwave radiation the technique does not rely on the processes of conduction, convection and radiation to transfer heat from outside the vessel to the sample core. In addition the vessels used are transparent to

microwaves and so energy is not consumed in heating them as is the case for conventional heating methods. No inertia both in the stopping and starting of the heating process is experienced and thus the amount of energy supplied to the sample can be accurately controlled. Microwave digestion is thus far more efficient and reproducible than traditional heating methods, resulting in substantial time savings. Another advantage is a decrease in blank levels due to less environmental contamination and the general requirement for less reagents. The blank level obtained is an important parameter to consider as it often determines the limit of detection of the technique. The microwave digestion technique also requires less operator attention, is more suited to smaller sample sizes and is particularly amenable to automation.

1.3.1 Theory of Microwave Heating

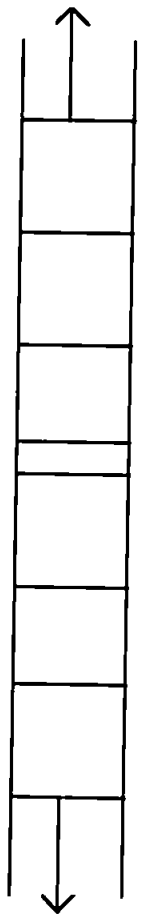
Microwaves are high frequency electromagnetic waves with a frequency of 300 to 300,000 MHz. Their position in the electromagnetic spectrum is given in Figure 1.1[14]. The most commonly used frequency for microwave appliances is 2450 MHz, assigned for industrial, and scientific use to conform to the International Radio Regulations adopted at Geneva in 1959. Microwave energy is a non-ionising source of radiation that causes molecular motion rather directly changing molecular structure. Samples absorb microwave energy according to their dissipation factor[12], tan δ which may be defined as:

$$\tan \delta = \frac{E''}{E'}$$

where E", the dielectric loss factor, is the amount of input energy which is lost to the sample by being dissipated as heat and E', the dielectric constant (real permittivity), is the ability of the sample to obstruct that energy as it passes through the sample.

Figure 1.1: The Electromagnetic Spectrum

Radiation	Wavelength	Frequency (Hz)	Atomic or Molecular Process
Cosmic rays	< 0.01 nm	$>10^{20}$	
Gamma rays	0.01-1 nm	10^{17}-10^{20}	Nuclear transformations
X-rays	0.1-100 nm	10^{15}-10^{18}	Inner electron shell transitions
Ultraviolet	100-400 nm	7.5×10^{14} - 10^{16}	Outer electron shell transitions
Visible light	400-800 nm	3.8×10^{14} - 7.5×10^{14}	Outer electron shell transitions
Infrared	800 nm-0.03 cm	10^{12}-3.8×10^{14}	Molecular vibrations
Microwaves (radar)	0.1-10 cm	10^{9}-10^{12}	Molecular rotations and electron spin orientation transitions
Radiowaves	10 cm-1000 m	10^{5}-10^{9}	Nuclear spin orientation transitions
A.C. generators	> 1000 m	$<10^{5}$	



The greater the dissipation factor the quicker the energy is absorbed and dissipated and therefore the lower the penetration of the energy into the sample will be. Penetration is considered infinite in materials transparent to microwave energy and is considered zero in reflective materials such as metals. For absorptive samples the dissipation factor has a finite value. Table 1.1 lists the dissipation factors (tan δ) of some common materials[12,15].

Table 1.1: Comparison of the Dissipation Factors of Different Materials

Material	Tan δ (x 10^4)[1]
Water	1570
Borosilicate glass	10.6
Fused quartz	0.6
Polyethylene	3.1
Polyvinyl chloride	55.0
Polystyrene	3.3
Nylon 66	128.0
Teflon PFA	1.5

Notes:
1: Measurements taken at 3000 MHz and 25°C except for polyvinyl chloride (20°C).

Digestion vessels should be constructed from a material with a low dissipation factor so that the microwaves are not absorbed by the vessel, but pass through to reach the sample inside. Quartz, glass and plastics are essentially transparent to microwave energy and are poor conductors of heat, thus are good insulators for vessels for microwave heating. However two other basic considerations exist, the necessity for good chemical and good thermal stability. Most plastics have good chemical resistance to acids but do not exhibit good thermal stability at the temperatures required for acid dissolutions. Teflon (polytetrafluoroethylene) however has a melting point of 306°C and thus is suitable for the

majority of microwave digestion applications, except dissolutions with sulphuric or phosphoric acid due to their high boiling points. Quartz and glass vessels exhibit better thermal stabilities, however are attacked by hydrofluoric acid and thus for such applications Teflon vessels are usually preferred.

It has been demonstrated that microwave heating takes place as a consequence of two main mechanisms; dipolar rotation and ionic conduction[12]. These processes mechanically agitate and rupture the surface layers of the sample thereby exposing fresh surfaces to attack by the digestion reagents[13].

1.3.1.1 Ionic Conduction

The conductive migration of dissolved ions in the applied electromagnetic field is referred to as ionic conduction. This ionic migration is a flow of current that results in heat generation due to the resistance of the environment against the ion flow. All ions in solution contribute to the conduction process however the relative contribution of each depends on a number of factors, namely the ion concentration and its mobility in the solution. As the ionic concentration of the solution increases the dissipation factor also increases. Factors such as size, charge and conductivity of the dissolved ions and degree of ion interaction with the solvent molecules are also important parameters to consider.

1.3.1.2 Dipole Rotation

The effect of the electromagnetic field of the microwave energy is to align molecules which have permanent or induced dipole moments (Figure 1.2a). A very small amount of energy is associated with the molecules in the aligned position. When the electric field is removed thermal agitation returns the molecules to their unorganised state, in relaxation time t, and the

Figure 1.2a: Alignment of Polar Molecules e.g. Water with the Poles of an Electromagnetic Field

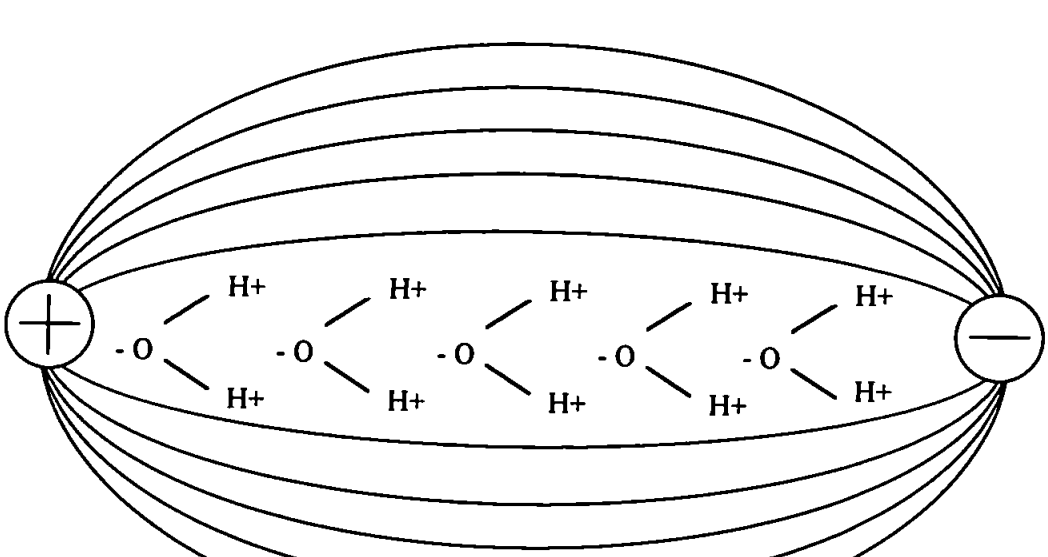
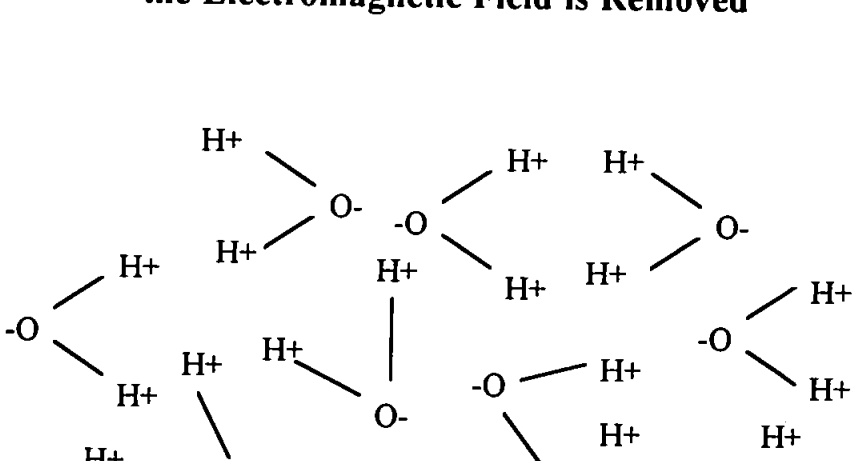


Figure 1.2b: Thermally Induced Disorder of Water Molecules as the Electromagnetic Field is Removed



energy is released in the form of heat (Figure 1.2b). At 2450 MHz the direction of the electric field changes direction 4.9×10^9 times per second[12]. Thus alignment and return to disorder also occurs 4.9×10^9 times per second, resulting in heating of the sample. The heating efficiency however is dependent upon the sample's dielectric relaxation time which is in turn dependent upon the temperature and viscosity of the sample.

1.3.1.3 Dielectric Relaxation Time

The dielectric relaxation time, τ, is defined as the time taken for molecules to achieve 63% of their return to disorder when the applied electromagnetic field is removed[12]. The maximum energy conversion per cycle (dielectric loss due to dipole rotation) by many materials will occur when $\omega = 1/\tau$, where ω is the angular frequency of the microwave energy in radians per second ($\omega = 2\pi f$ and f = microwave frequency). When $1/\tau$ is similar to ω such as in non-ionic polar samples the dissipation factor will be high, whereas when $1/\tau$ is considerably different from ω a low dissipation factor results and so heating time is increased. Obviously maximum energy conversion (high dissipation factor) is not desired for volumetric heating as only the outer layers will be heated and so the interior will only be heated by classical means, namely thermal conductance and convection.

The temperature of the solution also affects the dissipation factor. For example at room temperature $1/\tau$ for water is greater than 2450 MHz. With an increase in temperature, $1/\tau$ increases and therefore is further out of coincidence with ω. Therefore absorption of the input energy is decreased and deeper penetration results (dissipation factor decreases). However for organic liquids and solids the dissipation factor increases with temperature and thus care must be taken to control the procedure at high temperatures.

1.3.1.4 Viscosity

The viscosity of the sample affects molecular rotation and thus the ability of the sample to absorb energy. In very viscous liquids molecular mobility is restricted making it hard for molecules to align with the electric field, thus the dissipation factor will be low. With an increase in temperature, the dissipation factor will increase as viscosity decreases (molecular mobility will be higher). This is the case for the heating of ice, although as discussed in Section 1.3.1.3 following an increase in temperature $1/\tau$ will be further out of coincidence with ω and thus the dissipation factor will decrease. Initial heating of ice results in an increase in the dissipation factor as the viscosity of the ice is more influential on the dissipation factor than the dielectric relaxation time. However as viscosity decreases temperature becomes the more influential parameter and the dissipation factor decreases[12].

1.3.1.5 Sample Size

With decreasing sample size the amount of energy absorbed decreases as a considerable amount of microwave energy is reflected from the sample[12]. However in large samples possessing a large dissipation factor, heating may only occur beyond the penetration depth of the microwave energy by thermal conductance through molecular collisions. Thus temperatures near the surface will be higher than in the middle of the sample. Surface heating does not become a problem however unless penetration is very low because the action of boiling and other agitation increases thermal conductance, thereby distributing the heat throughout the sample.

1.3.1.6 Relative Contributions of Dipole Rotation and Ionic Conduction

Generally the temperature of the solution determines the relative contributions of dipole rotation and ionic conduction to energy conversion. For small molecules the dielectric loss to a sample due to dipole rotation decreases as the temperature increases, whereas the contribution from ionic conduction increases. The contribution of each mechanism depends upon the concentration and mobility of sample ions and the dielectric relaxation time. If the ion concentration and mobility are low, heating will be dominated by dipole rotation and will depend on the difference between $1/\tau$ and ω. However as the concentration and mobility of sample ions increase, heating will be dominated by ionic conduction and the relaxation time of the solution will become less important. However heating time is not totally dependent on the dielectric absorptivity of the sample but upon the sample size and shape and the microwave system used as well.

1.3.2 Instrumentation for Microwave Digestion

Microwave digestion instrumentation can generally be split into two broad categories:

a) Multimode microwave ovens i.e. domestic or commercial conventional design (large cavity).

b) Open focused microwave ovens (monomode).

The basic design of each are described in Sections 1.3.2.1 and 1.3.2.2 respectively.

1.3.2.1 Multimode Ovens

A design of a typical multimode microwave appliance is shown in Figure 1.3. The oven is generally composed of six main components: the microwave generator (magnetron) with its electric power supply, the wave guide, the microwave cavity, the mode stirrer, a circulator and a turntable.

The Magnetron

A magnetron (Figure 1.4a) is a vacuum device that converts direct current (DC) electrical energy into microwaves[16]. It is a circular symmetric tube containing a hollow cylindrical anode with a directly or indirectly heated cathode along its axis (Figure 1.4b). A constant potential is applied between the anode and the cathode and an axial magnetic field is produced by either a permanent magnet or an electromagnet. The electrons emitted from the cathode are accelerated towards the anode by the DC voltage between them. A ring of mutually coupled resonant cavities in the anode cause the emitted electrons, under the influence of the magnetic field, to resonate and thus the magnetron oscillates[12]. The oscillating electrons surrender energy to the microwave field that radiates from an antenna enclosed in the vacuum envelope of a tube (Figure 1.4a). The efficiency of the magnetron is generally between 60 and 65%[16], the remaining power of which contributes to the heating of the cathode or is removed by means of cooling fins or a circulating water jacket.

The Wave guide, Mode Stirrer and Turntable

The microwaves generated by the magnetron are channelled to the applicator (microwave cavity) by the wave guide which is constructed of a reflective material such as sheet metal. The microwaves entering the cavity are repeatedly reflected from wall to wall and a standing wave is produced. The electric field pattern can be very complex with some areas receiving a large amount of the energy, whereas others may be almost completely neglected[16]. To ensure an even distribution of incoming energy a mode stirrer is sometimes used to move the maxima of electromagnetic power around the cavity. This is essentially a fan-shaped blade which reflects and mixes the energy entering the microwave cavity[15]. Most ovens are equipped with a turntable to move the sample instead of the electromagnetic field in order to obtain an homogenous application of energy to each sample.

Figure 1.3: Layout of a Typical Multimode Microwave Oven

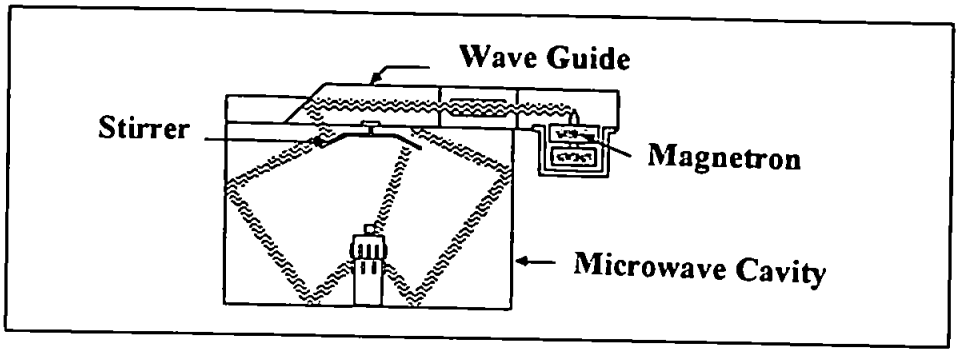


Figure 1.4a: Schematic of a Typical Magnetron

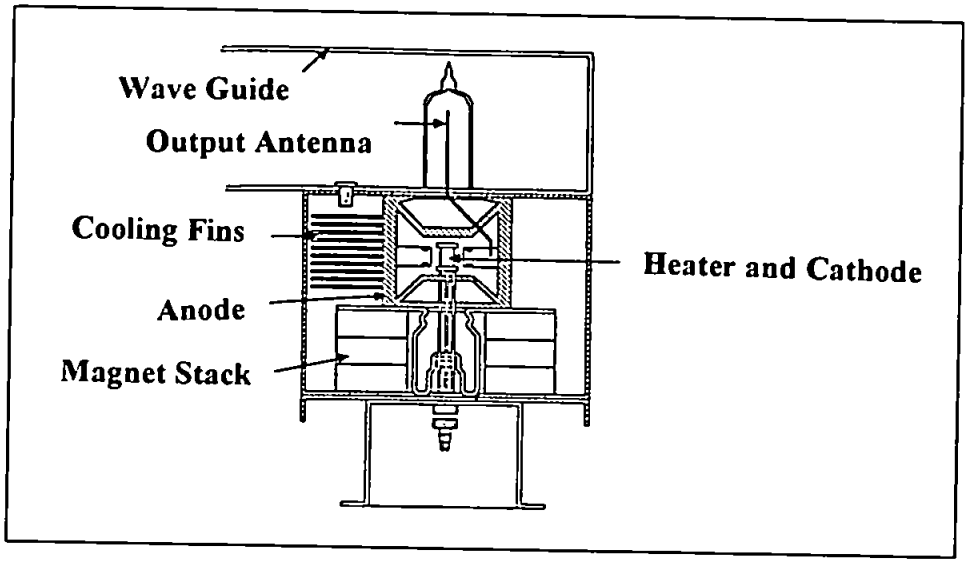
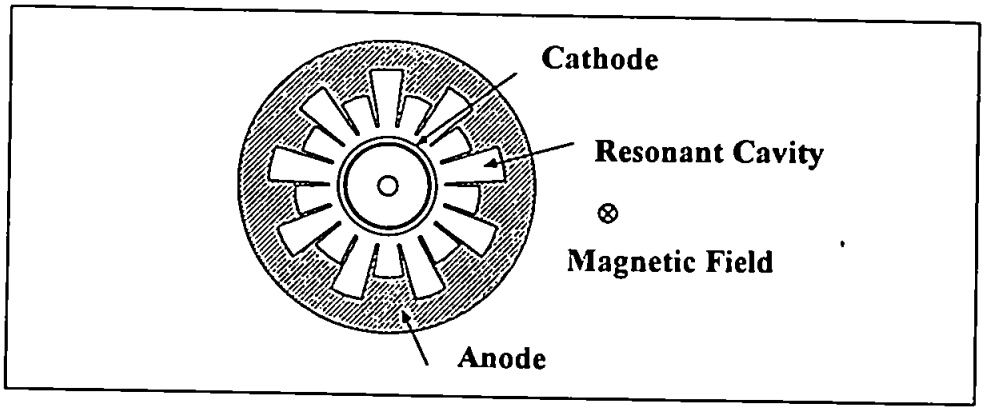


Figure 1.4b: Schematic of the Magnetron Anode and Cathode



The Circulator

A circulator is used in some appliances to prevent damage to the magnetron as a consequence of overheating from unabsorbed microwaves reflected back to the magnetron. This is of particular importance for microwave digestion appliances where the amount of sample/reagents heated are generally very small. The circulator is essentially a three-way ferromagnetic 'check valve' which allows microwaves to pass in the forward direction but diverts the reflected microwaves to a dummy load where the energy is harmlessly dissipated as heat[16].

1.3.2.2 Open Focused Microwave Digestion Systems

A diagram of an open focused microwave digestion unit is presented in Figure 1.5. The system is composed of a magnetron and a wave guide, as for multimode ovens. However in contrast to dispersing the microwaves into a large microwave cavity, which as described previously leads to an inhomogeneous and inefficient supply of energy to each sample (Figure 1.6a), the sample is placed directly in the path of the microwaves, within the wave guide (Figure 1.6b). The sample itself is placed in a digestion tube which is lowered into the vertical microwave cavity. Above the wave guide the cavity is surrounded by a metallic sheath which acts as a shutter to prevent microwaves from escaping. The focused microwave approach is a very efficient mechanism of transferring energy to the sample, resulting in a greater power density of more than ten times that obtained for multimode ovens[17]. As a result a far lower power setting is required.

Figure 1.5: Diagram of an Open Focused Microwave Oven

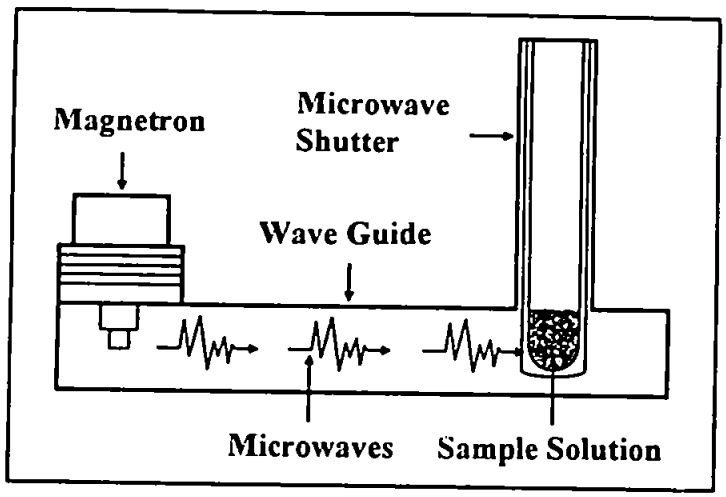
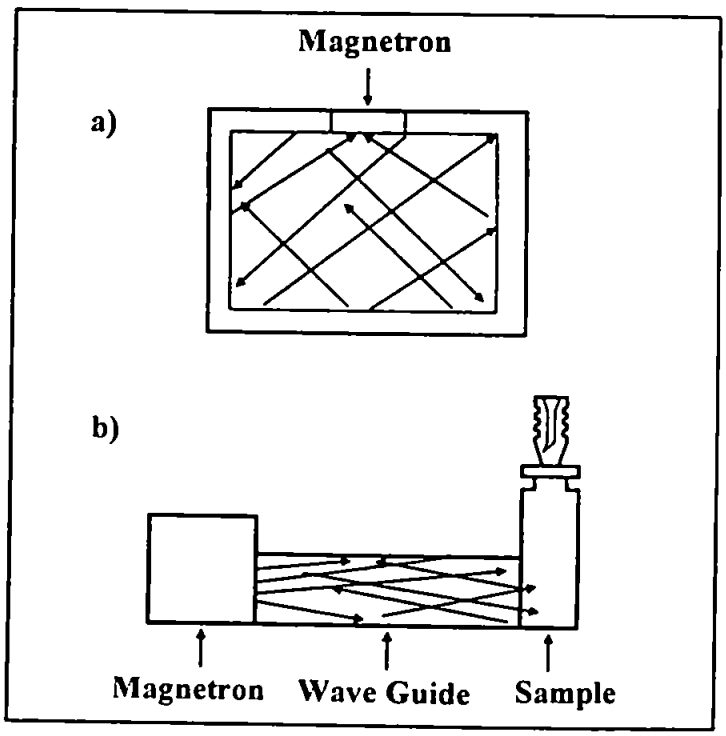


Figure 1.6: Dispersion of Microwave Energy Through a) a Multimode Oven and b) an Open Focused Microwave Oven



1.4 Literature Review of Microwave Digestion Techniques

The advantages of the microwave digestion technique have led to its application as an effective sample preparation method for a wide range of sample matrices. Each year more and more laboratories replace conventional digestion methods with the new technology, as is reflected in the ever increasing amount of material published on the subject. A number of review articles and books have been published[12,16,18-22] detailing the use of the technique for elemental analysis. Kuss[18] listed the applications of microwave digestion techniques for elemental analyses cited in the literature before 1992. Included were references for the digestion of biological, geological, environmental and metallic materials. A recent publication by Zlotorzynski[16] discusses the fundamental principles of microwave field interaction with the sample matrix. A review of the use of microwave assisted sample preparation in analytical chemistry has been undertaken by Smith *et al.*[22] and specifically for electrothermal atomic absorption spectrometry analysis by Chakraborty *et al.*[19].

This section includes a review of the application of microwave energy for the digestion of environmental samples (biological, geological and water) reported in the literature since 1992. Details of open and closed microwave digestion systems, including the advantages and disadvantages of each technique are discussed. The review also attempts to highlight any trends in the research and to identify universal digestion procedures for particular matrices or elements.

Tables 1.2-1.4 summarise the different microwave digestion procedures employed during the review period for biological, geological and water samples respectively. Each table characterises the matrix digested; elements determined; microwave system used; digestion method i.e. specific reagents and heating time; analytical technique and finally comments as to

Table 1.2: Summary of Published Microwave Digestion Procedures for Biological Samples

Matrix	Elements	Microwave System	Digestion Method	Analysis Technique	Comments	Ref.
Biological samples	Se	Prolabo Microdigest 301 (200 W)	Open focused HNO_3-H_2O_2 digestion for 20 min (n=1)	FI-CSV	Good recoveries were obtained for Se in BCR Lyophilised Pig Kidney.	23
Biological samples	As	Prolabo A320, (200 W)	Solubilisation: HNO_3 open focused digestion for 10 min. Mineralisation: HNO_3-H_2SO_4-HNO_3-H_2O_2 digestion for 70 min.	ICP-MS and HPLC-ICP-MS	Good results for total arsenic in BCR Cod Muscle after both solubilisation and mineralisation procedures.	24
Biological samples	Se	Prolabo Microdigest A-301 (200 W)	Open focused HNO_3 digestion at 30 W for 20 min, followed by addition of H_2O_2 and further heating for 25 min (n=1).	Se(VI) is reduced to Se(IV) prior to analysis by GC-MS.	Good agreement with certified values for NIST Bovine Liver and Mixed Diet (Finland), although for NIST Total Diet results were slightly low. Procedure was quicker than the conventional technique without needing $HClO_4$.	25
Fat-rich foods	Cu,Fe,Ni, Zn	Prolabo Microdigest 300M (200 W) and domestic oven (700 W)	Closed: HNO_3-H_2O_2 PTFE bomb digestion for 20 min. Open focused: HNO_3-H_2SO_4-HNO_3 digestion for 25 min (n=1).	FAAS and GF-AAS (for Ni).	Generally low results for the open digestion method. Reasonable agreement with results obtained from pressurised microwave and wet pressure autoclave digestion for soybean flour and linseed samples.	26
Foods	N	Prolabo Maxidigest MX-350 (350 W)	Open focused H_2SO_4-H_2O_2 Kjeldahl nitrogen digestion, for 20-45 min depending on food type (n=1).	NH_3 titration.	Substantial time savings on conventional methods without the need for a catalyst. However for new matrices each step of the procedure must be reoptimised separately.	27
Biological samples	Cd	Prolabo Microdigest A-301	Open focused HNO_3-H_2O_2 digestion for 42 min.	ET-AAS	Good results obtained for NIST Wheat Flour and Bovine Liver although for IAEA Fish Flesh Homogenate results were slightly low. Acceptable agreement with results from a conventional wet digestion for bovine muscle and liver, oyster, barley straw, cabbage, carnations, oak leaves, pine needles, apple-fruit and grass meal .	28
Bovine liver	Bi,Cd,Co, Cs,Cu,Fe, Hg,Mn,Mo, Pb,Rb,Sb, Sn,Sr,Tl,Zn	Prolabo Micro-digest A301 and Milestone MEGA 1200 (1200 W)	HNO_3-H_2O_2 digestion Closed vessel: 26.5 min. Open focused: 45 min.	ICP-MS	Open focused: Good results obtained for NIST Bovine Liver except for low Cu, Sr and Zn. Closed vessel: Good results except for low Cd, Pb and Sr.	29

For a key to the abbreviations used in Tables 1.2-1.4 see list following Table 1.4.

Table 1.2: Summary of Published Microwave Digestion Procedures for Biological Samples Continued

Matrix	Elements	Microwave System	Digestion Method	Analysis Technique	Comments	Ref.
Biological samples	As,Ba,Ca, Cd,Cu,Fe,K Mg,Mn,Na, P,Pb,Sr,Zn	Prolabo Microdigest M300 (300 W) and Floyd RMS-150 (600 W)	Open focused vessel: HNO_3-H_2SO_4-H_2O_2-NH_4EDTA digestion for 30 min (n=1). Closed vessel: HNO_3 digestion for 32 min.	ICP-AES and residual carbon content analysis.	Generally good results for the open digestion for NIST Bovine Liver and Oyster Tissue; IAEA Horse Kidney and NIES Mussel Tissue although Na recoveries were slightly low. Residual carbon content for the open system was far superior to the closed system.	30
Tea Leaves	Al,Ba,Ca, Cu,K,Mg, Mn,Zn	Prolabo Microdigest 301	Open focused HNO_3-$HClO_4$ digestion for 35 min (n=1).	ICP-AES	Good agreement with the certified values for NIES 'Tea Leaves'.	31
Fish samples	As	Prolabo Microdigest 301	On-line system incorporating HPLC separation; potassium persulphate/NaOH oxidation and L-Cysteine pre-reduction of As species in samples following enzymatic extraction.	On-line analysis by HG-AAS. Total As can be determined by removal of the HPLC column from the system.	The AsBet, DMA, MMA, As(V) and total arsenic content of NRCC TORT-1 and DORM-1 were determined by the on-line technique. Results for total arsenic were in good agreement with those obtained by ICP-MS analysis.	32
Fish samples	As	Prolabo Microdigest 301	Open focused HNO_3-H_2O_2 digestion for 15 min (n=1).	ICP-MS	Good results were obtained for As in NRCC DORM-1 and TORT-1.	32
Marine biological samples	Hg	Prolabo Microdigest 301	On-line digestion of 0.15% slurries (in 50% HCl) and Br^--BrO_3^- oxidation of organomercury species.	On-line analysis by CV-AFS	A recovery of 97% was obtained for a standard solution of methylmercury chloride. Results for Hg in NRCC DORM-2 were in good agreement with the certified value.	33
Fish samples	Hg	Prolabo Microdigest 301	Open focused HNO_3-H_2SO_4-H_2O_2 digestion for 25 min (n=1).	CV-AAS	Good results were obtained for Hg in NRCC DORM-2.	33
Fish samples	As,Cd,Co, Cr,Cu,Fe, Pb,Mn,Ni, Se,Sr,Zn	Prolabo Maxidigest M401 (300 W)	Open HNO_3 or HNO_3-H_2SO_4 digestion for 20-100 min (depending on sample size) followed by evaporation to a volume of 1 ml.	ET-AAS	Samples of up to 8 g were successfully digested. Generally good results were obtained for NRCC TORT-1 and LUTS-1.	34

Table 1.2: Summary of Published Microwave Digestion Procedures for Biological Samples Continued

Matrix	Elements	Microwave System	Digestion Method	Analysis Technique	Comments	Ref.
Biological samples	Al,As,Cd, Co,Cr,Cu, Hg,Mg,Mo, Ni,Pb,Zn	Prolabo Microdigest A300 (200 W)	Open focused digestion with: a) HNO_3-H_2O_2 b) HCl-HNO_3-H_2O_2. c) HNO_3-H_2SO_4-H_2O_2.	ICP-AES and ICP-MS	Good results obtained for procedure a) for BCR Spruce needles (except low Al, Mg); White Clover, Cod Muscle (except low Hg) and Plankton (except high Hg and low Mn). Procedure b) gave high As and low Mn and procedure c) low Hg in Cod Muscle. Results generally low for Plankton by procedure b) except for Cd and Zn.	35
Liver tissue	Sb(III) and Sb(V)	Prolabo Microdigest 301	Open focused digestion with: a) 1M acetic acid [for Sb(III)]. b) H_2SO_4-KI (total Sb).	HG-AAS	Good results were obtained for total Sb in spiked calve liver samples. Sb(V) is calculated as the difference between total Sb and Sb(III).	36
Fish muscle tissue	Hg	Prolabo Microdigest A301 and Superdigest (300 W)	Open: HNO_3-H_2SO_4-HNO_3-H_2O_2 digestion for 20 min (n=1) Closed: HNO_3 digestion.	CV-AAS	Good results were obtained for Hg in BCR Pig Kidney and IAEA Fish Tissue for both methods. For the open method, digestion with just HNO_3 and with HNO_3-H_2SO_4-HNO_3 resulted in low recoveries.	37
Biological samples	Cu,Zn,Cd	CEM MDS-81D (600 W)	HNO_3 low volume Teflon bomb digestion for 49 min (n=24).	FAAS and ET-AAS (for Cd)	Good results obtained for Cu, Zn and Cd in NRCC, DOLT-1 and TORT-1 and NIST Oyster Tissue; for Cu and Zn in NRCC DORM-1 and for Zn in NIST Albacore Tuna.	38
Botanical samples	B,Se	CEM MDS-2000 (630 W)	Se: HNO_3-H_2O_2-H_2O PTFE bomb digestion for 30 min following predigestion for 4 h. B: HNO_3-H_2O_2 PTFE bomb digestion for 45 min following predigestion for 4 h, (n=12).	FAAS (for Se) and ICP-AES (for B)	Se recoveries for NIST Wheat Flour: 23% with HNO_3; 30% with HNO_3-H_2O_2; 57% with HNO_3-H_2O_2 and 80% with HNO_3-H_2O_2-H_2O. B recoveries for NIST Apple Leaves: 60% with HNO_3 and 66-96% with HNO_3-H_2O_2. Longer digestion times or adding HCl (for Se) did not increase recoveries.	39
Cocoa	Cu,Fe	CEM MDS-81 with pressure transducer	HNO_3 PFA-PTFE bomb digestion for 30 min (n=7).	FAAS	The microwave digestion was 4 times quicker than the conventional hotplate method. Results from the two techniques were in good agreement.	40
Horse Kidney	Ca,Fe,Mg, Zn	CEM MDS-81	HNO_3 PFA-PTFE bomb digestion for 30 min (n=7).	FAAS	Good results were obtained for Fe and Mg in IAEA Horse Kidney, however Zn results were slightly high and Ca low.	41

Table 1.2: Summary of Published Microwave Digestion Procedures for Biological Samples Continued

Matrix	Elements	Microwave System	Digestion Method	Analysis Technique	Comments	Ref.
Biological samples	Ca,Cd,Fe, Mg,Zn	CEM MDS-81	On-line stopped-flow digestion of slurries (prepared in Triton X-100 and HNO_3) for 5 min.	Off-line analysis by AAS	Good results for Zn in IAEA Horse Kidney, but low Fe and Cd and high Mg. System unsuitable for Ca determinations. Good agreement between batch and on-line microwave and hot-plate digestion of cocoa powder. Unsuccessful attempts at slurrying NIST Pine Needles, Oyster Tissue and Bovine Liver at concentrations needed for FAAS detection.	42
Biological samples	Ca,Fe,Mg, Zn	CEM MDS-81	On-line digestion of HNO_3 slurries.	On-line analysis by FAAS	Good recoveries and precision for NIES Chlorella, Sargasso and Pepperbush and NIST Bovine Liver although precision slightly high for NIES Mussel. Zn was not detectable by FAAS in Chlorella and Sargasso, nor Ca in Bovine Liver at the maximum stable slurry concentration.	43
Botanical samples	As,Se	CEM MDS-2000	H_2O-HNO_3-H_2O_2 PTFE bomb digestion for 25 min	HG-AAS	Good results for BCR FD8 Maize Leaves and spike recoveries of 95 and 105% were obtained for As and Se respectively.	44
Botanical samples	Al,Ca,Cu, Cr,Fe,K,Mg Mn,Si,Ti, Zn	CEM MDS-81D	Teflon PFA bomb digestion with: a) HNO_3-HCl b) HNO_3-HF-H_2O_2.	FAAS and DCP-AES (for Cu).	a) Good Ca, K, Mg, Mn and Zn results but low Al, Cu and Fe recoveries for NIST Citrus Leaves and Pine Needles and IAEA Mixed Diet. b) Good results obtained for Al, Fe and Mg in Citrus Leaves, however Cu results were slightly low.	45
Bio-moniters	Ca,Cd,Cr, Cu,Fe,K, Mg,Mn,Ni, Pb,Zn	CEM MDS-2000	HNO_3-H_2O_2 PFA bomb digestion for 15 min (moss and rye grass) and 17 min (humus and hay) (n=10).	FAAS and GF-AAS	Good results were obtained for BCR 281 Rye Grass and IAEA V-10 Hay, except for low Fe recoveries. Moss and Humus samples were also successfully digested.	46
Botanical samples	Ca,Cu,Fe,K Mn,Mg,Na, P,Zn	CEM MDS-81D	PTFE bomb digestion with: a) HNO_3. b) HNO_3-H_2O_2 for 60 min (n=12).	AAS and AES (for Na and K).	No significant difference between the results of the two microwave procedures, a conventional dry ashing and a wet ashing technique for Lucerne Leaves.	47
Botanical samples	Ba,Ca,Cu, Mg,Mn,Zn	CEM MDS-81 with IR probe	HNO_3-HF PTFE bomb digestion for 20 min (n=6).	ICP spectrometry	Good agreement with certified values for NBS Citrus Leaves except for Cu (slightly low).	48
Food samples	Na	CEM MDS-2000	PTFE bomb HNO_3-H_2O_2 digestion for 1.5 h (n=12).	AAS, CIE and IC analysis.	No CRMs were analysed.	49

Table 1.2: Summary of Published Microwave Digestion Procedures for Biological Samples Continued

Matrix	Elements	Microwave System	Digestion Method	Analysis Technique	Comments	Ref.
Botanical samples	Mn	CEM MDS-81D	HNO_3 Teflon PFA bomb digestion a) without pre-digestion; b) with pre-digestion at room temperature (18 h); c) with microwave pre-digestion and reflux (7 min); d) with microwave pre-digestion without reflux.	FAAS	No significant differences in results generated from procedures a) to d) in sweet bay powder. Procedure c) enables a fast, safe digestion without large evaporation of acids in the pre-digestion step.	50
Food samples	Decompo-sition products	CEM MDS-81D	Bomb digestion with HNO_3; HNO_3-H_2O_2 or HNO_3 followed by H_2O_2/ $HClO_4$ treatment on a hotplate.	Carbon content analysis, IR, TLC	Results illustrated the necessity of employing different sample decomposition methods according to the sample matrix and analytical technique of choice.	51
Biological samples	Ca,Cu,Fe,K Mg,Mn,P,S, Zn	CEM MDS-81D	After initial heating with HNO_3-H_2O_2 in a PTFE bomb the reaction continues spontaneously without further irradiation.	ICP-AES and FAAS	Good recoveries were obtained for Ca, Fe, K, Mg, Mn and S in NIST Bovine Liver. P, Zn and Cu results were just outside the certified range. The heating time required depended on the sample size and amount of H_2O_2 used.	52
Fish samples	As,Cd,Pb	CEM MDS-2000	Teflon PFA HNO_3 digestion for up to 2 h depending on the sample (n=12).	ICP-AES and ICP-MS	Good agreement with certified values for NRCC TORT-1 and DORM-1 except for high As in the former. Spike recoveries were in the range 75%-117%.	53
Botanical samples	Ca,Fe,K, Mg,Mn,P,S	CEM MDS-2000	Closed digestion with a)HNO_3-$HClO_4$ for 1 h 23 min b)HNO_3-H_2O_2.	ICP spectrometry	Generally good results were obtained for NIES Citrus Leaves and Pine Needles and for Corn Leaves.	54
Biological samples	Ag,As,Cd, Cr,Cu,Ni, Pb,Zn	CEM SpectroPrep system (550 W) and Floyd RMS-150	On-line: 0.5% (m/v) slurries were digested in 20% HNO_3-3% H_2O_2. Batch: Teflon bomb HNO_3-HF digestion for 70 min with cooling step half way through.	ID-ICP-MS (standard additions) and ET-AAS (for As and Cr).	On-line system: good results obtained for NRCC LUTS-1 for Cd,Cu,Ni and Pb (Ag and Zn were low and Cr very high). For lobster hepatopancreas results were in agreement with the closed vessel technique. Slurries of Pacific oyster tissue were not amenable to direct uptake by the SpectroPrep unless prior digestion was undertaken.	55
Fish samples	Cd,Pb	CEM MDS-2000	HNO_3 digestion for 20-25 min (n=12).	GF-AAS	Good results were obtained for Cd and Pb in NRCC DORM-1.	56

Table 1.2: Summary of Published Microwave Digestion Procedures for Biological Samples Continued

Matrix	Elements	Microwave System	Digestion Method	Analysis Technique	Comments	Ref.
Biological samples	Hg	CEM MDS-2000	HNO_3 PTFE bomb digestion for 70s.	CV-AAS	Good agreement with the certified value and spike recoveries of 99-102% for NIST RM 50 'Albacore Tuna'.	57
Marine biological samples	As,Cd,Co, Cr,Cu,Hg, Pb,Zn	CEM MDS-81D	Teflon bomb HNO_3 digestion for 2 min followed by preconcentration on Chelex-100 column.	NAA and GF-AAS	Good agreement with certified values for NRCC DORM-1.	58
Botanical samples	Lanthanides and actinides	Milestone 1200 (1200 W)	HNO_3-H_2O_2 PTFE bomb digestion for 14 or 26 min depending on sample size.	ICP-MS	Good results obtained for Ce, Eu, Sm, Tb and U-238 in NIST Apple Leaves (low U-238) and for U-238 in Orchard Leaves (low Th-232).	59
Biological samples	Ru	Milestone 1200	HNO_3-H_2O_2 PTFE bomb digestion of homogenised samples (in water) for 30 min.	ET-AAS	Spike recoveries of 95-101% in liver and kidney samples were obtained but no CRMs were analysed.	66
Botanical samples	B,Cd,Cu,Fe Mn,P,Pb,Zn	Milestone MLS-1200 MEGA and domestic oven (665W),	Teflon bomb digestion with: a) HNO_3; b) HNO_3-HF-hotplate evaporation to dryness-HNO_3; c) HNO_3-H_2O_2; d) HNO_3-$HClO_4$.	ICP-AES, FAAS, ETA-AAS	a) Poor precision. Generally high P and low Fe results for Commite Inter-Instituts botanical reference materials. b) Acceptable precision. Fe results were improved but most still outside certified range. c) and d) Mixed Fe, Cu, Mn, Zn results for NIST Total Diet, NIES Mussel, Pepperbush and BCR Wholemeal Flour (many results too high).	61
Biological samples	Ca,Cu,Fe,K Mg,Mn,Na, P,Zn	Milestone MLS-1200	HNO_3-H_2O_2 PTFE bomb digestion.	ICP-AES and AAS	For NBS Orchard Leaves, Bovine Liver and Spinach low Fe but good Cu, Mn, Na, P and Zn results. Some Ca, K and Mg recoveries were slightly low.	62
Food stuffs	Al	Milestone MLS-1200	Teflon bomb HNO_3-H_2O_2 digestion for 23 min (n=3).	GF-AAS	Good results were obtained for NIST Wheat Flour, but results slightly high for Rice Flour. Reasonable agreement obtained with informational values for Total Diet and IAEA Fish Tissue. HF addition did not increase recoveries.	63
Food samples	Si	Floyd RMS 150 (850 W)	HNO_3-H_2O_2-HF Teflon bomb digestion for 30 min following overnight predigestion. HF is neutralised with H_3BO_3.	A tertiary amine mixture is added before ICP-AES analysis.	Good results obtained for NIST 1566 Oyster Tissue. Results in acceptable agreement with $LiBO_2$ fusion results for a range of food samples.	64

Table 1.2: Summary of Published Microwave Digestion Procedures for Biological Samples Continued

Matrix	Elements	Microwave System	Digestion Method	Analysis Technique	Comments	Ref.
Biological samples	As	Floyd RMS 150	HNO_3-H_2O_2 bomb digestion for 32 min (n=6).	ICP-MS	Good results were obtained for NIST Oyster Tissue and Orchard Leaves.	65
Biological samples	Ag,Al,As, Cd,Cr,Co, Cu,Fe,Hg, Mn,Ni,Pb, Se,SnTh	Floyd	HNO_3-HF digestion for 42 min, followed by cooling, re-heating for 42 min, evaporation to dryness on a hotplate (with/without H_2O_2) and redissolution in HNO_3/H_2O.	ID-ICP-MS and ICP-MS	Good results were obtained for Ag, Al, As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Se, Sn, Th and Zn in NRCC DORM-2 and DOLT-2.	66
Biological samples	Ca,Cu,Fe, Mn,Pb	Floyd RMS-150	21 different digestion procedures using HCl-HNO_3-HF in different ratios.	DCP-AES	Best procedure chosen by fractional factorial design. For chosen procedure good results were obtained for NRCC TORT-1 and NIST Pine Needles. Spike recoveries were 96-105%. Detrimental effect of *aqua regia* was reported (HNO_3 and HCl most effective in equal quantities).	67
Biological samples	As,Cd,Co, Cu,Ni,Pb	PMD, Paar	Quartz tube closed HNO_3-$HClO_4$ digestion. The procedure is dependent on the sample.	DP-ASV and HG-AAS (for As)	Mixed results obtained for Cd, Cu and Pb in BCR Bovine and Cod Muscle, Bovine Liver, Mussel Tissue and Brown Bread. Results for Co in NRCC TORT-1 were good but for Ni were slightly low. For determination of As in fish and cooking oil (by HG-AAS) addition of H_2SO_4 was needed. Good results were obtained for BCR Cod Muscle, Mussel Tissue, NIST Orchard Leaves and for NRCC TORT-1.	68
Biological samples	Ni	Domestic oven (700 W)	HNO_3-HCl PTFE bomb digestion for 14 min (n=3).	ICP-AES analysis after extraction of Ni complex formed with DPTH into butan-1-ol.	Generally good agreement with certified values for BCR Olive Europea, Lagarosiphon Major, Platihpnidium Ripariodides; NRCC DORM-1, DOLT-1, TORT-1; NIST Citrus Leaves; BCR Pig Kidney and Bovine Muscle.	69
Biological samples	Ni	Domestic oven (700 W)	HNO_3-HCl PTFE bomb digestion for 14 min (n=3).	ICP-AES analysis after extraction of Ni complex formed with BPTH into IBMK.	Good agreement with certified values for NIST Citrus Leaves and NRCC DORM-1 and TORT-1.	70
Shellfish	Al	Domestic oven (800 W)	On-line HNO_3 digestion of slurries (in 0.2% HNO_3).	Off-line analysis by ET-AAS	90% recovery for Al in NIST Oyster Tissue (SRM 1566a). Five fresh shellfish samples were also analysed.	71

Table 1.2: Summary of Published Microwave Digestion Procedures for Biological Samples Continued

Matrix	Elements	Microwave System	Digestion Method	Analysis Technique	Comments	Ref.
Shellfish	Se	Domestic oven (800 W)	On-line digestion of slurries (in 0.2% HNO_3) for 4 min (stopped flow).	Off-line analysis by ET-AAS	Good results obtained for NIST SRM Oyster Tissue 1566a. No significant results obtained between lyophilised and unlyophilised samples.	72
Botanical samples	Hg	Domestic oven (800 W)	HNO_3 PTFE bomb digestion for 3 min at 800 W (n=5).	FANES after reduction with $SnCl_2$ and in situ pre-concentration.	Good agreement with certified values for NIST 'Citrus Leaves' and 'Pine Needles'.	73
Biological samples	Cu,Fe,Zn	Domestic oven (700 W)	HNO_3-H_2O_2 open PTFE digestion for 14 min (n=100).	FI-AAS	Good results were obtained for Cu, Fe and Zn in NBS 'Bovine Liver' and for Zn in BCR 'Bovine Muscle' although results for Fe and Cu were just outside the certified range.	74
Biological samples	Pb	Domestic oven (700 W)	On-line HCl-HNO_3 digestion of samples (dispersed in Triton X-100 solution).	On-line analysis by FI-ETA-AAS	Good recoveries for BCR Bovine Muscle, Pig Kidney and NIST Bovine Liver. For NIST Pines Needles and BCR Olea Europea results were slightly low.	75
Fruit slurries and juices	Pb	Domestic oven (650 W)	On-line HNO_3 digestion of liquid and slurried samples (dispersed in Triton X-100).	FI-HG-AAS	No significant difference between results obtained by FI-MW-HGAAS and conventional Al heating block digestion (ETA-AAS analysis).	76
Food and feed crops	Pb	Domestic oven (600 W)	HNO_3 PTFE bomb digestion with V_2O_5 catalyst for 90 s.	ET-AAS	Good results obtained for NIST Citrus Leaves.	77
Fruit slurries	Cd,Cu,Fe, Pb,Se	Domestic oven (600 W)	HNO_3 PTFE bomb digestion with V_2O_5 catalyst for 90 s.	ET-AAS	Good agreement with results of a slurry procedure.	78
Biological samples	Hg	Domestic oven (700 W)	HNO_3 PTFE bomb digestion for 20 min after leaving samples overnight to partially digest (n=10)	CV-AFS, ICP-MS and ID-ICP-MS	Good agreement with certified values for BCR Cod Muscle after analysis by CV-AFS, ICP-MS (standard additions) and ID-ICP-MS (spike added prior to overnight digestion).	79
Botanical samples	Cu,Mn	Domestic oven (650 W)	On-line digestion of HNO_3-H_2O_2 slurries for 2 min.	On-line analysis by FAAS	Good agreement with certified value for Mn in NIST Tomato Leaves.	80
Botanical samples	Cr	Domestic oven (650 W)	*Aqua regia*-H_2O_2 PTFE bomb digestion.	ET-AAS with $NaVO_3$ modifier	Spike recoveries 98-103%. Good results obtained for Cr in NIST Tomato Leaves and Citrus Leaves.	81

Table 1.2: Summary of Published Microwave Digestion Procedures for Biological Samples Continued

Matrix	Elements	Microwave System	Digestion Method	Analysis Technique	Comments	Ref.
Biological samples	Cd	Domestic oven (650 W)	Closed vessel HNO_3 (14M) - H_2O_2 digestion for 12.5 min.	ET-AAS	Good results obtained for BCR Pig Kidney and for MA-M-2/TM Mussel Tissue (IAEA).	82
Botanical samples	Cu,Mn,Pb, Zn	Domestic oven (650 W)	Slurries (prepared in HNO_3) are merged on-line with H_2O_2 and digested at 100% power.	On-line FAAS analysis	Good agreement with certified value for Mn in NIST Tomato Leaves.	83
Botanical samples	B	Domestic oven	HNO_3-H_2O_2 PTFE bomb digestion for 1 h (n=2).	Photometry, fluorimetry, ICP-MS and ICP-AES	Good results obtained for NBS Tomato Leaves and Pine Needles although recoveries for Bovine Liver were low.	84
Botanical samples	Ca,Co,Cr, Cu,Fe,K, Mg,Mn,Ni, Pb,Zn	Domestic oven (550W)	HNO_3-H_2O_2 teflon bomb digestion with water cooled spiral for 6 min (n=1).	FAAS, FAES	Good results for Co and Cr in ISS/MMM certified Green Alga. Pb and Ni results were just outside the certified range. Acceptable results were obtained for the 'in-house' reference material 'Lucerne' except for Fe and Zn.	85
Octocorals	Cd,Cu,Ni Pb,Zn	Domestic oven (600 W)	Microwave pre-drying for 20-50 min. HNO_3 digestion in pyrex tube for 1 min (4-6 times) with cooling between heating steps.	ET-AAS and FAAS (for Zn)	Good recoveries were obtained for a synthetic CRM prepared from a mixture of 61% NIES Mussel and 39% $CaCO_3$. 8 octocoral species were also analysed.	86
Biological samples	Ca,Cu,Fe, Mg,Mn,Zn	Domestic oven (500 W)	HNO_3-$HClO_4$-HCl-HF PTFE bomb digestion (with polypropylene jacket) for 14 min (n=6).	'One-drop' FAAS	Good agreement with certified values for NIST Bovine Liver, NIES Pepperbush and Mussel samples. For NIES Tea Leaves Fe results were high and Ca low (also for NIES Sargasso).	87
Botanical samples	Cd	Domestic oven	HNO_3-$HClO_4$-HCl-HF PTFE bomb digestion (with polypropylene jacket) for 9 min (n=6), followed by hotplate evaporation to dryness and dissolution in $HClO_4$.	Fe was removed with HIPT in benzene and the Cd complex formed with APDC was extracted into chloroform for 'one-drop' FAAS analysis.	Good agreement with certified values for NIES Pepperbush and Rice Flour (low and medium); and for NIST Pine Needles, Orchard and Citrus Leaves.	88
Fish samples	Se	Domestic oven (600 W)	HNO_3-H_2SO_4-H_2O_2 PTFE bomb digestion.	Se(VI) is reduced to Se(IV).The complex $Se(O)SO_3^{2-}$ is formed and analysed by DPP.	Results were in good agreement with the certified value for Se in NIES No. 6 Mussel sample.	89

Table 1.2: Summary of Published Microwave Digestion Procedures for Biological Samples Continued

Matrix	Elements	Microwave System	Digestion Method	Analysis Technique	Comments	Ref.
Fish samples	Se	Domestic oven (600 W)	PTFE bomb digestion with: a) HNO_3-H_2O_2 b) HNO_3-H_2SO_4-H_2O_2 c) HNO_3-H_3PO_4-H_2O_2 d) HNO_3-$K_2S_2O_8$-H_2O_2	Se(VI) is reduced to Se(IV) for analysis by HG-AAS.	Good results were obtained for NIES Mussel following digestion procedure b) whereas procedures a), c) and d) gave low recoveries.	90
Fish samples	As	Domestic oven (700 W)	On-line potassium persulphate-NaOH oxidation following HPLC separation of As species in sample extracts.	On-line analysis by HG-AAS	As(V), MMA, DMA, arsenocholine and arsenobetaine levels were determined in a synthetic fish extract although no CRMs were analysed. Spike recoveries of 96-110% were obtained.	91
Seafood	As	Domestic oven (750 W)	Sample was heated with HCl-KI in a PTFE bomb for 8 min (n=1). The distilled $AsCl_3$ was collected in hydroxylamine hydrochloride.	HG-AAS	Good spike recoveries were obtained for inorganic arsenic in a mussel sample. Organoarsenic compounds were not however decomposed.	92
Botanical samples	Ca,K,Mg,P, S	Domestic oven (750 W)	HNO_3-$HClO_4$ open digestion for 15-30 min depending on the sample (n=25).	ICP-AES analysis	Good results were obtained for P in NBS Pine Needles and Citrus Leaves although results for Ca and K in the former were slightly outside the certified range.	93
Biological samples	As	Domestic oven (650 W)	HNO_3-H_2SO_4-H_2O_2 PTFE bomb digestion.	HG-AAS with a modified electrical heating system.	Good results for As in NIST Bovine Liver and spike recoveries of 91-108% were obtained.	94
Biological samples	Hg	Domestic oven (750 W)	PTFE bomb HNO_3-H_2O_2 digestion for 5 min following predigestion overnight.	CV-AAS	Good results were obtained for total Hg in NRCC DORM-1. Tuna fish samples were also analysed and spike recoveries of 91-93% were obtained.	95
Fish samples	As	Domestic oven (600 W)	HNO_3 PTFE bomb digestion with catalyst of V_2O_5 for 90 s.	HG-AAS	Good agreement with certified value for BCR Mussel Tissue and spike recoveries of 93-101% were obtained.	96
Fish samples	Hg	Domestic oven (600 W)	HNO_3 bomb digestion of samples and standards for 90 s.	CV-AAS	Good results for BCR Mussel Tissue and spike recoveries of 95-106% were obtained.	97
Botanical samples	As	Domestic oven (600 W)	HNO_3 bomb digestion with catalyst of V_2O_5 for 90 s.	HG-AAS	Good agreement with certified values for NBS Citrus Leaves.	98

Table 1.2: Summary of Published Microwave Digestion Procedures for Biological Samples Continued

Matrix	Elements	Microwave System	Digestion Method	Analysis Technique	Comments	Ref.
Total diet samples	Al,Ca,Cu, Fe,K,Mg Na,P,Zn	Domestic oven (750 W)	Digestion (quartz vessel) with: a) HNO_3; b) HNO_3-H_2O_2 c) HNO_3-H_2SO_4; d) HNO_3-HCl.	ICP-AES	Good results were obtained for NIST Total Diet except for: a) slightly low K and Zn; b) slightly low K; c) slightly high P and slightly low K; d) low Al, K and Zn.	99
Biological samples	As	Domestic oven	HNO_3 teflon bomb digestion for 90 s.	ET-AAS	Good results were obtained for NIST Oyster Tissue	100
Biological samples	Se	Domestic oven (650 W)	HNO_3 Teflon bomb digestion (3 min) followed by evaporation to dryness with $HClO_4$ (x 2) and redissolution in H_2O.	SW-CSV following reduction of Se(VI) to Se(IV)	Good agreement with certified values for NIST Bovine Liver for a sample weight of 5 mg.	101
Food samples	Al	Domestic oven	HNO_3 PTFE bomb digestion (32 min)	ICP-AES	Generally low results were obtained for total diet samples. Higher recoveries obtained by a HNO_3-HF-HNO_3-$HClO_4$ digestion in a drying oven.	102
Botanical samples	P	Not specified	HNO_3-H_2O_2-HCl digestion.	ICP-AES	Good agreement with the certified value in NIST Citrus Leaves.	103
Botanical samples	Cd,Cu,Pb, Zn	Domestic oven (850 W)	HNO_3-$HClO_4$ digestion in quartz crucible placed inside teflon bomb for 29 min (n=4) followed by hotplate evaporation to dryness.	DP-ASV	Good results for NIST Citrus Leaves, Lucerne P-alfalfa (Slovakia) and CL-1 Cabbage leaves (Poland) (except low Cu). Cu, Pb and Zn results for NIST Apple Leaves were low.	104
Biological samples	Cd,Cu,Pb, Zn	Domestic oven	HNO_3-$HClO_4$ digestion in quartz crucible placed inside Teflon bomb for 11 min (n=10).	DP-ASV	Generally good results obtained for CL-1 Cabbage Leaves (Poland), P-alfalfa Lucerne (Slovakia), BCR Rye Grass, SRM Apple Leaves, BCR and SRM 1577b Bovine Liver.	105
Biological samples	Po	Domestic oven (650 W)	HNO_3 PFA bomb digestion (1 h for plants and 2 h for animal tissue, n=4), evaporation to dryness and redissolution in HCl.	Alpha spectrometry after plating onto silver discs.	A good level of precision was achieved with no loss of Po during the digestion.	106

Table 1.2: Summary of Published Microwave Digestion Procedures for Biological Samples Continued

Matrix	Elements	Microwave System	Digestion Method	Analysis Technique	Comments	Ref.
Biological samples	Cd,Co,Cu, Ni, Pb	Domestic oven	HNO_3-HCl-$HClO_4$-HF PTFE bomb digestion.	ICP-AES	Good Cu and Pb, but low Cd, Co and Ni results were obtained for NIES Pepperbush. Results were also good for Cd, Cu and Pb in Chlorella. For NIES Mussel, Cd, Co and Cu results were good, however Ni and Pb were low. Generally low results were obtained for NIES Tea Leaves.	107
Botanical samples	Co	Domestic oven	HNO_3-HCl bomb digestion for 10 min followed by hotplate evaporation to dryness.	Fl spectro-photometric determination following complexation with PSAA	Good agreement with certified values were obtained for NIES Pepperbush.	108
Biological samples	Cd,Cu,Fe, Mn,Pb	-	Open HNO_3-H_2O_2-digestion following overnight predigestion.	GF-AAS	Generally good results for BCR Bovine Mussel, Olea Europea (except high Cu), Lagarosiphoumajur (except low Cu) and Pig Kidney (except low Cu, Fe, Zn) and for NBS Citrus Leaves (except high Cu), Pine (except low Pb) and Wheat Flour.	109
Biological samples	As	-	H_2SO_4-HNO_3-H_2O_2 closed digestion for 30 min.	ICP-AES	A selection of fish and shellfish samples were analysed however no CRMS were included.	110
Botanical samples	Al,Fe,Si	-	HNO_3-H_2O_2-HF digestion for 13 min.	ICP spectrometry	UCD 155 (Avacado); 176 (Citrus); 124 (Barley Hulls) and 190 (Rice Straw) and NIST 1547 (Peach) samples were analysed.	111
Duck Eggs	Cu,Cd,Hg, Pb	-	H_2SO_4-HNO_3 digestion.	FAAS and CV-AAS (for Hg)	-	112
Food samples	Amino acids	-	Hydrolysis performed by heating with 6 M HCl in a closed vessel.	-	Amino acid sequence from microwave digestion and conventional method were compared in Bovine Serum Albumen and Durum Wheat samples.	113
Food samples	Bromide ions	-	Removal of Na ions by cation exchange chromatography following closed PTFE digestion.	Ion exchange chromatography with UV detection.	Recoveries of 87-119% obtained at concentrations of 25 and 50 ppm.	114
Botanical samples	Phenolic acids	-	Teflon bomb NaOH digestion at 700 W for 90 s.	HPLC	The liberation of β-ether bound phenolic acids from plant cell walls of maize, wheat, oilseed rape stems and barley is an order of magnitude more effective than with a dioxane-HCl procedure and as effective, but far quicker than with high-temperature alkaline digestions.	115

Table 1.2: Summary of Published Microwave Digestion Procedures for Biological Samples Continued

Matrix	Elements	Microwave System	Digestion Method	Analysis Technique	Comments	Ref.
Biological samples	Ag,Ba,Cd, Cs,Hg,Mo, Pb,Rb,Sb, Sn,Sr	-	HNO_3 PTFE bomb digestion for 40 min.	ICP-MS	Good results obtained for Ag, Mo, Pb and Rb in NIST Bovine Liver (Sr slightly high); for Rb in NIST Wheat Flour (Mo slightly high) and for Cd and Hg in BCR Pig Kidney.	116
Biological samples	Al	-	HNO_3 PTFE bomb digestion for 4 min (x 2), 8 min, 10 min (x 4) with cooling between each step.	ET-AAS	Results were in good agreement with the certified values for Al in NIES Mussel; NIST Citrus Leaves and Oyster Tissue. Spike recoveries of 92-104% were also obtained.	117

Table 1.3: Summary of Published Microwave Digestion Procedures for Geological Samples

Matrix	Elements	Microwave System	Digestion Method	Analysis Technique	Comments	Ref.
Sediment samples	Hg	Prolabo Microdigest 301	On-line digestion of 0.15% slurries (prepared in 50% HCl) with Br^--BrO_3^- oxidation of organomercury species.	On-line analysis by CV-AAS	A recovery of 97% was obtained for a methylmercury standard. Good results obtained for Hg in NRCC PACS-1.	33
Sediment samples	Hg	Prolabo Microdigest 301	Open focused HNO_3-H_2SO_4-H_2O_2 digestion for 10 min (n=1).	CV-AAS	Results were in agreement with the certified value for NRCC PACS-1.	33
Soil and sediment samples	As,Cd,Co,Cr Cu,Hg,Mo, Ni,Pb,Zn	Prolabo Microdigest A300 and CEM MDS-81D	a) Closed vessel HCl-HNO_3-HF digestion and open focused digestion with: b) HNO_3-H_2O_2; c) HCl-HNO_3-H_2O_2.	ICP-AES and ICP-MS	Good results obtained for procedure b) for BCR Amended Soil and for c) and a) for Estuarine Sediment (except high Hg and Pb).	35
Soil samples	Hg	Prolabo Maxidigest MX350 and CEM MDS-81D	Open and closed digestion with: a) 1M HCl b) 50% HNO_3 c) HNO_3 d) *Aqua-regia*	CV-AAS and ET-AAS	For the open digestion results were low for NIST Montana Soil whereas good results were obtained for the closed digestion (procedures a, b and d).	118
Sediment samples	Hg	Prolabo Maxidigest (300 W)	On-line digestion of slurries prepared in aqua regia-$KMnO_4$.	On-line analysis by FI mercury system.	Good results were obtained for NIST Buffalo Sediment although those for NRCC BCSS-1 were slightly high. Good spike recoveries were also obtained.	119
Soil, sediment sewage sludge	Hg	Prolabo Microdigest 301	On-line digestion of slurries prepared in nitric acid.	On-line FI-CV-AFS analysis.	Good results were obtained for BCR Sewage Sludge (Domestic); State Bureau of Metrology (China) Polluted Farm Soil and Canadian Centre for Mineral and Energy Technology Lake Sediment. Results for BCR Sewage Sludge Amended Soil were slightly low.	120
Soil samples	Cu,Fe,Zn	Prolabo Microdigest 301	Automated DTPA-$CaCl_2$-triethanolamine extraction using a robotic station (5 samples h^{-1}).	Automated centrifugation and transport to FAAS.	The extraction efficiency of Zn is comparable to the conventional technique whereas a greater efficiency resulted for Fe and Cu.	121
Coal samples	Ca,Cd,Fe, Mg,Zn	CEM MDS-81	On-line stopped-flow digestion of slurries (in Triton X-100 and HNO_3) for 5 min per sample.	Off-line analysis by AAS	Incomplete digestion of coal resulted.	42

Table 1.3: Summary of Published Microwave Digestion Procedures for Geological Samples Continued

Matrix	Elements	Microwave System	Digestion Method	Analysis Technique	Comments	Ref.
Soil samples	As,Se	CEM MDS-2000	H_2O-HNO_3-HCl-HF PTFE bomb digestion for 30 min.	HG-AAS	Good results for MRG-1 silicated rocks (Canada Centre for Mineral and Energy Technology). Spike recoveries of 95% were obtained.	44
Sediment samples	Hg	CEM MDS-2000	HNO_3 PTFE bomb digestion for 70 s.	CV-AAS	Good agreement with the provisional value for NIES No. 2 Pond Sediment.	104
Sediment samples	As,Cd,Co,Cr Cu,Mn,Ni, Pb,Zn	CEM SpectroPrep system	On-line digestion of 1% slurries (in 20% HF, 50% HNO_3 and 10% HCl).	ICP-MS, ICP-AES (for Fe and Al) and ET-AAS (for As).	Good results were obtained for As, Cd, Co, Cu, Fe, Mn, Ni, Pb and Zn in NRCC BCSS-1, however results for Cr and Al were low.	55
Phosphatic fertilisers and animal feed-stuffs	As,B,Ba,Bi, Cd,Co,Cr,Cu Hg,Mn,Mo, Ni,Pb,Se,Sb, V,W,Zn	CEM MDS-81D	PTFE bomb digestion with: a) HNO_3 b) HNO_3-$HClO_4$-HF.	ICP-MS	For NIST Buffalo River Sediment: a) Good As, Cd, Co, Cu, Ni and Pb results but low Ba, Cr, Mn, Sb, V, Zn and high Hg. b) Good As, Co, Cu and Ni results. Ba, Hg and V results were improved but Ni, Zn and Sb were high and Cr low. Results for NBS Florida Phosphate Rock by procedure a) generally agreed well with the certified and informational values.	122
Sediment samples	Co,Cu,Mn, Pb,Zn	CEM MDS-81D	18 digestion procedures with different combinations of HNO_3, H_2O_2, HF and HCl.	FAAS and Lvov platform (for Co)	PCA and multicriteria decision making methods PROMETHEE and GAIA selected a HF-HNO_3-HCl digestion as the best for NBS Buffalo River Sediment.	123
Sediment and rock samples	Co,Cr,Cu,Ni Pb,Zn	CEM MDS-81D	14 digestion procedures with different combinations of HF, HNO_3, HCl, H_2O_2 and acetic acid.	FAAS	PCA, SIMCA, PROMOTHEE, GAIA and Fuzzy Clustering chemometric techniques selected an HNO_3-HF digestion as the best procedure for NBS Buffalo River Sediment and 'In House' secondary rock standard.	124
Sediment samples	Cd	CEM MDS-81D	HNO_3 Teflon bomb digestion for 80 min (n=12).	GF-AAS	Good results were obtained for NIST Sediments 1646 and 2704. Results were in good agreement with a conventional HF-$HClO_4$ digestion undertaken in platinum crucibles.	125
Sediment samples	Cr,Cu,Hg, Mn,Ni,Pb, Zn	CEM MDS-81D	PTFE bomb digestion (n=12) with: a) *Aqua regia* (80 min). b) *Aqua regia*-HF (80 min).	AAS and CV-AAS (for Hg)	For procedure a) results were generally low for NRCC MESS-1 and PACS-1. Results were slightly improved after digestion by procedure b) however most results are not in agreement with the certified values.	126

Table 1.3: Summary of Published Microwave Digestion Procedures for Geological Samples Continued

Matrix	Elements	Microwave System	Digestion Method	Analysis Technique	Comments	Ref.
Dust, ashes and sediments	Al,As,Ba,Be Ca,Co,Cr,Cu Fe,Mg,Mn, Ni,Pb,S,Sb, Ti,V,Zn	CEM MDS-81D	Teflon PFA bomb digestion for 22 min (n=6) with: a) HNO_3-HCl (acid soluble elements) b) HNO_3-HCl-HF with H_3BO_3 neutralisation (total digestion).	ICP-AES	a) Recoveries obtained for different elements in the range: NRCC MESS-1: 25-103%; NRCC PACS-1: 38-99% NIST Coal Fly Ash: 60-103%; 'In-house' Dust: 23-100% b) Good results for Coal Fly ash (except low Co) and for MESS-1 (except low Ti). For PACS-1 good results except for Al, Fe, Ca, Mg, Ni and S (just outside certified range).	127
Sludge samples	As,Se	CEM MDS-81D	PFA bomb digestion for 1-2 h with: a) HNO_3 -HCl; b) H_2O_2-HCl-H_2SO_4 c) H_2O_2-H_2SO_4; d) HNO_3-H_2SO_4.	FI-HG-AAS	Method d) gave the best recoveries (validated using NIST San Joaquin Soil). Good results obtained for As, but Se recoveries slightly high. For NIST Domestic Sewage Sludge results agreed well with those of a conventional reflux method but the method was faster and $HClO_4$ was not required.	128
Sediment samples	As,Hg,Se	CEM MDS-81D	PFA bomb digestion with: a) H_2SO_4-HNO_3-HCl (As and Se) b) HNO_3 (Hg)	FI-AAS (As and Se) and CV-AAS (Hg)	Good results for proposed NIST SRM Estuarine Sediment (1646a) and Buffalo River Sediment (2704). Results were in good agreement with those of a traditional reflux digestion.	129
Molyb-denite mineral	Os	CEM MDS-81D	HNO_3-H_2SO_4 PTFE bomb digestion for 45 min followed by heating with $K_2Cr_2O_7$.	Os distillation prior to ICP-MS analysis	Technique applied to Re-Os age determination in a natural molybdenite sample. Results in agreement with those obtained by a U-Pb method for zircon (associated mineral).	130
Geological samples	Ba,Be,Co,Cr Cs,Cu,Hf, Mo,Ni,Nb, Pb,Rb,Sb,Sc Sn,Sr,Ta,Th, Tl,U,W,Zn, Zr and REEs	CEM MDS-81D	PFA bomb HNO_3-HF-$HClO_4$ digestion for 63 min (n=4) followed by hotplate evaporation to dryness with $HClO_4$ and dissolution in HNO_3.	ICP-AES and ICP-MS	Generally acceptable results obtained for Ba, Be, Co, Cs, Cu, Ni, Nb, Pb, Rb, Sb, Sn, Sr, Ta, Th, Tl, U, W, Zn and for most of the REEs in a range of geological CRMs. The accuracy of Cr, Hf, Mo, Sc, Zr determinations varied with sample type whereas Y recoveries were low in the 9 CRMs analysed.	131
Geological samples	Au,Ir,Pd,Rh, Ru,Pt	CEM MDS-81D	a) Low pressure HNO_3-HCl-HF-$HClO_4$ digestion. b) High pressure *aqua-regia*-HF digestion. Residues were fused with Na_2CO_3/Na_2O_2.	ICP-MS	Procedure a) was employed for the digestion of sulphide-rich samples and procedure b) for silicate, sulphide and chromitite samples. A number of CRMs were also analysed.	132

Table 1.3: Summary of Published Microwave Digestion Procedures for Geological Samples Continued

Matrix	Elements	Microwave System	Digestion Method	Analysis Technique	Comments	Ref.
Airbourne particulates on teflon filters	Al,As,Ba,Cd Cr,Cu,Fe,K, Mg,Mn,Na, Ni, Pb,Sb,V, Zn	CEM MDS-2000	HNO_3-$HClO_4$-HF digestion (32 min) followed by open vessel evaporation of HF (25 min) (n=12).	ICP-MS	Generally low results were obtained for NIST Urban Particulate Matter [accredited in part to the low mass of sample used (0.1 mg)]. Higher recoveries were obtained with a conventional pressure bomb digestion although the digestion time was 10 times higher.	133, 134
Airbourne particulates on glass fibre filters	Al,Fe,K,Mg, S,Zn	CEM MDS 2000	HNO_3-$HClO_4$ PTFE bomb digestion for 9 min followed by cooling, removal of filter residue, heating with HF (9 min) and H_3BO_3 neutralisation of HF.	ICP-AES	Recoveries of between 90 and 101% were obtained for Al, Fe, K, K, Mg, S and Zn in NIST Urban Particulate Matter.	135
Sediment samples	Cd,Cr,Co,Cu Fe,Mn,Ni,Pb Se,Zn	CEM MDS-2000	PTFE bomb HF-HCl-HNO_3 digestion for 15 min (n=12).	FAAS and GF-AAS	Orthogonal array design was applied to the optimisation of digestion parameters. Generally good results obtained for NBS Buffalo River Sediment; NRCC BCSS-1 and NIES Pond Sediment.	136
Coral soil samples	Si	Floyd RMS-150	HNO_3-H_2O_2-HF teflon bomb digestion for 25 min.	ICP-AES analysis following addition of H_3BO_3 and a tertiary amine mixture.	Acceptable agreement with a $LiBO_2$ fusion procedure was obtained for the 5 coral soil samples investigated. Average precision of the method was determined as 7%.	30
Soil and dust	As	Floyd RMS-150	HNO_3-$H2O_2$ bomb digestion for 32 min (n=6).	ICP-MS	Good results were obtained for NIST Urban Particulate Matter and IAEA Soil 7.	65
Sediment samples	Cd,Cr,Cu,Pb Ni,Sb,Sn,Th	Floyd	Samples were digested with HNO_3-HF for 52 min, evaporated to dryness on a hotplate and redissolved in HNO_3/H_2O.	ID-ICP-MS, ICP-MS and GF-AAS	Good results were obtained for Cd, Cr, Cu, Pb, Ni, Sb, Sn and Th in a Mississippi River delta sediment sample (NOAA/7).	66
Dust samples	Cd,Pb	Floyd RMS-150	Teflon bomb digestion for 20 min with: a) HNO_3; b) HCl-HNO_3 c) HNO_3-HF.	DPASV and FAAS	Digestion efficiencies of 85-95% were obtained (RSDs= 10%) for NBS Urban Particulate Matter, BCR City Waste Incineration Ash and River Sediment. No significant differences were found between the 3 microwave methods and a standard hotplate digestion method.	137

Table 1.3: Summary of Published Microwave Digestion Procedures for Geological Samples Continued

Matrix	Elements	Microwave System	Digestion Method	Analysis Technique	Comments	Ref.
Geological samples	Al,Fe,K,Mg, Na,Si	Milestone MLS-1200	PTFE bomb digestion. Coal: HNO_3-H_2O_2-HF-$HClO_4$ Limestone: HCl-HF; Iron Ores: HCl.	ICP-AES and AAS	Good agreement with XRF results for limestone samples. Fe levels in BCS NIMBA Fe Ore, Fe Ore Sinter and Lincolnshire Fe Ore were within the certified range.	62
Sediment samples	50 elements	Milestone MLS-1200	Teflon HNO_3-HF bomb digestion for 19 min followed by evaporation to dryness (90 min) and dissolution in HCl.	ICP-MS, TXRF	Good results obtained for Al, Ca, Fe, K, Mg, Na, Pb, Rb, Sr, Ti and V in NRCC MESS-1, however Ba results were high and Zn low.	138
Rock and sediment samples	Th,U,Y and lanthanides	Milestone MLS-1200 MEGA	Teflon bomb HF-HNO_3-HCl digestion for 16 min followed by heating with H_3BO_3 and EDTA for 8 min.	ICP-MS	Results presented for USGS andesite (AGV-1), basalt (BCR-1, BHVO-1), diabase (W-2, DNC-1), granite (G-2), marine mud (MAG-1); for CCRMP syenite (SY-2), gabbro (MRG-1), lake sediments (LKSD-1,4), stream sediments (STSD-1,4) and for NIM-G (granite), BE-N (basalt), GSD-1,5,6 (stream sediment) and NBS 1645 (river sediment).	139
Airbourne particulates on PTFE filters	Al,As,Cd,Cr Cu,Fe,K,Mg Ni,Pb,S,Sb, V,Zn	Milestone MLS-1200	HNO_3-$HClO_4$-HF PTFE bomb digestion for 8 min.	FAAS, GF-AAS, ICP-AES and ICP-MS	Good results were obtained for As, Cd, Cu, Fe, Mg, Ni, S, Sb and Zn in NIST Urban Particulate Matter, however K, Pb and V results were slightly low and Al and Cr very low.	140
Coal samples	REEs	Milestone MLS-1200	PTFE bomb digestion with HNO_3-H_2O_2-HF-HCl.	HPIC with UV/Vis detection and on-line preconcentration	Acceptable agreement obtained with published values for NBS 1632a, SARM-18, 19 and 20 coal CRMs (not certified).	141
Baghouse Dust	Hg	Questron Q-Wave 1000	HNO_3 teflon bomb digestion.	CV-AAS	No statistical difference in data obtained for the traditional water bath and the microwave digestion technique.	142
Marine sediments	Pb	Portland DMR-140	HNO_3-HCl PTFE bomb digestion for 10 min.	ET-AAS	Good results for NRCC PACS-1 and spike recoveries of 95-99% obtained. Results compared to a slurry method.	143
Sediments, geological samples	Hg	Domestic oven (800 W)	HNO_3 PTFE bomb digestion for 3 min at 800 W (n=5).	FANES analysis after reduction with $SnCl_2$ and in situ preconcentration.	Results for NIST River Sediment were slightly low.	73

Table 1.3: Summary of Published Microwave Digestion Procedures for Geological Samples Continued

Matrix	Elements	Microwave System	Digestion Method	Analysis Technique	Comments	Ref.
Sewage sludge	Cu,Mn	Domestic oven (650 W)	On-line digestion of slurries prepared in HNO_3-H_2O_2 (4 min).	On-line analysis by FAAS	Generally good agreement with certified values for CBR Sewage Sludge-Domestic and Industrial samples.	80
Sediments, sewage sludge	Cr	Domestic oven (650 W)	*Aqua regia*-HF-H_2O_2 PTFE bomb digestion followed by 10 min heating with H_3BO_3 in a water bath.	ET-AAS (no modifier)	Good agreement with certified value in BCR River Sediment and with informational value in Sewage Sludge. Spike recoveries of 101-102% were obtained.	81
Sewage sludge	Cd	Domestic oven (650 W)	Closed vessel *aqua regia*-HF-H_2O_2 digestion followed by H_3BO_3 treatment.	ET-AAS	Good results obtained for BCR Sewage Sludge Domestic.	82
Sewage sludge	Cu,Mn,Pb, Zn	Domestic oven (650 W)	Slurries prepared in HNO_3 were merged on-line with H_2O_2 and digested at 100% power.	On-line FAAS analysis	Good results were obtained for Cu, Mn and Pb in CBR Sewage Sludge-Industrial and for Pb in Sewage Sludge-Domestic. Poor results for Cu, Mn in Sewage Sludge-Domestic and for Zn in both sewage CRMs.	83
Sediment samples	As	Domestic oven (700 W)	On-line potassium persulphate-NaOH oxidation following HPLC separation of As species (prior digestion of samples is necessary).	On-line analysis by HG-AAS	As(V), MMA and DMA species were determined in a sediment extract although no CRMs were analysed.	91
Sediment samples	Hg	Domestic oven (750 W)	PTFE bomb HNO_3-H_2SO_4-H_2O_2 digestion for 4 min following predigestion overnight.	CV-AAS	Good results were obtained for total Hg in MESS-2. Spike recoveries of 91-108% were obtained in River Mersey sediment samples.	95
Sewage sludge	Cd,Cr,Cu,Ni Pb,Zn	Domestic oven (662 W)	On-line digestion of 0.2-0.75% (w/v) slurry prepared in 1.5 M HNO_3.	On-line ICP-AES analysis	Generally good results obtained for BCR Sewage Sludge Industrial.	144
Dust wipe and air filters	Pb	Domestic oven (800 W)	Teflon PFA HNO_3 digestion for 6 min (n=12).	GF-AAS	Recoveries of 96-114% were obtained for the NIOSH and ELPAT wipe samples. For air filter samples spike recoveries of 94-103% were obtained.	145

Table 1.3: Summary of Published Microwave Digestion Procedures for Geological Samples Continued

Matrix	Elements	Microwave System	Digestion Method	Analysis Technique	Comments	Ref.
Sewage sludge	Cd,Cr,Pb	Domestic oven	HCl-HNO_3 Teflon bomb digestion (20 min) following predigestion overnight.	ET-AAS	Good results were obtained for Pb and Cr in BCR Sewage Sludge (CRM 145R) but Cd results were slightly low.	146
Soil and sediment samples	Cd,Cu,Pb	-	Open HNO_3-H_2O_2-*aqua regia*-HF digestion following overnight predigestion.	GF-AAS	Generally low results for BCR Calcareous Loam Soil and River Sediment and for NBS Urban Particulate Matter.	109
Silicate rocks	Fe oxidation states	-	HF-H_2SO_4 PTFE bomb digestion under Ar atmosphere.	Absorbance at 560 nm of Fe(III)-Tiron complex followed spectrophotometrically with time	FeO and Fe_2O_3 results compared with those from the static o-phenanthroline method.	147

Table 1.4: Summary of Published Microwave Digestion Procedures for Water Samples

Matrix	Elements	Microwave System	Digestion Method	Analysis Technique	Comments	Ref.
Water samples	Se(IV) and Se(VI)	Prolabo Microdigest 301	On-line microwave assisted HCl pre-reduction of Se(VI) to Se(IV).	On-line analysis by FI-CSV	Se(VI) is calculated as the difference between the Se(IV) concentration after pre-reduction and the initial Se(IV) concentration. Results were low for Se(IV) and therefore high for Se(VI) in a BCR candidate water sample. The total Se concentration is however in good agreement with the proposed value.	23
Water and waste water	Hg	Prolabo Maxidigest	On-line digestion of samples prepared in H_2SO_4-HNO_3-$KMnO_4$-$K_2S_2O_8$ with HCl carrier.	FI-mercury system	Generally good spike recoveries were obtained for inorganic and methylmercury in drinking water and waste water samples.	119
Water samples	Se	Prolabo Microdigest 301	On-line HCl (6M) reduction of Se(VI) to Se(IV).	On-line analysis by HG-AAS	Se(VI) concentration calculated as the difference between total Se and Se(IV) content. Good results obtained for NIST 1643c Trace Elements in Water.	148
Water samples	Se	Prolabo Microdigest 301	On-line HCl reduction of Se(VI) to Se(IV) following HPLC separation of Se(VI) and Se(IV).	On-line analysis by HG-AFS	Good agreement with the total Se content of NIST 1643c Trace Elements in Water was obtained.	149
Water samples	As,Bi,Hg, Pb,Sn	Prolabo Maxidigest MX-350	On-line digestion of samples mixed with a suitable oxidising agent (depending on element).	FI-CV-AAS and HG-AAS	Good Bi and Hg results were obtained although problems were experienced for As, Pb and Sn determinations.	150, 151
Water samples	Hg	Prolabo Maxidigest MX-350	On-line $KBrO_3$-KBr digestion (7 samples h^{-1}).	FI-CV-AAS in amalgamation mode	Good recoveries were obtained for the 7 Hg species investigated. Acceptable agreement with results from an external laboratory for 22 water samples.	152
Water samples	Total N and P	CEM MDS-81D	Teflon bomb potassium persulphate and NaOH digestion for 45 min (n=12).	Colorimetric determination	Good recoveries for all P and N compounds tested except for animoantipyrine (60-73%). Spike recoveries of 98.4-105.9% were obtained in real samples.	153
Waste water samples	Total P	CEM MDS-81D	On-line HNO_3 digestion with prior addition of pyrophosphatase (25 samples h^{-1}).	Colorimetric detection of molybdenum blue complex	Good agreement with batch 'block' digestion (3 h). Complete recoveries of tetrameta, trimeta, ortho and pyrophosphate as orthophosphate were obtained.	154
Water samples	COD	Milestone MLS 1200	On-line $K_2Cr_2O_7$-H_2SO_4 oxidation (3 min).	FI spectrophotometric detection	Good agreement with the standard COD method for a range of water samples, food industry waste and a sewage sample.	155

Table 1.4: Summary of Published Microwave Digestion Procedures for Water Samples Continued

Matrix	Elements	Microwave System	Digestion Method	Analysis Technique	Comments	Ref.
Water samples	As	Domestic oven (700 W)	On-line potassium persulphate-NaOH oxidation following HPLC separation of As species.	On-line analysis by HG-AAS	As(III), As(V) and arsenobetaine were determined in mineral, sewage and harbour sea water samples although no CRMs were analysed.	91
Water, waste water and sewage effluents	Total P	Domestic oven (700 W)	On-line potassium peroxydisulphate digestion.	FI colorimetric detection of phospho-molybdenum blue complex	Complete digestion of all P compounds tested except condensed phosphates. No significant difference between results for on-line and batch methods although the former has a small positive bias.	156

Key To Abbreviations Used

Analysis Techniques

AFS	Atomic fluorescence spectrometry
CIE	Capillary ion electrophoresis
CV-AAS	Cold vapour-atomic absorption spectrometry
DCP-AES	Direct current plasma-atomic emission spectrometry
DP-ASV	Differential pulse anodic stripping voltametry
DPP	Differential pulse polarography
ET-AAS	Electrothermal atomisation atomic absorption spectrometry
FAAS	Flame atomic absorption spectrometry
FAES	Flame atomic emission spectrometry
FANES	Furnace atomic non-thermal excitation spectrometry
FI	Flow injection
GF-AAS	Graphite furnace-atomic absorption spectrometry
HG-AAS	Hydride generation-atomic absorption spectrometry
HPIC	High performance ion chromatography
HPLC	High performance liquid chromatography
IC	Ion chromatography
ICP-AES	Inductively coupled plasma-atomic emission spectrometry
ICP-MS	Inductively coupled plasma-mass spectrometry
ID	Isotope dilution
IR	Infrared spectrometry
NAA	Neutron activation analysis
SW-CSV	Square wave cathodic stripping voltametry
TLC	Thin layer chromatography
TXRF	Total reflection X-ray fluorescence spectrometry
XRF	X-ray fluorescence spectrometry

Reference Material Suppliers

BCR	Community Bureau of Reference
CCRMP	Canadian Certified Reference Materials Project
ELPAT	Environmental Lead Proficiency Analytical Testing Program
IAEA	International Atomic Energy Agency
ISS/MMM	Istituto Superiore di Sanita Roma
NBS	National Bureau of Standards
NIES	National Institute of Environmental Studies
NIOSH	The National Institute for Occupational Safety and Health
NIST	National Institute of Standards and Technology
NRCC	National Research Council of Canada
USGS	United States Geological Survey

Compounds

APDC	Ammonium pyrrolidin-1-yldithioformate
BPTH	1,5-bis[phenyl-(2-pyridyl)methylene]thiocarbonohydrazide
DPTH	1,5-bis(di-2-pyridylmethylene)thiocarbonohydrazine
DTPA	Diethylenetriamine pentaaceticacid
EDTA	Ethylenediaminetetraaceticacid
HIPT	2-hydroxy-4-isopropylcycloheptatrienone
IBMK	Isobutyl methyl ketone
PSAA	2-(5-bromo-2-pyridylazo)-5-(N-propyl-N-sulphopropylamino)aniline

Others

COD	Chemical oxygen demand
CRM	Certified reference material
MW	Microwave
REEs	Rare earth elements
RM	Reference material

the effectiveness of the procedure. In many cases certified reference materials have been used for validation of the procedure. Results are often classed as 'good' when in fact they lie outside of the uncertainty limits of the certified value (usually defined as twice the standard deviation of the mean of the certified value). For clarification, in this review results described as 'good' indicate that they lie within the uncertainty limits of the certified value. Other results are classified as 'low' or 'high' accordingly.

1.4.1 Open and Closed Digestion Systems-Advantages/Disadvantages

During the review period most studies have concentrated on the development of closed digestion methods. Most commonly these are carried out in multimode (domestic or commercial microwave ovens), although one company has developed a commercial closed focused microwave digestion system[37]. Multimode ovens have also been used for open digestions however the majority of open vessel applications utilise focused microwaves. Each method has it's own advantages and disadvantages and so it is not possible to suggest either as being the most suitable for all applications.

1.4.1.1 Closed Microwave Digestion Techniques

The closed digestion technique involves placing the sample in a vial (or bomb), usually constructed of PTFE as discussed in Section 1.3.1. After the addition of the digestion reagents the bomb is tightly sealed before placing in the microwave oven for irradiation by microwave energy. Initial closed vessel research was undertaken in domestic multimode microwave ovens. Digestion vessels were placed inside evacuated dessicators or large plastic jars to contain the evolved acid vapours and improve safety in the event of an explosion. In order to prevent damage to the magnetron from reflected microwaves unabsorbed by the usually small samples, an additional load of water was commonly placed in the microwave

oven. However as these auxiliary loads reduced the amount of microwave energy reaching the sample a constant and reproducible supply could not be guaranteed and thus results were often irreproducible. Further disadvantages of the use of domestic microwave ovens is that the power output of the magnetron is static, the power output of the oven being controlled by cycling the magnetron off and on to obtain an average power level. Domestic ovens typically have a high time base, generally between 10 and 30 s. Thus to obtain 50% power the magnetron will only be on for half of the time base. For example with a base of 20 s the magnetron is operated at maximum power for 10 s, followed by zero power for a further 10 s, rather than emitting a continuous output of 50% power for the full heating period. This approach is highly undesirable for analytical work as significant heat losses can occur during the periods of zero power output. As a result of the unsuitability of domestic ovens for use in analytical chemistry a number of commercial systems have been specially developed to overcome the problems of acid fume damage, sample power reflection, field inhomogeneity and long time bases[38,59,64,68,142,143].

The major advantage of the closed microwave digestion technique is the high heating efficiency due to the elevated temperatures and pressures which can be attained as a consequence of the evaporation of digestion acids and the gases evolved during the decomposition of the sample matrix. However the excessive build up of pressure, especially during the digestion of samples with a high organic content, can lead to the rupture of sealed vessels. For this reason most digestion bombs are fitted with pressure relief valves, designed to open when the pressure becomes too great, and thus maintain safety. If venting does occur sample losses are likely and due to the reduction in acid vapours a less active digestion may result. Considerable research has therefore been undertaken to find ways of controlling or reducing pressure build up during the digestion process[39,41,48,50,79,85,95,102,109,146,157].

One method of achieving this aim is to predigest the sample, and thus enable the gases evolved from the decomposition of easily oxidised organic matter to escape before commencing the closed digestion procedure. This has been carried out by a number of workers by leaving the samples to predigest overnight[39,50,79,95,109,146]. However if high sample throughput is required this extra step must be taken into account since a large number of digestion vessels will obviously be required. Predigestion can also be carried out by microwave heating in an unsealed vessel prior to the capping and digestion of the sample in the usual manner. However it is important that this step should not be too lengthy since it may counteract the benefit of rapid digestion using microwave systems. Also excessive evaporation of digestion reagents and volatile elements must be avoided. Reid *et al.*[50] overcame these problems by employing an open vessel heptane-cooled reflux predigestion step during which oxidation products could escape whilst retaining the analytes and digestion reagents for the ensuing closed digestion.

Heltai *et al.*[85] investigated the idea of controlling the vapour pressure by means of a water cooled spiral inserted into the closed space of the digestion bomb. During the digestion the acid vapours evolved are condensed on the spiral producing a reflux action which continuously renews the liquid phase over the sample for effective digestion. A different approach involves leaving the closed digestion to continue spontaneously after initial heating to induce the reaction. Using this method temperatures greater than 150°C and pressures in excess of 150 psi were achieved, sufficient for example to digest NIST Bovine Liver[102].

Another technique reported in order to control pressure build up is to monitor the pressure or temperature throughout the course of the reaction and subsequently only apply microwave power when the readings are below a preset level. In this way the pressure can be controlled and so venting will not occur unless a very rapid reaction evolving copious amounts of gas takes place. Clearly monitoring can not be achieved using a thermocouple, due to it's incompatibility with microwave radiation. One commercial company has overcome this problem by placing a pressure transducer inside one of the bombs to continually monitor the pressure[41]. Other workers[48] have taken temperature measurements using a non-invasive infra red probe attached to the bottom of the microwave oven. In this case the output from the probe is fed to a computer which switches the magnetron on and off to achieve a preset time-temperature programme.

Reid *et al.*[157] described a method for the rapid cooling of Teflon pressure vessels using liquid nitrogen. Cooling in the microwave unit itself, although considerably decreasing reaction rates, was found to be useful in some cases to prevent uncontrollable increases in pressure. However a more effective method involved cooling subsequent to or in between heating cycles. This approach saved considerable time and additionally prevented pressure build up occurring after the microwave process had ceased which could otherwise lead to venting of the vessel.

1.4.1.2 Open Digestion Techniques

Open digestion systems operate at atmospheric pressure and thus do not suffer from the problems associated with pressure build up. However they do require an effective fume removal system. Most of the open vessel work has been carried out using commercial focused microwave systems[23-37,118-121,148-152] in which heating is more efficient than with

conventional microwave designs because the microwave beam is directly focused on the sample (see Section 1.3.2.2). The open vessel approach also allows the delivery of digestion reagents at any stage of the procedure. This may be beneficial for the effectiveness of the digestion, and is a distinct advantage over closed digestion methods where the further addition of reagents can not be achieved unless the vessels are cooled before opening. Also the system can quickly and effectively evaporate to dryness which can be a particular advantage during the digestion of geological samples for the removal of HF. Another advantage is that the power output of the magnetron can be controlled more readily (in comparison with domestic ovens). For example at 50% power the output of the magnetron is actually reduced to 50% rather than pulsing on and off to produce an overall mean of 50% power (see Section 1.4.1.1). Further details of this system are given in Section 3.1.2.1. Direct temperature measurements and temperature control to follow a previously defined programme are also possible[158]. A potential disadvantage however is that only one sample can be digested at a time, although this can be overcome by use of an autosampler unit with the ability to run up to 16 samples[159]. A multicavity focused system is also available for the digestion of up to four samples for the determination of Kjedahl nitrogen[160]. A more recent addition to the commercial market is a two or six cavity open microwave digestion unit with the ability to program the power output/desired temperature to each sample independently[161]. However the microwave beam is not strictly focused on the sample.

A less common approach is the use of multimode microwave ovens[74,93,109] for open digestions. Burguera *et al.*[74] demonstrated that the digestion of biological samples could be effectively achieved for 100 samples placed in polyethylene test tubes (covered in a Teflon sheet) in only 14 minutes.

1.4.2 On-line Microwave Digestion Techniques

There is a growing trend towards the development of on-line microwave digestion and analysis techniques, both for solid[33,42,43,55,71,72,75,76,80,83,119,120,144] and liquid[32,91,148-152,154-156] samples. Such techniques can lead to considerable time savings when compared to batch microwave digestions and thus the benefits over conventional techniques are even more impressive. However for solid samples it is usually necessary to prepare a slurry of the sample before analysis. This is necessary in order for the effective transferral of the sample into the digestion manifold. Most workers have reported the necessity of further grinding of the sample, sometimes with the addition of surfactants[42,76], in order to produce a stable slurry. Samples can then be digested in either a continuous or stopped flow system for on-line analysis[33,43,75,76,80,83,119,120,144] or collected for separate treatment[42,55,71,72].

On-line microwave digestion of slurries has been successful for the digestion of biological, soil, sediment and sewage sludge samples for elements such as Al, As, Cd, Cu, Co, Cr, Fe, Hg, Mg, Mn, Ni, Pb and Zn, although incomplete digestion has been reported for some samples such as coal[42]. It has also been noted that for some detection systems e.g. flame atomic absorption spectrometry (FAAS)[42,43] that the mass of sample required for trace analysis may be incompatible with the slurry approach. Other workers have reported blocking of the transfer lines for some samples therefore necessitating a pre-digestion before on-line treatment[55]. An alternative method to the slurry approach was reported by Legere *et al.*[162] who proposed encasing the sample with a digestible capsule for easy transferral into the digestion tube. Once in place the reagents could be added, the tube sealed and the digestion allowed to continue in a fully automated system. Torres *et al.*[121] developed a microwave-assisted robotic method for the extraction of Cu, Fe and Zn from soil samples. The system

was capable of the weighing, extraction, centrifugation and transport of the sample to the flame atomic absorption spectrometer.

In contrast to the problems associated with solids, water samples are more compatible with the on-line digestion process. Techniques suitable for the determination of the chemical oxidation demand (COD)[155], total P[154,156], As[91], Bi[150,151] and Hg[119,150-152] and the speciation of Se[148,149] have been developed. These procedures result in considerable time savings on the batch techniques, especially if the system can be combined with an autosampler. Open focused microwave digestion systems are particularly suited to on-line applications, having been successfully used by a number of workers for this purpose[32,33,119,148-150,152].

1.4.3 Chemometrics

Chemometrics[123,124,136] and factorial designs[67] have been used to select the best digestion technique for a particular purpose i.e. to chose the best combination of reagents, reagent volumes, digestion times and power settings. This is of particular value in a multielement situation when no single digestion procedure gives good results for all the elements required and a method is required to obtain the best overall performance. In addition Feinberg[60] has related digestion programmes to the nature of the sample matrix using an empirical modelling approach. A preliminary study has been performed using Kjedahl nitrogen determinations in food samples to define reference digestion procedures, and was found to be very effective for precisely defined samples, but for complex foods the model needed further development.

1.4.4 'Universal' Digestion Procedures

The following section discusses the use of microwave digestion systems for the digestion of biological, geological and water samples in order to identify any potential 'universal' digestion procedure for a particular matrix or element. Tables 1.5-1.7 summarise the different reagent combinations that have been used for the determination of different elements in biological, geological and water samples respectively.

1.4.4.1 Biological Samples

Many papers have been published reporting the use of microwave digestion procedures for biological samples[23-117] (Table 1.2). A wide range of samples have been investigated, the diversity of which is nearly matched by the number of different digestion methods used. A wealth of different combinations of acids and oxidising agents are commonly employed for the determination of different elements in biological samples (Table 1.5). Few trends seem to exist with good results being obtained for the same element in the same matrix after digestion with a range of different reagents. Conflicting evidence also exists as to the efficacy of the same reagent combination for the digestion of a particular matrix.

For the determination of aluminium in botanical samples there is disagreement as to whether digestion with HF is necessary. In support is the work undertaken by Lajunen *et al.*[45] who report low Al recoveries in NIST Citrus Leaves, Pine Needles and IAEA Mixed Diet after a closed nitric-hydrochloric acid digestion. Recoveries were improved by employing nitric and hydrofluoric acids in combination with hydrogen peroxide. This finding was reinforced by the low results obtained for total diet samples after a closed digestion with just nitric acid[102]. Again results were improved by using a HF, nitric and perchloric acid digestion (in a drying oven). Incomplete recoveries were also obtained for Al in BCR Spruce Needles following a

Table 1.5: Reagents Used for the Digestion of Different Biological Samples

Reagents Used	Elements Determined
Fish Samples	
HNO_3	Al,As,Ca,Cd,Co,Cr,Cu,Fe,Hg,Mg,Mn,Ni,Pb, Se,Sr,Zn
HNO_3 with V_2O_5 catalyst	As
HNO_3-H_2O_2	Ag,As,Cd,Cr,Cu,Hg,Ni,Pb,Zn
HNO_3-HCl	Ni
HNO_3-H_2SO_4	As,Cd,Co,Cr,Cu,Fe,Mn,Ni,Pb,Se,Sr,Zn
HNO_3-HF	Ag,Al,As,Cd,Co,Cr,Cu,Fe,Hg,Mn,Ni,Pb, Se,Sn,Th,Zn
HCl-HNO_3-H_2O_2	Cd,Fe,Zn
HNO_3-$HClO_4$	Co,Cu,Fe,Pb
HCl-HNO_3-HF	Ca,Cu,Fe
HNO_3-H_2SO_4-H_2O_2	As,Hg,Se
HNO_3-$HClO_4$-HCl-HF	Ca,Cd,Co,Cu,Fe,Mg,Mn,Zn
HNO_3-H_2SO_4-H_2O_2-NH_4EDTA	Ca,Cd,Cu,Fe,K,Mg,Mn,P,Sr,Zn
HNO_3-$HClO_4$-H_2SO_4	As
HNO_3-H_2O_2-HF	Si
HCl-Br^-/BrO_3^-	Hg
HNO_3-H_2SO_4-HNO_3-H_2O_2	Hg

Reagents Used	Elements Determined
Terrestrial Plants	
HNO_3	Al,Ca,Cd,Ce,Cu,Eu,Fe,Hg,K,Mg,Mn,Na,P,Pb, Po,Rb,Se,Sm,Tb,Th,U,Zn
HNO_3-H_2O_2	Al,As,B,Ca,Cd,Cr,Cu,Fe,K,Mg,Mn,Na,Ni,P,Pb, S,SeZn
HNO_3-HCl	Ca,Co,Cu,Fe,K,Mg,Mn,Na,Ni,Pb,Zn
HNO_3-$HClO_4$	Al,Ba,Ca,Cd,Cu,Fe,K,Mg,Mn,P,Pb,S,Zn
HNO_3-$HClO_4$-HCl-HF	Ca,Cd,Cu,Fe,Mg,Mn,Pb,Zn
HCl-HNO_3-HF	Ca,Cu,Fe
HNO_3-HF-H_2O_2	Al,Fe,Mg
Aqua regia-H_2O_2	Cr
HNO_3-H_2SO_4	Al,Ca,Cu,Fe,Mg,Na,Zn
H_2SO_4-H_2O_2	Kjedahl nitrogen
HNO_3-H_2O_2-HF	Al,Fe,Si
HNO_3-HF	Ba,Ca,Mg,Mn,Zn
HNO_3 with V_2O_5 catalyst	As
HNO_3-H_2O_2-HCl	P

Table 1.5: Reagents Used for the Digestion of Different Biological Samples Continued

Reagents Used	Elements Determined
Other Biological Tissue Samples	
HNO_3	Ag,Cd,Fe,Hg,Mg,Mo,Po,Pb,Rb,Zn
HNO_3-HCl	Ni,Pb
HNO_3-$HClO_4$	Cd,Cu,Pb,Se
HNO_3- H_2O_2	Bi,Ca,Cd,Co,Cs,Cu,Fe,Hg,K,Mg,Mn,Mo,Na, P,Pb,Rb,Ru,S,Sb,Se,Sn,Sr,Tl,Zn
HNO_3-H_2SO_4-H_2O_2	As
HNO_3-$HClO_4$-HCl-HF	Ca,Cu,Fe,Mg,Mn,Zn
H_2SO_4-KI	Sb
HNO_3-H_2SO_4-HNO_3-H_2O_2	Hg
HNO_3-H_2SO_4-H_2O_2-NH_4EDTA	Ca,Cd,Cu,Fe,K,Mg,Mn,P,Sr,Zn

Reagents Used	Elements Determined
Marine Plants	
HNO_3	Cd,Fe,Mg
HNO_3-H_2O_2	Co,Cr
HNO_3-$HClO_4$-HCl-HF	Cd,Cu,Pb

Table 1.6: Reagents Used for the Digestion of Different Geological Samples

Reagents Used	Elements Determined
Sediments	
HNO_3	As,Cd,Co,Cu,Hg,Ni,Pb
HCl	Cd,Pb
HNO_3-H_2O_2	Cd
HCl-HNO_3	Cd,Pb
HNO_3-HF	Al,Ca,Co,Cr,Cu,Fe,K,Mg,Na,Ni, Pb,Rb,Sr,Ti,V,Zn
HNO_3-$HClO_4$-HF	As,Ba,Co,Cu,Hg,Ni,Nb,Pb,Rb,Sb,Sn,Sr,Ta,Th,Tl,W, V,Zn,REEs
HNO_3-HCl-HF	Al,As,Ca,Cd,Co,Cr,Cu,Fe,Mg,Mn,Ni,Pb,S,Se,Ti,V, Zn
HNO_3-HF-HNO_3	Cd,Cr,Cu,Ni,Pb,Sb,Sn,Th
HF-HNO_3-HCl	As,Cd,Co,Cu,Fe,Mn,Ni,Pb,Th,U,Y,Zn and lanthanides
HCl-HNO_3-H_2O_2	As,Cd,Cr,Cu,Ni,Zn
HCl-Br^-/BrO_3^-	Hg
H_2SO_4-HNO_3-HCl	As,Se
Aqua regia-$KMnO_4$	Hg
Aqua regia-HF-H_2O_2	Cr

Reagents Used	Elements Determined
Rocks/Minerals	
HNO_3	Co,Cr,Cu,Mn,Mo,Ni,V
HCl	Fe
HNO_3-HF	Co,Cr,Cu,Ni,Pb,Zn
HCl-HF	Al,K,Mg,Si
HNO_3-$HClO_4$-HF	Ba,Be,Co,Cs,Cu,Ni,Nb,Pb,Rb,Sb,Sn,Sr,Ta,Th,Tl,U, W,Zn,REEs
HF-H_2SO_4	Fe
HNO_3-H_2SO_4-$K_2Cr_2O_7$	Os
HF-HNO_3-HCl	Th,U,Yand lanthanides
HNO_3-HCl-HF-$HClO_4$	Au,Ir,Pb,Pt,Rh,Ru
HNO_3-H_2O_2	Cd,Cu,Ni,Pb,Zn
HNO_3-H_2SO_4	As
HNO_3-H_2O_2-HNO_3-HCl-HF	Cd,Cu,Pb
HNO_3-H_2O_2-HF	Si
Aqua-regia	Hg

Table 1.6: Reagents Used for the Digestion of Different Geological Samples Continued

Reagents Used	Elements Determined
Soil	
HNO_3	Hg
HNO_3-H_2O_2	As,Cd,Cu,Ni,Pb,Zn
HNO_3-H_2SO_4	As
HCl	Hg
HNO_3-H_2O_2-HF	Si
Aqua regia	Hg
Sludge	
HNO_3	Cd,Cr,Cu,Hg,Ni,Pb,Zn
HCl-HNO_3	Cr,Pb
HNO_3-H_2O_2	Cu,Mn,Pb
HNO_3-H_2SO_4	As,Se
Aqua regia-HF-H_2O_2	Cd,Cr

Reagents Used	Elements Determined
Dust and Ashes	
HNO_3	Cd,Pb
HNO_3-H_2O_2	As
HCl-HNO_3	Cd,Pb
HNO_3-HF	Cd,Pb
HNO_3-HCl-HF	Al,Ba,Ca,Cr,Cu,Fe,Mg,Mg,Ni,Ti,V,Zn
HNO_3-$HClO_4$-HF	Al,As,Ba,Cd,Cr,Cu,Fe,K,Mg,Mn,Na,Ni,Pb,S,Sb,V,Zn
HNO_3-H_2O_2-HNO_3-HCl-HF	Cu,Pb
Coal	
HNO_3-H_2O_2 -HF-HCl	REEs

Table 1.7: Reagents Used for the Digestion of Different Water Samples

Reagents Used	Elements Determined
$K_2S_2O_8$	Total P
$K_2S_2O_8$-NaOH	As, Total P and N
H_2SO_4-HNO_3-$KMnO_4$-K_2SO_8	Hg
HNO_3 and pyrophosphatase	Total P
$KBrO_3$-KBr	Bi, Hg
HCl	Se
$K_2Cr_2O_7$-H_2SO_4	COD

nitric acid-hydrogen peroxide digestion[35] and in shellfish following a simple nitric acid digestion[71].

Evidence also exists however to suggest that digestion with HF is unnecessary for some samples. For example good results have been obtained for Al in NIST Total Diet[99] and in NIST Citrus Leaves[117] after a nitric acid digestion. A combination of nitric acid and hydrogen peroxide was used for the digestion of NIST Wheat Flour although results for Rice Flour were slightly high[63]. Recoveries were not however increased by the inclusion of HF in the procedure. The determination of aluminium in tea leaves was successfully determined after an open nitric and perchloric acid method, although it was reported that low recoveries were obtained with nitric acid, alone and in combination with hydrogen peroxide[31].

Similar discrepancies also exist for the determination of iron. A simple nitric acid digestion was used successfully for the determination of iron in Cocoa[40], IAEA Horse Kidney[41], Leucerne[47], NIST Total Diet[99], NIST Bovine Liver, NIES Mussel, Chlorella, Sargasso and Pepperbush samples[43]. However Mingorance *et al.*[61] obtained low and imprecise results using a similar method. Slight improvement was obtained with a nitric and hydrofluoric acid digestion but results were still outside the certified range. Good results were finally obtained with nitric acid and hydrogen peroxide for NIST Total Diet and with nitric and perchloric acid for NIES Pepperbush and Mussel samples. However low results were obtained for both procedures for BCR Wholemeal Flour. A nitric acid and hydrogen peroxide digestion was also employed by Burguera *et al.*[74] to give good results for NBS Bovine Liver, but for BCR Bovine Muscle results were slightly high. Using a similar procedure good results were obtained by Chakraborti *et al.*[109] for BCR Pig Kidney, Bovine Muscle and Liver, NBS Citrus Leaves and Pine Needles and Wheat Flour and in Bovine Liver by Sah *et al.*[52] and Krachler *et*

al.[29]. However low iron recoveries have been reported using the same reagent combination for NBS Orchard and Spinach Leaves and Bovine Liver[62], Leucerne[85], BCR Rye Grass[46], IAEA Hay[46] and for fat-rich foods[26]. For the latter, recoveries were improved by use of a closed nitric-sulphuric-nitric acid digestion. Sulphuric acid has also been used by Krushevska *et al.*[30] in combination with nitric acid, hydrogen peroxide and NH_4EDTA to give good results for NIES Mussel Tissue, IAEA Horse Kidney, NIST Bovine Liver and Oyster Tissue.

A number of workers however have reported that to obtain complete iron recoveries, digestion with HF was necessary. For example Lajunen *et al.*[45] found a hydrogen peroxide, nitric and hydrofluoric acid digestion far more effective than a simple nitric and hydrochloric acid digestion for the decomposition of NIST Citrus Leaves. A nitric, perchloric, hydrochloric and hydrofluoric acid digestion was also successfully developed by Kojima *et al.*[87] for the determination of iron in NIES Pepperbush and Mussel samples and for NIST Bovine Liver although in NIES Tea Leaves results were slightly high. Mohd *et al.*[67] used a chemometrics technique to select the best reagent combination for the digestion of NRCC TORT-1 and NIST Pine Needles. The chosen procedure involved digestion with hydrochloric, nitric and hydrofluoric acids in which the hydrochloric and nitric acids were present in equal quantities rather than as *aqua regia*.

A number of different techniques for the determination of selenium have also been suggested. Banuelos *et al.*[39] investigated a number of different reagent combinations with and without a pre-digestion stage. Using a simple nitric acid digestion a selenium recovery of only 23% was obtained for NIST Wheat Flour. Recoveries were improved to 80% after a nitric acid and hydrogen peroxide digestion with a 4 hour pre-digestion step (only 57% without pre-digestion). However further heating or the addition of hydrochloric acid did not

increase recoveries. Selenium determinations have also been successfully carried out using an open nitric acid and hydrogen peroxide digestion for BCR Lyophilised Pig Kidney[23], BCR Maize Leaves[44], NIST Bovine Liver[25] and Mixed Diet but results were slightly low for NIST Total Diet[25]. An alternative technique, for the digestion of NIST Bovine Liver, was offered by Prasad *et al.*[53] which involved a closed nitric acid digestion followed by evaporation to dryness with perchloric acid in order to remove all traces of organics for analysis by square wave cathodic stripping voltametry. However for analysis by hydride generation-atomic absorption spectrometry (HG-AAS) a similar procedure was found ineffective. This was also the case when phosphoric acid or potassium persulfate were added[90]. Good results however were obtained for NIES Mussel tissue by using nitric and sulphuric acids with hydrogen peroxide[89,90]; for NRCC DORM-2 and DOLT-2 after digestion with nitric and hydrofluoric acids[66] and in a semi-on-line nitric acid digestion for NIST Oyster Tissue[72].

For arsenic, good results have been obtained with a nitric acid digestion for BCR Cod Muscle[24], NIST Oyster Tissue[100] and NRCC DORM-1, although for TORT-1 results were slightly high[53]. Yusof *et al.*[58] and Liu *et al.*[34] however obtained good results for TORT-1 using a similar digestion procedure. A nitric acid digestion was also carried out by Navarro *et al.*[96,98], in combination with a catalyst of V_2O_5, to obtain good results for BCR Mussel Tissue and NBS Citrus Leaves. A nitric acid and hydrogen peroxide digestion was successfully employed for the digestion of NIST Oyster Tissue and Orchard Leaves[65], for NRCC TORT-1 and DORM-1[32], for BCR Maize Leaves[44] and for BCR Spruce Needles, White Clover, Cod Muscle and Plankton samples[35]. However El Moll *et al.*[110] required the use of a more vigorous multi-step procedure with nitric, sulphuric acid and hydrogen peroxide for the open vessel digestion of a range of fish samples. Krushevska *et al.*[30] also employed a mixture of nitric acid, sulphuric acid and hydrogen peroxide but in combination NH_4EDTA for a range

of biological samples. Schramel *et al.*[68] reported that a nitric acid digestion was successful for the digestion of NIST Orchard Leaves, although for the determination of arsenic in fish by HG-AAS a nitric, perchloric and sulphuric acid digestion was necessary. Presumably this step was required to break down the organoarsenic compounds present in the sample. Similar work by Mayer *et al.*[94] employed a nitric acid, sulphuric acid and hydrogen peroxide mixture for the determination of As in NIST Bovine Liver by HG-AAS. McLaren *et al.*[66] developed a nitric and hydrofluoric acid digestion followed by hotplate evaporation to dryness and dissolution of the sample in nitric acid for the determination of a number of elements including arsenic in NRCC DORM-2 and DOLT-2. An alternative approach for the breakdown of organoarsenic compounds is to undertake an on-line potassium persulfate/sodium hydroxide digestion[32,91]. Speciation of arsenic may then be achieved by the coupling of a high performance chromatography (HPLC) column to the system. In the former system[32] the determination of total arsenic was also possible by virtue of an L-cysteine pre-reduction step.

The more readily released elements from biological matrices such as Cu and Zn have been determined after digestion with a vast number of different reagents ranging from nitric acid alone[30,34,38,40-43,46,47,58,78,83] and in combination with hydrogen peroxide[26,29,35,47,52,62,74,80,83,85,99], to combinations of nitric, perchloric, hydrochloric and hydrofluoric acids[87,105].

Methods for the determination of mercury are however a little more in agreement, with many workers employing a closed nitric acid digestion procedure. Good results have been obtained for BCR Pig Kidney[37,116], Mussel Tissue[97] and Cod Muscle[79] ; for NIST Citrus Leaves, Pine Needles[73] and Albacore Tuna[57]; for IAEA Fish Tissue[37] and for NRCC TORT-1[58] using this procedure. However a number of workers have reported that the use of strong oxidising reagents such as sulphuric acid and hydrogen peroxide are necessary for the

determination of mercury using open[33,37] and closed microwave digestion systems[95]. An on-line system for the determination of Hg in biological and sediment samples has been reported[33]. The system was suitable for the analysis of samples containing organomercury compounds by the utilisation of a bromide/bromate oxidation reaction.

1.4.4.2 Geological Samples

Less work has been carried out for the digestion of geological samples than for biological samples, although a wide range of matrices have been digested by a number of different digestion procedures (Table 1.3)[30,33,35,42,44,55,62,65,66,73,80-83,91,95,104,109,118-147]. Included are sediment, soil, rock, sludge, ash and dust samples. For the determination of some elements simple nitric or hydrochloric acid digestions will suffice for some samples. For example good Fe recoveries in Fe ore samples were obtained using a simple hydrochloric acid digestion but for limestone samples the additional use of hydrofluoric acid was required[62]. Lead in dust wipe and air filters[145] was determined after a nitric acid digestion. Mercury were also determined using a similar procedure in NIST SRM Estuarine Sediment and Buffalo River Sediment[129], in NIES Pond Sediment[104] and in baghouse dust[142], although in NIST Buffalo Sediment results were high[122] and in NIST River Sediment results slightly low[73]. Results were also low for mercury in NIST Montana Soil following both an open and closed nitric acid digestion[118]. However Morales-Rubio *et al.*[120] found on-line digestion of slurries prepared in nitric acid successful for the determination of Hg in sewage sludge, soil and sediment samples. An alternative system has been proposed by Hanna and McIntosh[119] in which sediment slurries prepared in *aqua regia* and potassium permanganate were digested on-line for analysis in a flow injection mercury system. In addition an on-line system employing a bromide/bromate oxidation reaction has been developed for the determination of mercury in sediments[33].

Feng *et al.*[137] found no significant differences between the results obtained after digestion with nitric acid, with hydrochloric and nitric acid or with nitric and hydrofluoric acid for the determination of Cd and Pb in NBS Urban Particulate Matter, BCR City Waste Incineration Ash and River Sediment. Good results were obtained for Cr and Pb in BCR Sewage sludge after digestion with *aqua regia*, although Cd results were slightly low[146]. However using a nitric acid and hydrogen peroxide digestion, low Cu and Pb (but good Cd) results were obtained in the latter two samples and BCR Calcareous Loam Soil by Chakraborti *et al.*[109]. The results were improved by employing an extra heating step with *aqua regia* and HF. Good Cu and Mn results have also been obtained in sewage sludge using a nitric acid and hydrogen peroxide digestion[80,83], and for cadmium in sediment samples after digestion with just nitric acid[125]. A nitric acid digestion was also employed by Averitt *et al.*[122] to obtain good results for As, Cd, Co, Cu, Ni and Pb in NIST Buffalo River Sediment however Ba, Cr, Mn, Sb, V and Zn results were low and Hg results were high. Barium, Hg and V results were improved using a nitric, perchloric, hydrofluoric acid digestion procedure although Cr was still low and Zn and Sb too high. Marr *et al.*[126] reported a benefit from the addition of HF to an *aqua regia* digestion for the analysis of the sediments NRCC MESS-1 and PACS-1, however results for Cr and Mn were still low.

In environmental analysis it is often useful to acquire information on the bioavailable rather than the total elements present. This can often be achieved by using an acid leach procedure. Paudyn *et al.*[127] carried out such a procedure for the samples NRCC MESS-1 and PACS-1 and NIST Coal Fly Ash. This work also demonstrated how the digestion conditions required for the total release of different elements varies from sample to sample. For example, complete recoveries of Mn were obtained for Fly Ash compared to only 63% for MESS-1. Total recoveries were also obtained for Cu and Zn in all samples whereas for other elements

recoveries were much lower, e.g. only 35-60% for chromium, thus warranting a more vigorous digestion procedure. Low Cr and Al results were obtained in NRCC BCSS-1 following an on-line hydrofluoric, nitric and hydrochloric acid digestion although recoveries for As, Cd, Co, Cu, Fe, Mn, Ni, Pb and Zn were good. Low Al and Cr results were also obtained in NIST Urban Particulate Matter after digestion with nitric, perchloric and hydrofluoric acids, however As, Cd, Cu, Fe, Mg, Ni, S, Sb and Zn results were in agreement with the certified values[140]. Complete recoveries of Cr and a range of other elements except for low Ti in NRCC MESS-1, low Co in Fly Ash and slightly low Al, Ca, Fe, Mg, Ni and S in PACS-1 were obtained using a nitric, hydrochloric and hydrofluoric acid digestion. Good recoveries were also obtained for Cr in Mississippi River delta sediment following a nitric and hydrofluoric acid digestion[66] and by an *aqua regia*, hydrofluoric acid and hydrogen peroxide digestion for BCR River Sediment and Sewage Sludge samples[81]. However good Cr recoveries were obtained without the use of HF following an open hydrochloric, nitric acid and hydrogen peroxide digestion (and in a closed hydrochloric, nitric and hydrofluoric acid digestion) in BCR Estuarine Sediment[115]. Good As, Cd, Cu, Ni and Zn recoveries were also obtained, although Hg and Pb levels were high. Totland *et al.*[131,132] also employed the use of HF in combination with nitric and perchloric acid for the successful digestion of nine rock and sediment samples.

For the determination of As and Se in soil and sewage sludge by HG-AAS, digestion with nitric and sulphuric acids was found to be the most effective procedure[128]. Lasztity *et al.*[65] employed a nitric acid and hydrogen peroxide digestion for the determination of As in NIST Urban Particulate Matter and IAEA Soil 7. A nitric, hydrochloric and hydrofluoric acid digestion was successfully employed by Jimenez De Blas *et al.*[44] for the digestion of soil samples.

For the determination of rare earth elements in coal Watkins *et al.*[141] employed a closed nitric acid, hydrogen peroxide, hydrofluoric and hydrochloric acid digestion. Sen Gupta *et al.*[139] developed a hydrofluoric, nitric and hydrochloric acid digestion for the determination of Th, U, Y and the lanthanides in a large number of sediment and rock samples.

The use of a chemometrics technique for the selection of the best technique for the determination of Co, Cu, Mn, Pb and Zn in NBS Buffalo River Sediment was carried out by Kokot *et al.*[123]. A digestion procedure with hydrofluoric, nitric and hydrochloric digestion was selected whereas for the elements Co, Cr, Cu, Ni, Pb and Zn a nitric and hydrofluoric acid digestion was found to be most effective[124]. A hydrofluoric, hydrochloric and nitric acid digestion procedure was selected by an orthogonal array design for the digestion of sediment samples[136].

1.4.4.3 Water Samples

Although only a few publications have reported the application of microwave digestion to the determination of elements in water samples (Table 1.4)[23,91,119,148-156] there is a little more agreement over the digestion reagents required. For example Benson *et al.*[156] employed an on-line potassium persulfate digestion successfully for the determination of total phosphorus, although incomplete digestion of condensed phosphates was observed. A similar digestion procedure but in batch mode, has been carried out for the determination of total phosphorus and nitrogen by Johnes *et al.*[153]. Full recoveries for phosphorus were obtained but for nitrogen the breakdown of aminoantipyrine was incomplete. The determination of P was also carried out using a nitric acid digestion with prior addition of pyrophosphate to give complete recoveries of tetrameta, trimeta, ortho and pyrophosphate[154]. Arsenic speciation has been achieved by on-line HPLC separation followed by a potassium persulfate and sodium

hydroxide microwave digestion and analysis by HG-AAS[91]. Pitts *et al.*[148] developed an on-line microwave reduction system for the conversion of Se(VI) to Se(IV) prior to analysis by HG-AFS, which in a later publication enabled the speciation of Se(VI) and Se(IV) following separation by HPLC[149]. A similar system for the pre-reduction of Se(VI) to Se(IV), but for analysis by FI-CSV was reported by Bryce *et al.*[23]. For the determination of mercury by CV-AAS, digestion with $KBrO_3$-KBr is commonly used. Welz *et al.*[152] developed an on-line system for Hg and Bi, although problems were encountered for the determination of As, Pb and Sn. As described previously a system for the on-line determination of Hg in sediments, water and waste water samples was proposed by Hanna and McIntosh[119]. For COD determinations Balconi *et al.*[155] developed an on-line method employing a $K_2Cr_2O_7$-H_2SO_4 digestion.

1.4.5 Conclusions

Biological samples consist of a complex mixture of carbohydrates, proteins and lipids and so are not completely soluble in water or organic solvents. Before analysis it is therefore necessary to decompose the organic matter and release the metals from the sample matrix. The majority of the digestion procedures used to date employ the initial use of strong oxidising agents such as nitric acid to decompose the organic matrix of the sample. Many elements are then liberated as soluble nitrate salts. Other acids can then be employed to break down the sample further according to the elements that need to be determined and the chosen analysis technique. For example hydrochloric acid is a good solvent for many metal oxides, for metals that are oxidised more easily than hydrogen and for some organometallic compounds. The use of hydrofluoric acid is necessary for the determination of a number of elements which are associated with siliceous minerals.

For the determination of iron and aluminium in biological samples a wide variety of reagents have been successfully employed. For the former, digestions with nitric acid alone and in combination with hydrogen peroxide are very common and generally effective for the digestion of biological samples. However the requirement for HF during the digestion of some botanical samples has been reported. For the determination of aluminium many workers have reported that the low results generated from digestion with just nitric acid or nitric acid and hydrogen peroxide can be improved by the addition of HF. However other workers have found this step unnecessary. Therefore for the determination of Al and Fe it would seem that no steadfast rules can be made regarding the need for HF. This seems to be dependent on the exact sample type, according to the amount of siliceous material present. The determination of Al is also hindered by high background levels which can be prohibitive

for trace analysis. Thus measures to control background contamination, in addition to the preparation of sample blanks, should be routinely adopted to minimse this problem.

For the determination of arsenic, digestion with nitric acid is in many cases successful, although for the analysis of fish samples by HG-AAS more vigorous conditions are usually required. Sulphuric acid is often employed to break down organoarsenic compounds which are not hydride forming upon reaction with sodium borohydride. Similar findings have also been observed for the determination of Hg and Se. For the latter, nitric acid and hydrogen peroxide digestions have been used successfully to replace the conventional nitric and perchloric acid and sulphuric acid procedures. However when analysis by hydride generation is desired, sulphuric acid is still required to break down the more resistant organoselenium compounds. For the less strongly bound elements such as Cu and Zn the digestion procedure seems less critical with a wide range of different reagent combinations giving good results.

The wide range of sample compositions represented by geological materials preclude the use of any one digestion procedure. For example sediment samples consist of a combination of different materials e.g. clay, organic material, siliceous and other minerals and are thus one of the most difficult sample matrices to digest. Therefore in many cases to attain a complete digestion the use of HF is necessary to decompose resistant minerals, in addition to strong oxidising reagents such as nitric acid and sometimes perchloric acid to break down the organic matter. From the literature there is evidence to suggest that a number of elements such as Cd, Cu, Hg, Pb and Zn can be easily released after digestion with just nitric or hydrochloric acids. This is because in many cases they are sorbed onto clay minerals or are in other readily decomposed phases, rather than within the resistant framework-lattice silicates. However other elements are more strongly bound either as part of resistant minerals or

associated with other minerals and so in such cases the use of HF may be required. For example some Cr bearing minerals notably chromite are very difficult to decompose even with the use of HF/$HClO_4$ under pressure, although complete Cr recoveries have been reported without the use of HF. The need for HF is therefore very much dependent on the exact minerals present in the samples. Due to the fact that real samples will probably vary in exact composition to that of the certified reference materials used for validation of a procedure it would seem prudent to suggest that for the determination of all but the most weakly bound elements in sediments that digestion with HF is recommended.

The digestion reagents required for efficient digestion of a particular sample type are very much dependent on the exact sample matrix as well as the elements to be determined. Excluding water samples there is generally little agreement in the literature, with often conflicting evidence as to which reagent combinations are most effective for the same matrix. This may reflect the fact that the digestion is influenced by other factors other than just the choice of reagents e.g. the relative proportions of each reagent, heating times and the pressure and temperature reached during the procedure. In many cases good results for the same matrix have been reported by a number of different methods. In addition the literature suggests that no standard digestion procedures can be employed for the determination of a specified element in all samples of the same type e.g. Fe in all biological samples or for the determination of all the elements in a particular sample e.g. all the elements in Mussel Tissue. Therefore it may not be justified to extrapolate a technique designed for the determination of just a few elements to a multielement determination.

The choice of sample preparation method may however be influenced by a number of practical considerations in addition to the type of sample and elements to be determined.

These may include the number of samples to be analysed, method of analysis, safety aspects, capital and operating cost of equipment, operator skill and degree of accuracy and precision required. The method of final analysis is an important factor, influencing the extent of the digestion required e.g. for electroanalytical techniques complete break down of the organic components is necessary whereas ICP-AES can tolerate dissolved solid contents of up to 1-2%. Also the addition of certain reagents during the reaction can be considered. For example the addition of boric acid for HF neutralisation may cause the final solution to possess a high solids content which can give problems in sample introduction systems as well as increasing the background signal and thus degrading sensitivity. ICP-MS suffers from a number of spectroscopic interferences particularly polyatomic ion interferences. Thus the presence of a number of acids including hydrochloric and sulphuric in the final solution are not recommended for the analysis of some elements. Therefore adapting a digestion method for analysis by a different analytical technique to that originally intended may not prove successful.

When considering the speed of a particular procedure it is not just the time for the actual digestion that should be considered. Other factors should also be examined such as sample preparation before analysis such as grinding and slurry formation, predigestion and cooling times, including those necessary between reagent additions/heating cycles and the washing of digestion vessels. These factors are often overlooked.

One standard method of validating a procedure is by the use of a suitable certified reference material. However as discussed previously there seems to be a lack of consistency in the "grading" of these results. Often results are classed as 'good' even though they do not lie within the uncertainty limits of the certified values.

As discussed above it is not just digestion procedures which lend themselves to automation but also the choice of digestion method. Chemometrics and factorial designs have been used effectively to help choose the best digestion procedure for a particular purpose. Models have also been developed to predict digestion methods depending on the composition of the sample of interest. This undoubtedly will become more useful in the future especially to predict digestion conditions for new samples.

For batch digestions many closed digestion procedures are developed in terms of heating at a particular power setting for a certain period of time, usually optimised to maximise energy input without causing venting of the vessels. These procedures are therefore operational i.e. specific to the particular microwave system and bomb design used. Adaptation for use in a different laboratory may not be straight forward unless the same equipment is used as reoptimisation of the original power settings and heating times will probably be necessary. The optimum power and time settings may also vary considerably according to the exact nature of the sample due to the amount of organic matter present which influences the amount of gaseous products evolved during the reaction.

It has been shown that direct temperature and pressure measurements during the course of the digestion are possible. Such measurements can then be fed to a computer controlling the magnetron to achieve a preset temperature or pressure programme. This technology offers the potential to produce far more reproducible and controllable procedures, reducing the possibility of venting of digestion vessels. In closed systems it also enables the system to be operated to it's full digestion potential, i.e. at it's maximum pressure level without venting, for each sample regardless of it's exact organic matter content. Following this approach digestion procedures can be adapted far more easily between similar samples, between different

workers and could potentially lead to the establishment of standard digestion procedures, in addition to improving the overall safety of this technique.

There has been a growing trend in recent years towards the development of fully automated on-line microwave digestion and analysis techniques. This area is well suited to water and waste water samples of which a large number are routinely analysed in many laboratories. Open focused systems have been found to be particularly useful for on-line applications. The adaptation of standard batch digestion methods to fully automated systems can easily be achieved and is an area with much scope for development. The digestion of solids is complicated by the method of introducing the sample into the system. Introduction in the form of a slurry is one approach, however the determination of low levels of analyte may be problematic due to limitations in the maximum stable slurry concentration. However, despite the initial problems encountered with on-line microwave digestion systems for solid samples good results have been obtained for a number of biological and geological materials. This approach to sample digestion would seem to offer much potential for further development and could result in dramatic time savings over batch microwave and conventional digestion techniques.

1.5 Research Objectives

The need for the determination of a wide range of trace elements in environmental samples has been outlined above. The use of microwave digestion is rapidly expanding as an efficient sample preparation technique and has largely revolutionised conventional approaches to sample digestion. The aim of this project was to utilise the advantages of open focused microwave digestion systems for the digestion of a wide range of environmental matrices particularly those that have proved time consuming or troublesome to digest by conventional techniques. Sample matrices of interest in this study included biological, botanical and geological materials. Particular emphasis was assigned to the task of developing techniques capable of on-line microwave digestion followed by on-line analysis in a fully automated system. Techniques suitable for speciation studies were also addressed. Following sample preparation, trace element determinations were undertaken by a number of analytical techniques including ICP-AES, ICP-MS, HG-AAS and cold vapour-atomic fluorescence spectrometry (CV-AFS).

Specific aims of this research:

1 The development, characterisation and optimisation of a number of novel batch microwave digestion procedures for the determination of a range of trace elements in various samples including tea leaves, sediments and seaweed.

2 To address the well documented problems associated with extraction procedures to support the analysis of organotin species in sediment samples by HPLC-ICP-MS.

3 The development and utilisation of an on-line microwave digestion technique for the decomposition of 'non-reducible' arsenic species in biological samples. The ultimate aim of this part of this study was to develop a technique capable of the separation of the arsenic species arsenobetaine (AsBet), dimethylarsinic acid (DMA), monomethylarsonic acid (MMA) and inorganic arsenic [(As(V) and As(III)] by HPLC prior to on-line decomposition by microwave digestion and analysis by HG-AAS. Simple modification of the technique was required to allow the total arsenic content of the sample to be determined. In addition quantification of the total 'reducible' arsenic species [As(V), As(III), MMA and DMA] by on-line pre-reduction HG-AAS was addressed.

4 To evaluate the suitability of an open batch microwave digestion technique for the determination of the total mercury content of sediment and biological samples. To underpin this study with more detailed investigations of the factors influencing the digestion procedure, temperature measurements were taken throughout the course of the digestion with the aim of correlating them with the results obtained for a biological certified reference material.

5 The development of an on-line microwave digestion technique for the determination of total mercury in solid environmental samples. The possibility of oxidation of organomercury compounds in slurried solid samples followed by analysis by CV-AFS was investigated and applied to the analysis of samples containing high levels of organomercury compounds such as marine biological samples.

2. Analytical Instrumentation

2 ANALYTICAL INSTRUMENTATION

2.1 Principles of Atomic Emission, Absorption and Fluorescence Spectrometry

Atomic emission spectrometry (AES), atomic absorption spectrometry (AAS) and atomic fluorescence spectrometry (AFS) derive analytical information from atomic spectra in the optical region of the electromagnetic spectrum. The optical region encompasses the ultraviolet (UV), visible and near infrared wavelengths of light (see Figure 1.1). The atomic spectra originate from energy transitions in the outer electronic shells of free atoms or ions. Figure 2.1 shows the radiation processes that form the basis of the three techniques[163]. The horizontal lines represent different energy levels in an atom where E_0 is the term used for the lowest energy level (ground state) and E_i and E_j represent other energy levels with E_j being higher (greater energy) than E_i. A solid vertical line refers to a transition involving the absorption or emission of energy as radiation whereas a wavy line refers to a non-radiative transition. The energy of the absorbed or emitted radiation is quantified according to Planck's equation (Equation 2.1).

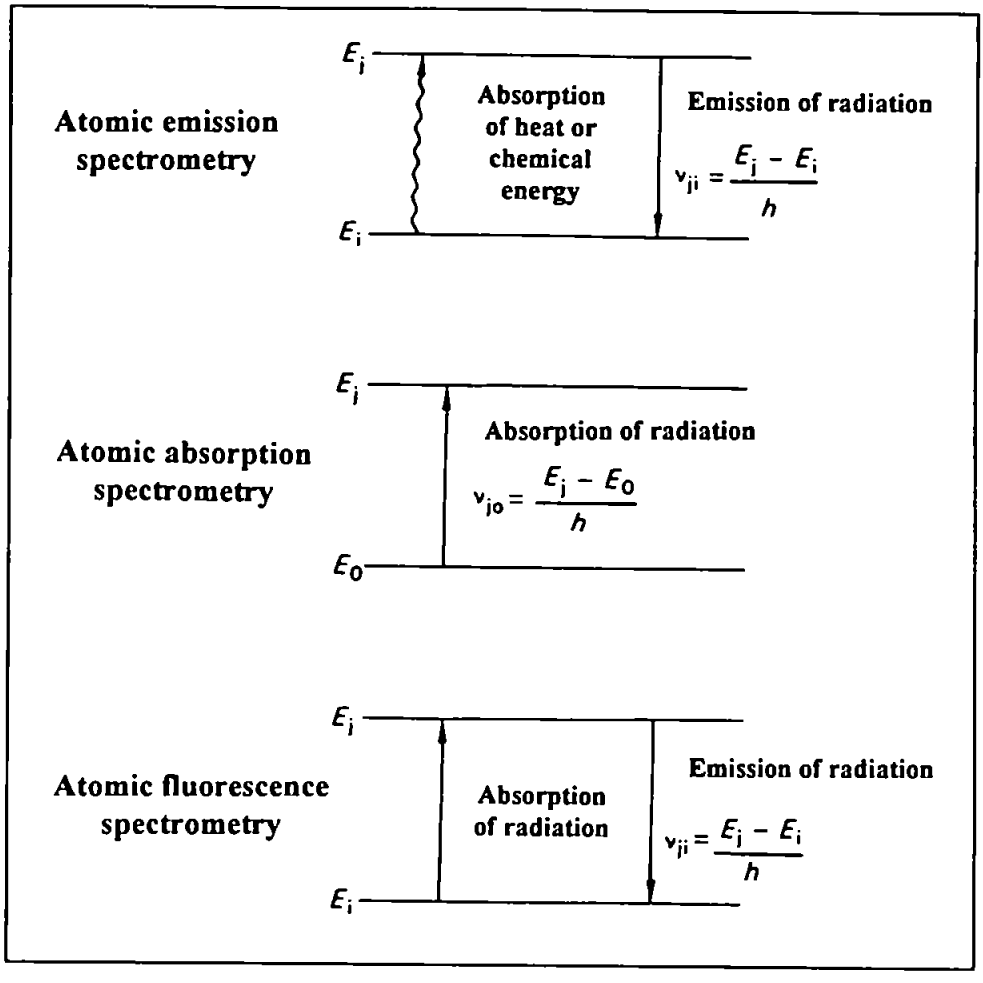
$$E = h\nu \qquad \textbf{(Equation 2.1)}$$

where E is the energy difference between the two energy levels in the atom, h is Planck's constant (6.624×10^{-34} J s^{-1}) and ν is the frequency of the radiation. The frequency is also related to wavelength by Equation 2.2.

$$\lambda = \frac{c}{\nu} \qquad \textbf{(Equation 2.2)}$$

where λ is the wavelength (in m) and c is the speed of light in a vacuum (2.9979×10^8 m s^{-1}).

Figure 2.1: Radiation Processes for AES, AAS and AFS



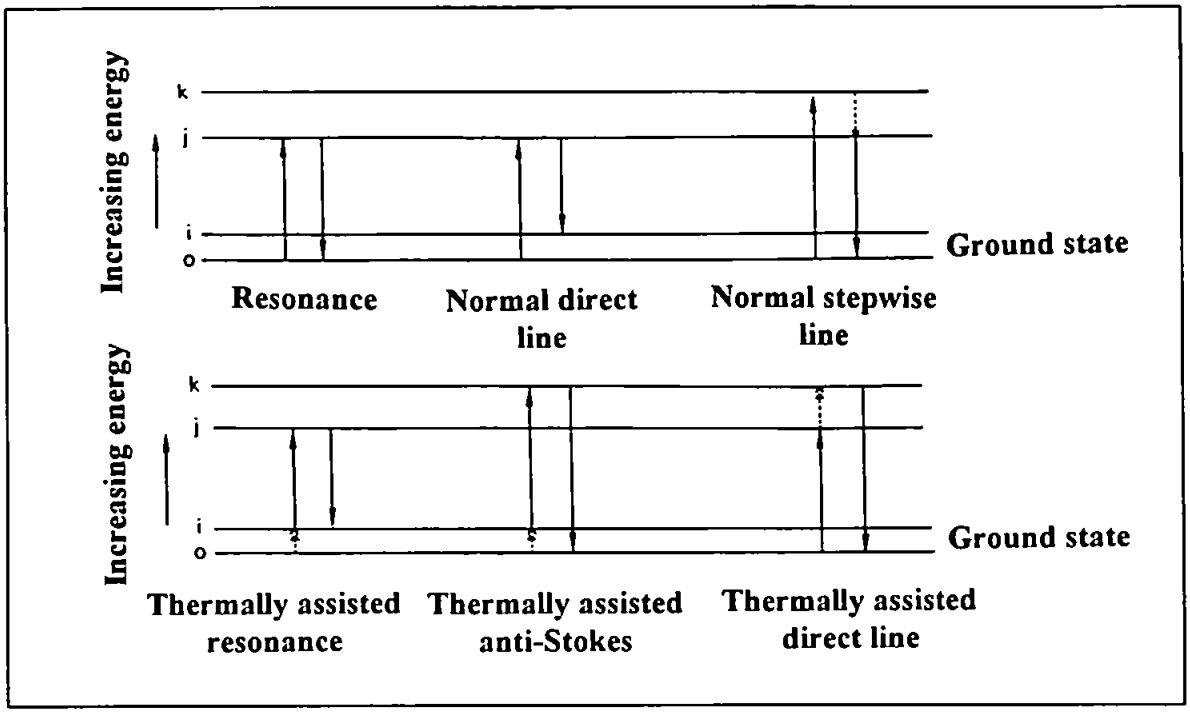
In AES, the absorption of thermal energy by an atom (on collision with another particle) has the effect of promoting one or more electrons to a higher energy level. In this state the atom is said to be in an energy-enriched or 'electronically excited' state[164]. However excitation is only possible if the energy of the radiation is exactly equal to the energy difference between the initial and final energy levels. In the excited state atoms are generally unstable and thus de-excitation occurs spontaneously by the emission of a "particle" of electromagnetic radiation (known as a photon), returning the electrons to the original energy level[165]. Every element has its own characteristic set of energy levels and thus its own unique set of absorption and emission wavelengths. It is this property that makes atomic spectrometry useful for element-specific analytical techniques[165].

In AAS and AFS, atoms are excited by the absorption of radiation from an external light source. An atom will only absorb exactly defined wavelengths of energy which are of a characteristic wavelength for that element. In flames, atoms are most commonly encountered in the ground state and therefore virtually all practical absorption and fluorescence measurements originate from atoms in the ground state[163]. The excitation energy is lost by collisional deactivation with other particles for AAS, whereas in the case of AFS de-excitation involves emission of light. For AAS the light source is viewed directly and the attenuation of radiation measured, whereas for AFS the source is not viewed directly and quantification is achieved by measurement of the re-emitted radiation[163]. Atomic fluorescence radiation is emitted isotropically by the atomic vapour and can be observed from all directions around the atom cell. For quantification the emission profile is usually measured in a direction perpendicular to the incident beam so that light from the source does not reach the detector. There are several types of atomic fluorescence, each characterised by the pathways followed by atoms in the excitation and de-excitation processes. Six types of flame AFS processes are summarised in Figure 2.2[163] where i, j and k represent energy levels with increasing energy. The solid lines represent radiational processes and the dashed lines non-radiational processes. For the latter, a single headed arrow represents non-radiational deactivation and a double-headed arrow a thermal activation process. The term 'anti-Stokes' is used when the radiation emitted is of shorter wavelength, i.e. greater energy than that absorbed.

The most intense fluorescence process, resonance fluorescence, has been the most useful type to exploit analytically. In this case the radiational excitation and de-excitation of the atomic system are between the same upper and lower energy levels and the emitted light (fluorescence) is at the same wavelength as the excitation source. Direct-line fluorescence

mechanisms have also been exploited as the emission is at a different wavelength to that of excitation and thus by using filters the effects of scattering can be eliminated.

Figure 2.2: Types of Atomic Fluorescence



As previously stated, during excitation the amount of light emitted (as in AES and AFS) or absorbed (as in AAS) by an element is of a characteristic wavelength for that element. In addition over a defined range, known as the linear dynamic range, the absorption/emission profile is proportional to the concentration of the element. Thus by comparison with a set of external calibrants the concentration of the analyte in the sample can be accurately determined. The dynamic linear range of the technique is defined by the concentration at which 'self absorption' occurs. As the concentration of atoms in the flame/plasma increases, the possibility increases that photons emitted by excited atoms in the hot region in the centre will collide with atoms in the cooler, outer regions of the flame/plasma, and thus be absorbed[163]. In AES and AFS this weakens the emission/fluorescence intensity reaching the

detector and thus destroys the proportionality between net analyte signal and the concentration of the element present. For a uniform source of given temperature and a spectral line of given shape and wavelength, the degree of self absorption increases with the intensity of the emission line[166]. In AAS self absorption in the hollow cathode lamp is caused by gaseous atoms in the source absorbing at the resonance wavelength emitted by excited atoms. This alteration of the line profile caused by self absorption means that only part of the resonance lines emitted from the radiation source can be absorbed by the atoms in the atomiser. The absorption curve does not then approach 0% transmission asymptotically, but a value corresponding to the residual radiation. In practice this leads to a non-linear analytical curve. In AAS, AFS and AES self absorption leads to curvature of the calibration curve towards the x-axis.

A pre-cursor of the AAS, AES and AFS techniques is atomisation of the analytes of interest, that is dissociation into free atoms and/or ions. This is commonly undertaken by aspiration of the sample into a suitable heating source. This results in desolvation of the aerosol particles to form solid salt particles, vaporisation to yield gas phase molecular species, followed by atomisation to give free atoms which may, given sufficient energy, lose an electron to form free ions[167]. For the AAS and AFS techniques, excitation of the atoms or ions can then occur by absorption of radiation from an external light source. In AES the heating source must not only provide sufficient energy for atomisation but must also result in excitation of the elements of interest. Inductively coupled plasmas (ICP) are commonly used as atomisers for AES although a number of different sources are also available such as flames, lasers, microwave induced plasmas, furnaces, glow discharges etc. In addition ICPs are also used as ion sources in mass spectrometry.

2.2 Inductively Coupled Plasma-Atomic Emission Spectrometry

The first major application of an ICP was reported in 1961 by Reed[168,169] who used it to grow crystals of refractory compounds such as alumina. He found that refractory powders introduced into the central axis of an ICP were completely vaporised. Following publication of his work, Greenfield[170], Wendt and Fassel[171] independently developed ICP systems for spectrochemical analysis. The most significant advance in this work came with the development of an ICP torch that permitted the injection of a sample aerosol into a central channel in the plasma and achieved limits of detection of 1-10 ng ml^{-1} for many elements[172]. The first commercial instruments for inductively coupled plasma-atomic emission spectrometry (ICP-AES) became available in 1974 and since then ICP-AES has become a well established technique for routine analysis.

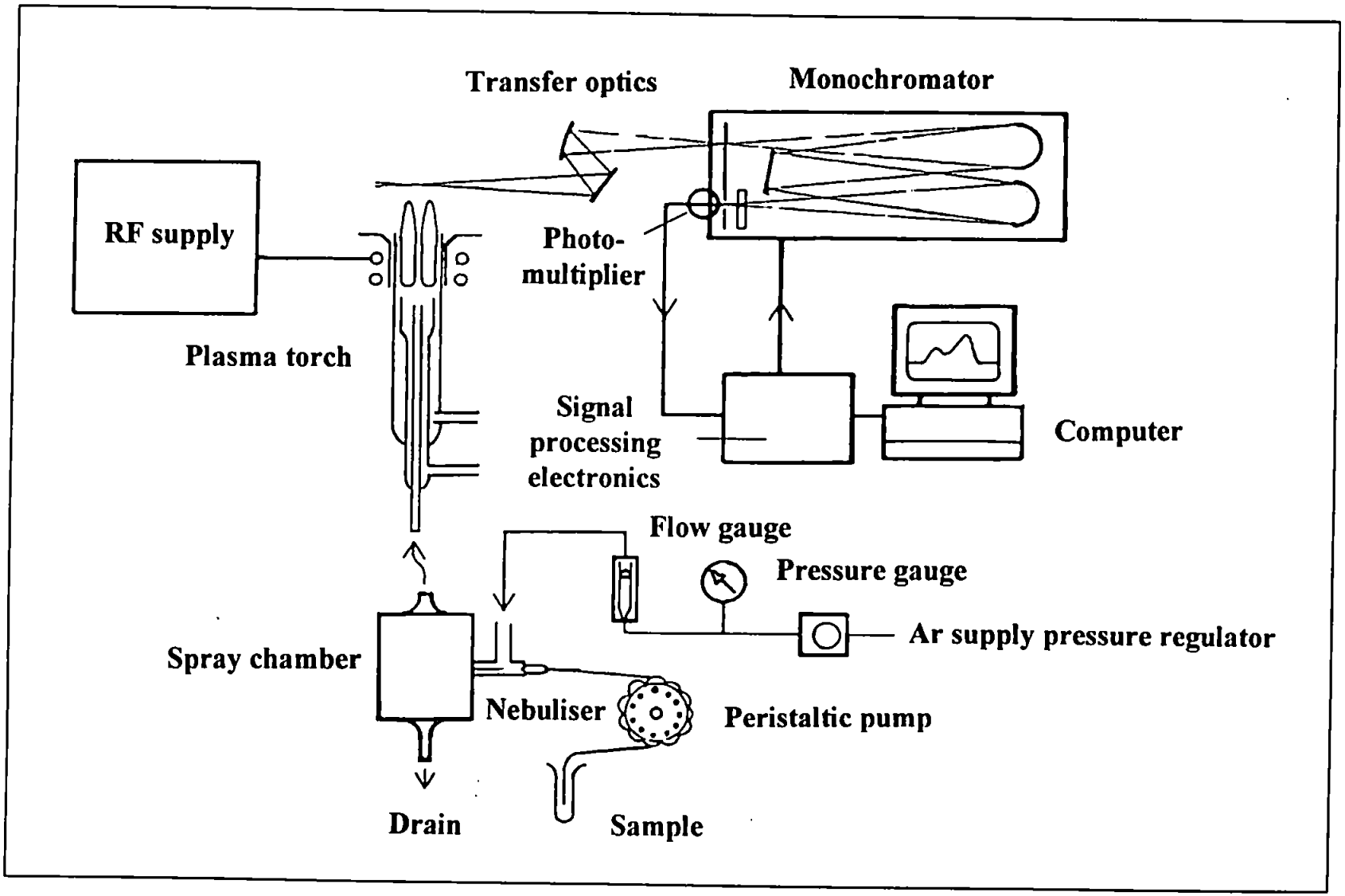
Today ICP-AES is widely used for the analysis of more than 70 elements utilising emission lines in the wavelength range 160-900 nm. Benefits of the technique include multielement capability, selectivity and long linear calibration ranges, generally of a factor of 10^4-10^5 [167]. Limits of detection are generally in the range 0.1-100 ng ml^{-1} [166], for many elements a factor of 1 to 10 times better than for flame atomic absorption spectrometry (FAAS). For the alkali metals however sensitivity for FAAS is far superior as these elements are almost completely ionised in the high temperature of the ICP (6000-10000 °K[173] *cf* to 2000-3000 °K for FAAS). The resulting ions have extremely stable electronic configurations and thus do not produce intense emission spectra. For ICP-AES detection limits for elements such as B, Ge, Hf, Nb, Re, Ta, Th, U, W, Zr and most of the lanthanides far exceed those possible by FAAS, which is limited by the formation of refractory oxides or carbides that resist dissociation to free atoms. This problem is not so prevalent in ICP-AES due to the high temperature of the plasma and the inert argon atmosphere. ICP-AES is less prone to chemical interferences than

FAAS however it does suffer from a range of spectral interferences. A comparison of limits of detection for the FAAS, ICP-AES, inductively coupled plasma-mass spectrometry (ICP-MS) and hydride generation-atomic absorption spectrometry (HG-AAS) techniques for the elements determined throughout this thesis are given in Table 2.1. A schematic design of an ICP-AES instrument is shown in Figure 2.3 and the various components discussed in Sections 2.2.1-2.2.4.

Table 2.1: Limits of Detection for Selected Trace Elements (ng ml^{-1})

Element	FAAS[163]	ICP-AES[174]	ICP-MS[175]	HG-AAS
As	110	12	0.001-0.01	0.8[176]
Al	18	4	0.01-0.1	-
Ca	2	0.03	0.1-1.0	-
Cr	5	4	0.001-0.01	-
Cu	2	2	0.001-0.01	-
Fe	6	1.5	0.01-0.1	-
Hg	160	8.5	<0.001	0.04[163] (CV-AAS)
K	2	10	0.1-1	-
Mg	0.2	0.1	0.01-0.1	-
Mn	2	0.3	0.001-0.01	-
Pb	15	14	<0.001	0.6 [177]
Sn	31	16	0.001-0.01	0.5
Ti	50	0.6	0.01-0.1	-
V	50	2	0.001-0.01	-
Zn	1	0.9	0.001-0.01	-

Figure 2.3: Components of an Inductively Coupled Plasma-Atomic Emission Spectrometer



2.2.1 Sample Introduction Systems

The ICP requires samples to be introduced in the form of a gas, vapour or aerosol of fine droplets or as a slurry of solid particles. For aerosol formation various types of nebuliser are currently available including thermospray vaporisers and pneumatic, ultrasonic and direct injection nebulisers. For routine analysis pneumatic nebulisers are the most common choice due to their convenience, reasonable stability and ease of use. Pneumatic nebulisers use a jet of gas (usually argon) to break the liquid sample into small droplets. A number of designs are available including concentric, Babington and cross-flow nebulisers[178]. Diagrams of a V-groove (Babington type) and a Meinhard (concentric type) nebuliser are shown in Figures 2.4a and 2.4b respectively.

For V-groove nebulisers the liquid sample flows down a V-shaped groove or channel. A jet of gas is issued from a capillary hole in the middle of this groove which effectively disrupts the sample flow to cause nebulisation. Conceptually the V-groove nebuliser evolved from the Babington nebuliser[179] in which a thin film of water flows over the surface of a sphere, to be nebulised by an emerging jet of gas. The major advantage of the V-groove nebuliser is its resistance to blockage as the sample does not have to pass through any extremely narrow passages. Thus this design is particularly suited for the analysis of samples possessing a high dissolved or suspended solid content.

Concentric nebulisers make use of the 'Venturi' effect, in which the reduced pressure resulting from the nebuliser gas passing through a small orifice causes the solution to be drawn into the gas jet and to be broken up into droplets of various sizes[173]. Although this means that the sample does not need to be pumped into the nebuliser, a peristaltic pump is often used to standardise the sample uptake rate for solutions with different viscosities[173].

Figure 2.4a: Schematic of a V-Groove Nebuliser

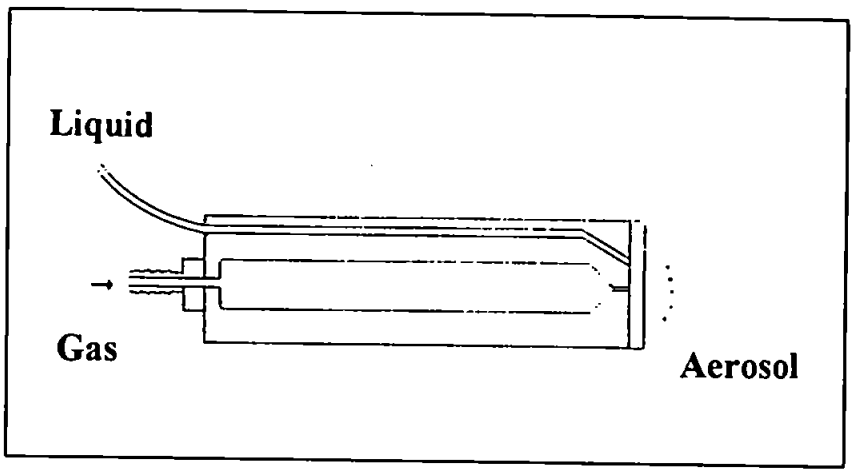
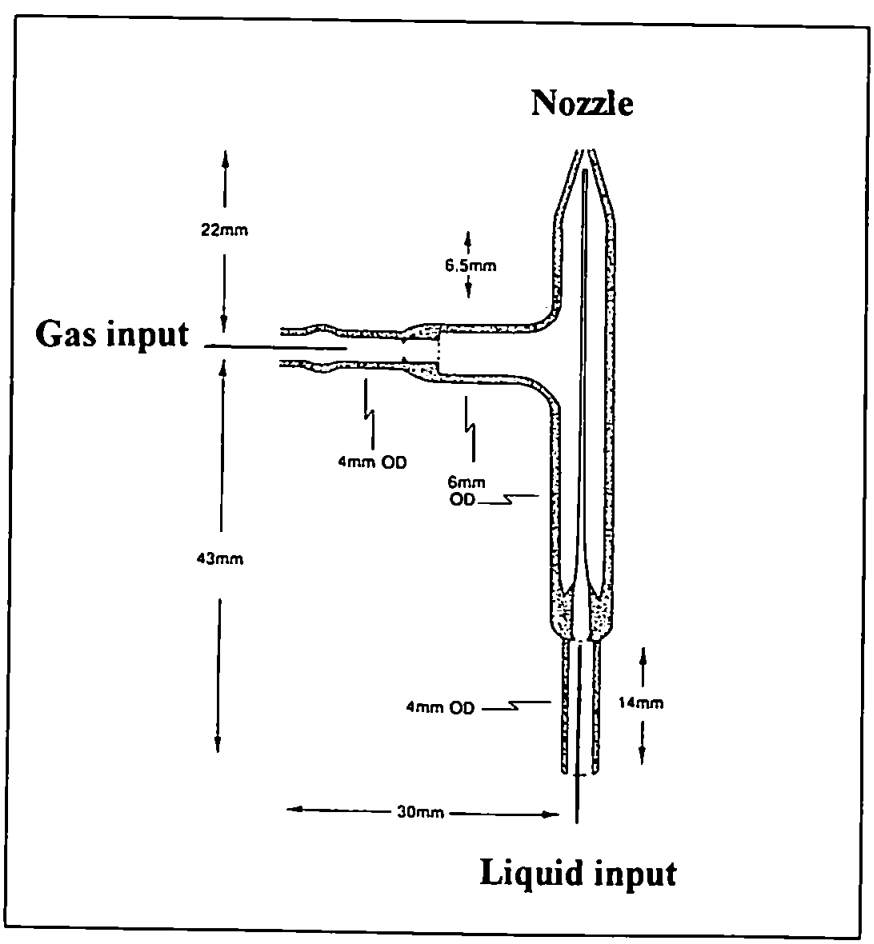


Figure 2.4b: Schematic of a Meinhard Concentric Nebuliser



A peristaltic pump is also needed for V-groove nebulisers. Although the concentric nebuliser is widely used as a general purpose nebuliser it is more prone to blocking than the V-groove design due to the narrow diameter of the inner sample capillary tube (about 0.3 mm i.d.)[178].

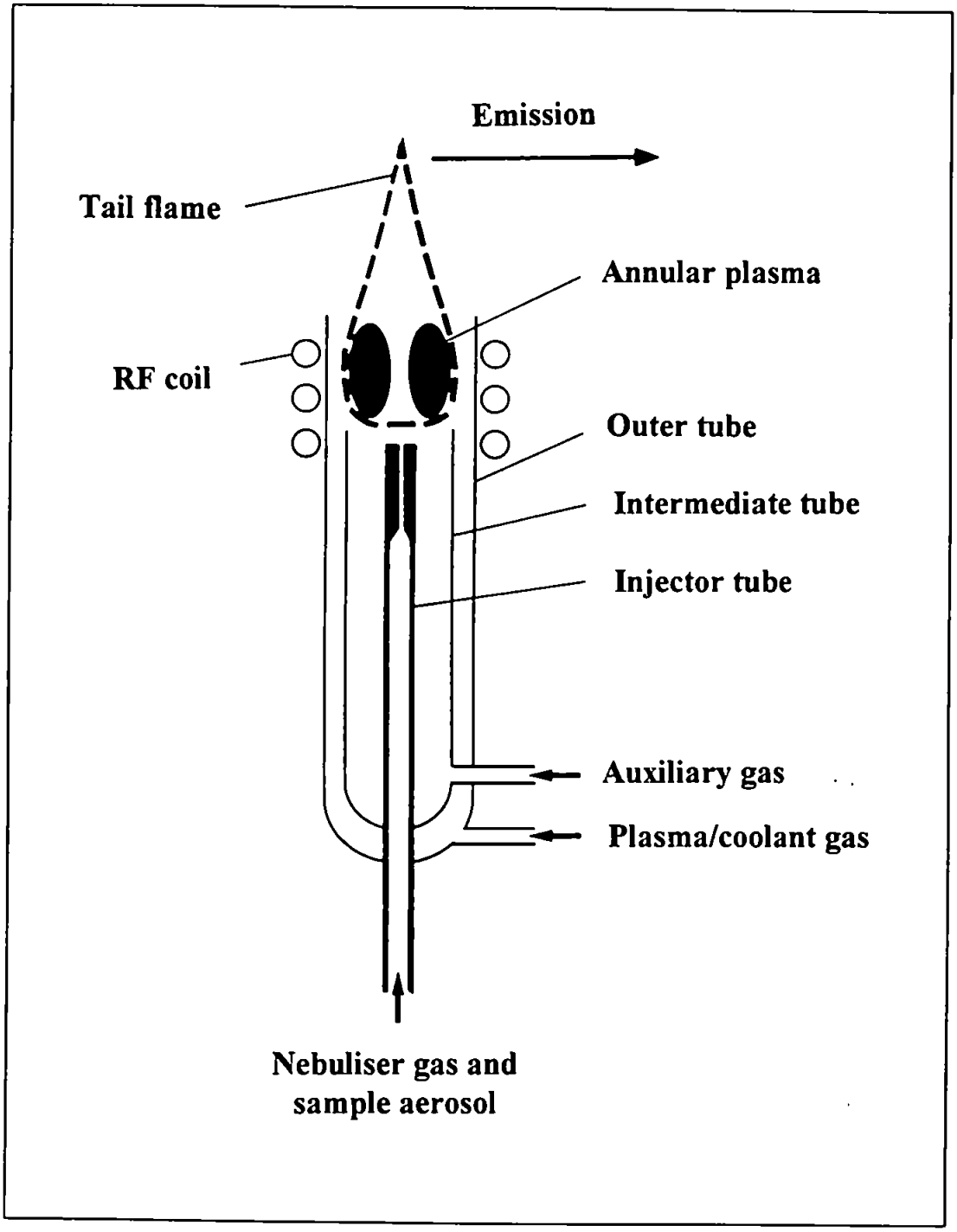
Following nebulisation the resulting aerosol is not suitable for direct passage into the plasma as it contains unfavourably large droplets (up to a diameter of 100 μm) which can cause instability in the ICP[173]. Ideally the droplet size distribution of particles reaching the plasma should be between 0.1 and 5 μm[174] to ensure the rapid desolvation, volatilisation and atomisation of the aerosol particles. The removal of the larger aerosol particles can be achieved by passage through a spray chamber. This works on the basis that as the gas flow carrying the aerosol enters the spray chamber it undergoes sharp changes in direction which the larger droplets cannot follow[178]. These droplets are thus removed by impaction on the walls of the spray chamber and subsequently run to waste. Passage of the sample through a spray chamber also helps to even out some of the fluctuations in the rate of aerosol generation and transport which can lead to high analytical noise levels[174]. Generally the overall transport efficiency of samples into the plasma is only about 1-3%[175], although this is dependent on a number of factors including the viscosity, dissolved solid content and surface tension of the sample, nebuliser gas and sample flow rates as well as the design of the nebuliser.

2.2.2 The Inductively Coupled Plasma

The ICP is an electrodeless discharge in a gas at atmospheric pressure maintained by energy coupled to it from a radio frequency generator[178]. The most commonly used gas is argon although other gases such as nitrogen and oxygen are sometimes used as additions to the main argon supply[178]. The plasma is generated inside and at the open end of an assembly of three concentric quartz tubes known as a torch as shown in Figure 2.5. The main argon stream (the plasma or coolant gas) is introduced tangentially between the intermediate tube and the outer tube, generally at a flow of 10-15 l min^{-1} [178]. This confines and stabilises the plasma and helps prevent the torch from overheating[174]. The auxiliary gas (intermediate tube) is present to lift the plasma from the exit of the nebuliser flow to again prevent melting of the torch and to minimise build-up of salt or carbon on the tip of the injector tube[178]. The innermost of the three tubes (injector) carries the aerosol from the sample introduction system and punches a hole in the base of the plasma to form a distinct axial channel through the plasma[174]. This confines the atoms to the centre of the plasma, to reduce dispersion. The central tunnel of the plasma is cooler than the rest of the plasma but at 5000-6000 °K is hot enough to atomise most samples and cause varying degrees of ionisation of the constituent elements.

The ICP torch is surrounded by a load coil of 2-4 turns of hollow copper coil, cooled by a flow of water[178]. This induction coil is connected to a high-frequency (radiofrequency) generator generally operated at a power of 1-2 kW and a frequency of 27 or 40 MHz[178]. Energy is transferred into the ICP by the interaction of ionised argon with the electromagnetic field of the induction coil[174]. The positive argon ions and the electrons formed are accelerated by the high-frequency field of the coil, however the electrons reach much higher velocities due to their smaller mass. Thus energy transfer into the plasma is dominated by

Figure 2.5: Inductively Coupled Plasma Torch



processes involving electrons. Since a gas is a poor conductor until it is ionised, the plasma must be initiated by being 'seeded' with a sufficient supply of energetic electrons to ionise the argon gas[173]. This can be achieved by the generation of a spark discharge into this gas stream. In the presence of the RF coil some electrons in the spark gain sufficient energy to undergo inelastic collisions with argon atoms, yielding an avalanche of charged particles in sufficient numbers to absorb the energy from the RF field[174]. Once the electrons reach the ionisation potential of the plasma support gas (argon), further ionisation takes place and a stable self-sustaining plasma at a temperature of 6000-10000 °K is formed[173].

2.2.3 Optical Spectrometer

Measurement of the analytical emission lines of interest can be achieved by either simultaneous or sequential detection[173]. Simultaneous instruments use a polychromator with a detector for each wavelength and although are capable of impressive speed are limited to measurements at pre-selected wavelengths[174]. Sequential instruments on the other hand use a monochromator and although are generally slower are more versatile in the wavelengths they can measure. Both approaches use focusing optics to collect the light from the plasma and focus it, through the use of mirrors, onto the entrance slit of the spectrometer[174]. The light is then reflected onto the diffraction grating by a collimating mirror and further reflected by a second mirror to focus the spectrum in the plane of the exit slit. The spectrum is scanned by moving it across the exit slit by rotating the diffraction grating. For measurements below 190nm the monochromator is evacuated and the optical path between the monochromator and the plasma purged with argon to minimise attenuation of the light by oxygen in the air[174].

2.2.4 Detectors and Readout Systems

Photomultiplier tubes are the most common detectors for use in ICP-AES. These work following the principle that when photons of light hit the photocathode, electrons are emitted and are accelerated towards another electrode (the first dynode) which is held at a positive potential relative to the cathode. One or more electrons are emitted from the first dynode for each electron leaving the photocathode. In a similar fashion, these electrons are accelerated towards the second dynode etc. until they reach the last dynode[164]. During this process the signal intensity is sufficiently magnified so that the emission intensity can be calculated electronically for digital display or fed to a chart recorder or computer.

Recent developments in detector technology have included the introduction of charge transfer devices (CTDs) such as the charge-coupled device (CCD) and charge-injection devices (CID). CTDs are multichannel photon-integrating detectors that accumulate signal information as light strikes them[177] and offer sensitivity and dynamic linear range capabilities that exceed all other types of light detector[180].

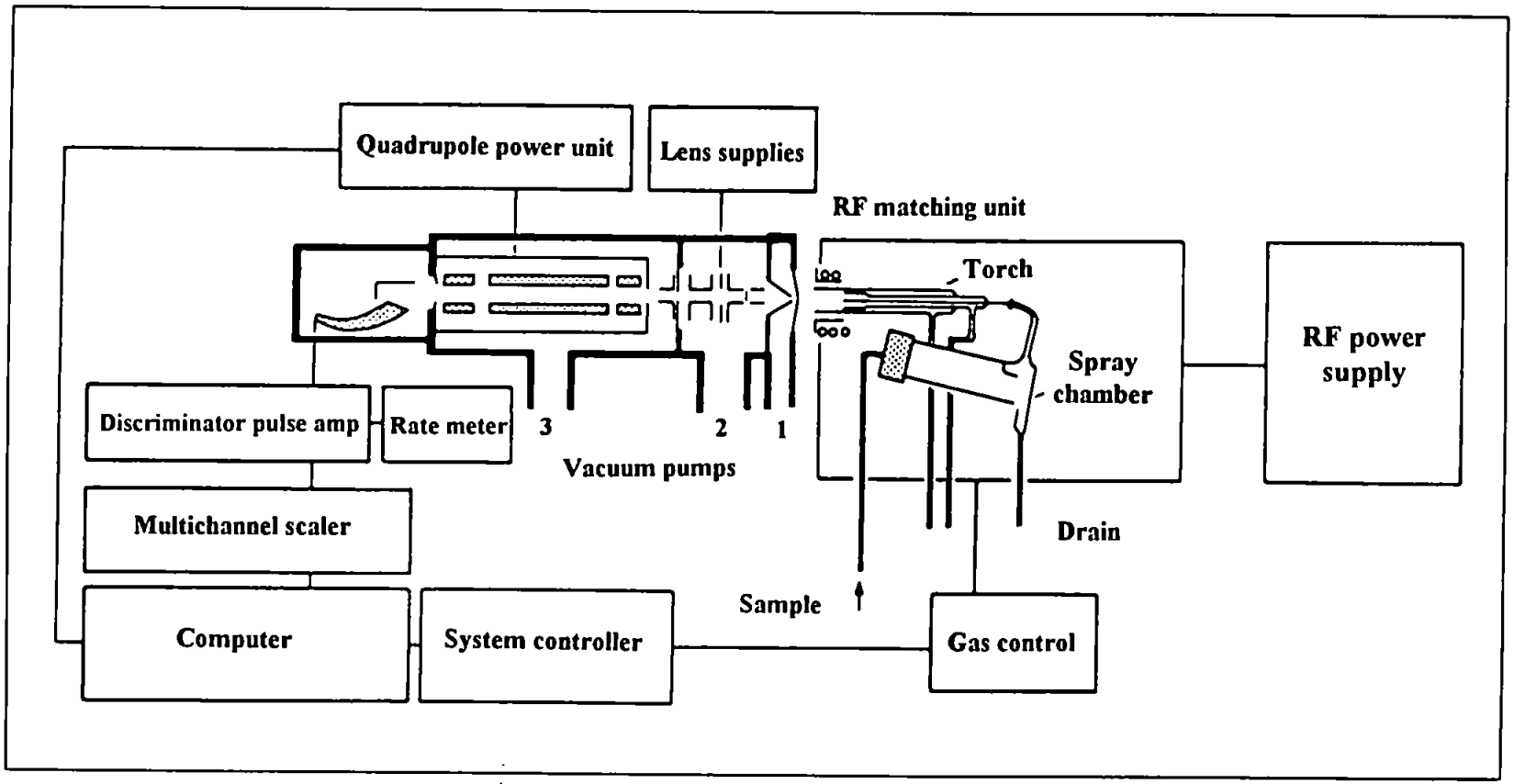
2.3 Inductively Coupled Plasma-Mass Spectrometry

The development of inductively coupled plasma-mass spectrometry (ICP-MS) began in the early 1970s, however a number of problems were initially encountered. These included the extraction of ions from the high temperature plasma (at atmospheric pressure) into the MS (under vacuum) and the effects of RF electric fields on mass analysis and ion detection of the ICP. Collaboration between Houk *et al.* in the USA and Gray *et al.* in the UK[181], in addition to the work of Douglas and French[182] overcame these problems and led to the development of the system and interface now employed in commercial ICP-MS instrumentation.

ICP-MS combines the benefit of plasma excitation as an ion source with mass spectrometry to achieve fully quantitative determinations across the mass range. The main benefits of the techniques are high sensitivity and selectivity, large dynamic linear ranges and multielement and isotopic capabilities[175]. Limits of detection for many elements are in the range 0.01-0.1 ng ml^{-1}, in many cases a factor of a thousand times better than for ICP-AES[175]. Disadvantages include high purchasing and operating costs and long term stability and sensitivity problems which as a result of environmental temperature fluctuations require operation in a temperature controlled laboratory. However the major limitation is a susceptibility to a range of non-spectroscopic (matrix) and spectroscopic interferences, particularly polyatomic ion interferences[178].

A typical design of an ICP-MS instrument is shown in Figure 2.6. The sample introduction system and plasma are fundamentally the same as those described in Section 2.2 for ICP-AES. Most commonly liquid samples are introduced into the ICP using a pneumatic nebuliser in conjunction with a spray chamber[175]. Processes occurring in the plasma include desolvation, vaporisation, atomisation, excitation and ionisation. Extraction of ions from the plasma into the vacuum system of the mass spectrometer is achieved using a two stage rotary pumped interface. The interface consists of two cones (usually nickel), called the sampler and skimmer, positioned end-on to the ICP. The supersonic jet of ions formed passes through a series of chambers held at increasingly lower pressures and is focused by a series of electrostatic ion lenses. Finally the ion beam enters the mass analyser which can be either a quadrupole or magnetic sector design[178]. Positive ions are then separated with respect to their mass to charge ratio (m/z), the latter usually being equal to one. Detection is usually performed using an electron multiplier and the data then transferred to a computer via a multi-channel analyser.

Figure 2.6: Components of an Inductively Coupled Plasma-Mass Spectrometer

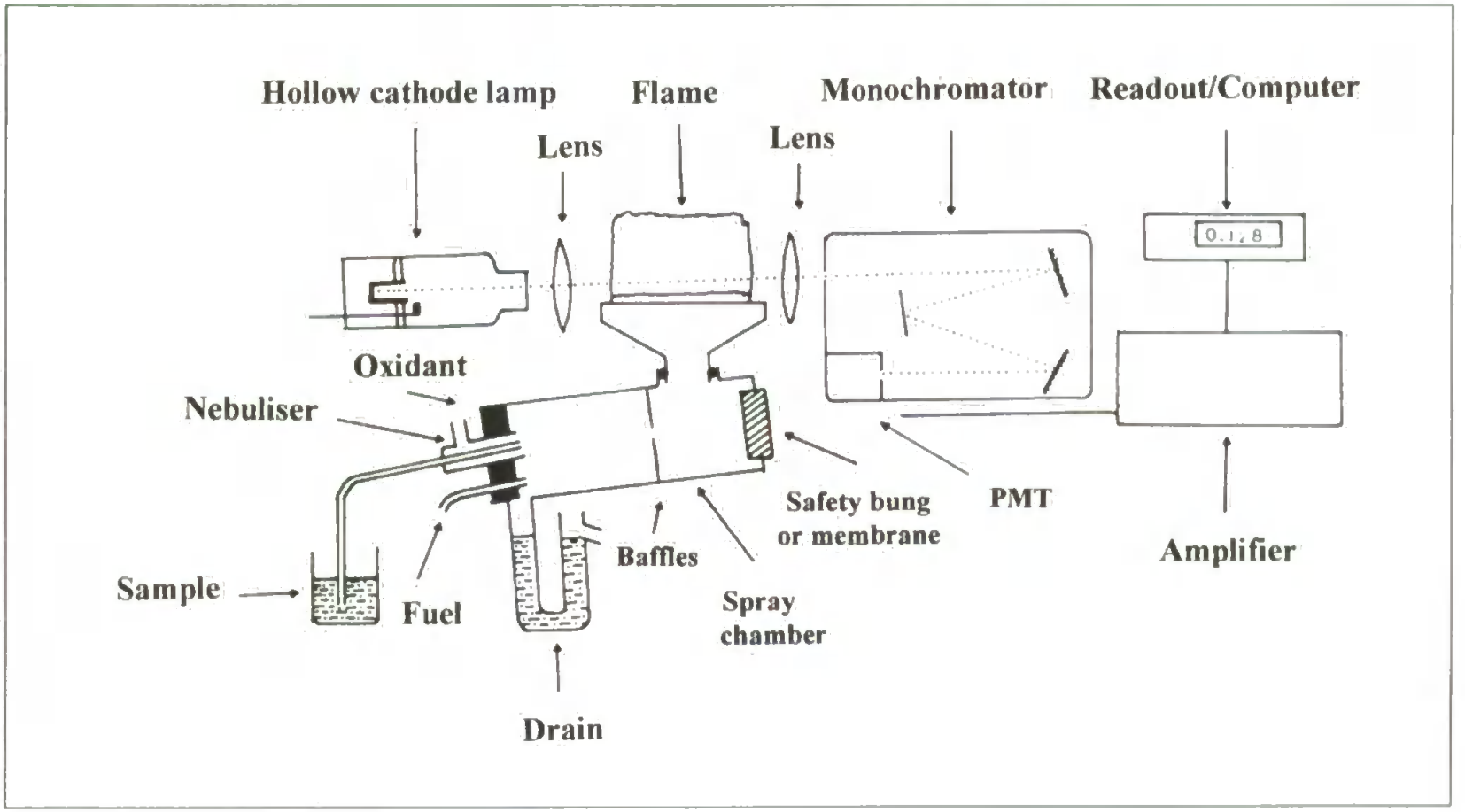


2.4 Flame Atomic Absorption Spectrometry

Theoretically the AAS technique was considered possible for many years however its development was considerably delayed because of the apparent need for high resolution to achieve quantitative measurements[163]. Initial ideas involved the use of a continuum light source however a monochromator capable of a resolution of 0.001 nm would then be necessary to isolate the atomic absorption lines of interest. In 1955 Walsh[183] overcame this obstacle by the realisation that light sources were available for many elements which emitted atomic spectral lines at the same wavelengths as those at which absorption occurred[164]. The utilisation of a hollow cathode lamp as a light source from which light is emitted at discrete wavelengths only, required a monochromator capable only of filtering out the other lines produced by the lamp, (e.g. from the filler gas). In any case these other lines would be well separated from the lines of interest and thus a high resolution monochromator was not required. Walsh further pursued this original idea and by 1965 the AAS technique was in general use for many different applications[173].

Today AAS is one of the most widely used analytical techniques with a capability of determining about 65 metal and metalloids[184] with limits of detection commonly in the ng ml^{-1} range. Limitations of the technique include limited linear calibration ranges of generally 10^2-10^3 and a number of interferences, mainly due to chemical and ionisation effects. A typical design of a flame atomic absorption spectrometer is shown in Figure 2.7 and an explanation of the individual components given in Sections 2.4.1-2.4.6.

Figure 2.7: Components of a Flame Atomic Absorption Spectrometer



2.4.1 Sample Introduction

Nebulisation of the sample is commonly achieved by use of a pneumatic nebuliser[185]. The oxidant (usually air) doubles as the nebuliser gas, issued at high velocity from a narrow jet which concentrically surrounds a central capillary through which the sample solution is aspirated[164]. The high velocity of the gas creates a drop in pressure which sucks up, draws out and shatters the liquid into tiny droplets[163]. In addition an impact bead may be placed in the path of the aerosol to provide a secondary fragmentation and thus improve nebulisation efficiency[163]. After mixing with the fuel and oxidant the aerosol passes into the spray chamber. Here the larger droplets collect on the walls of the chamber and only the finest particles are transferred to the burner head. The transport efficiency is generally between 4 and 8%[164].

2.4.2 Flame Conditions

The flame of choice must be sufficiently hot in order to desolvate, vaporise and atomise the samples repeatably to the same extent in order to avoid erroneous results[164]. The most routinely used flame is the air-acetylene flame providing a temperature of ca. 2200 °C[164]. However this is not sufficient to break the element-oxygen bonds of some elements such as aluminium and silicon which form refractory oxide compounds. In these cases a hotter nitrous oxide-acetylene flame, providing a temperature of 3000°C, can be employed[164]. It is also important to remember that flame chemistry plays a vital role and that the break down of refractory metal oxides is also aided by the chemically reducing environment maintained in the fuel-rich atmosphere.

2.4.3 Hollow Cathode Lamp

As previously stated the external light source is commonly a hollow cathode lamp[164], where the element of interest or one of its alloys lines the cathode positioned at the centre of the lamp. The lamp is filled with inert gas, usually neon or argon at low pressure[163]. When a voltage of about 500 V[163] is applied between the cathode and anode, positively charged ions, generated from the filler gas, are attracted to the surface of the cathode[185]. The bombardment of these ions on the inner surface of the cathode cause metal atoms to be dislodged (sputtered) from the cathode. These atoms pass into the region of the intense discharge where they meet a concentrated stream of gas ions and excited noble gas atoms and are hence excited to radiate their spectral lines[185]. In order to achieve high intensity and stability the discharge is confined to the central cathode region by means of a number of sheets of insulator material, commonly composed of mica, glass or ceramic material. The end window of the lamp is commonly made from quartz or optical silica if necessary to transmit UV light[164]. The light beam is positioned parallel to the burner head, the exact position of which is optimised to give maximum signal intensity in the detector.

2.4.4 Monochromators

A grating monochromator is necessary to isolate the wavelengths of interest from the other lines emitted from the flame/sample and from the hollow cathode lamp (e.g. from the filler gas). The emitted radiation enters the monochromator through the entrance slit and is focused by a mirror onto the diffraction grating. The grating disperses the radiation to produce a spectrum of light across the exit slit which acts as a window to isolate the particular wavelength of interest[164]. By rotating the grating, the analytical wavelength of interest can be passed through the exit slit and be focused onto the detector[184].

2.4.5 Background Correction

When using a line source such as a hollow cathode lamp, in addition to atomic absorption, absorption from molecular species and non-specific absorption including scattering from particulates are also observed. Non-specific absorption is a particular problem at shorter wavelengths (< 300 nm) and can lead to positive errors in the analytical measurement[163]. However this problem can be overcome by the use of background correction techniques. The simplest form involves passing a continuum source of radiation, such as that provided by a deuterium arc, through the flame. When a sample is atomised, broad-band non-specific absorption will occur across the entire spectral slit and absorption of the deuterium lamp emission will take place. In addition although the analyte atoms will also absorb a proportion of the continuum signal, atomic lines are very narrow (10^{-3} nm) and so the total amount of energy absorbed across the whole spectral slit (for example 0.1 nm) is very small when compared to the non-specific absorption[184]. Even if 100% absorption of the radiation from the hollow cathode lamp occurred a maximum of only 1.5% of the continuum radiation would be absorbed[185]. Having such a small contribution the atomic absorption is effectively ignored and the absorption of the deuterium lamp is taken to represent the non-specific absorption of the sample. In practice background correction is achieved by employing a beam splitter to switch between the hollow cathode and deuterium lamp signals. This approach enables subtraction of the background signal (deuterium lamp) from the analyte plus background signal (hollow cathode lamp) to give a corrected analyte measurement.

2.4.6 Detection

A photomultiplier is usually employed for conversion of the light to an electrical signal, the principles of which are explained in Section 2.2.4. During this process the signal intensity is sufficiently magnified so that the absorbance can be calculated electronically for digital display or fed to a chart recorder or computer.

2.5 Atomic Fluorescence Spectrometry

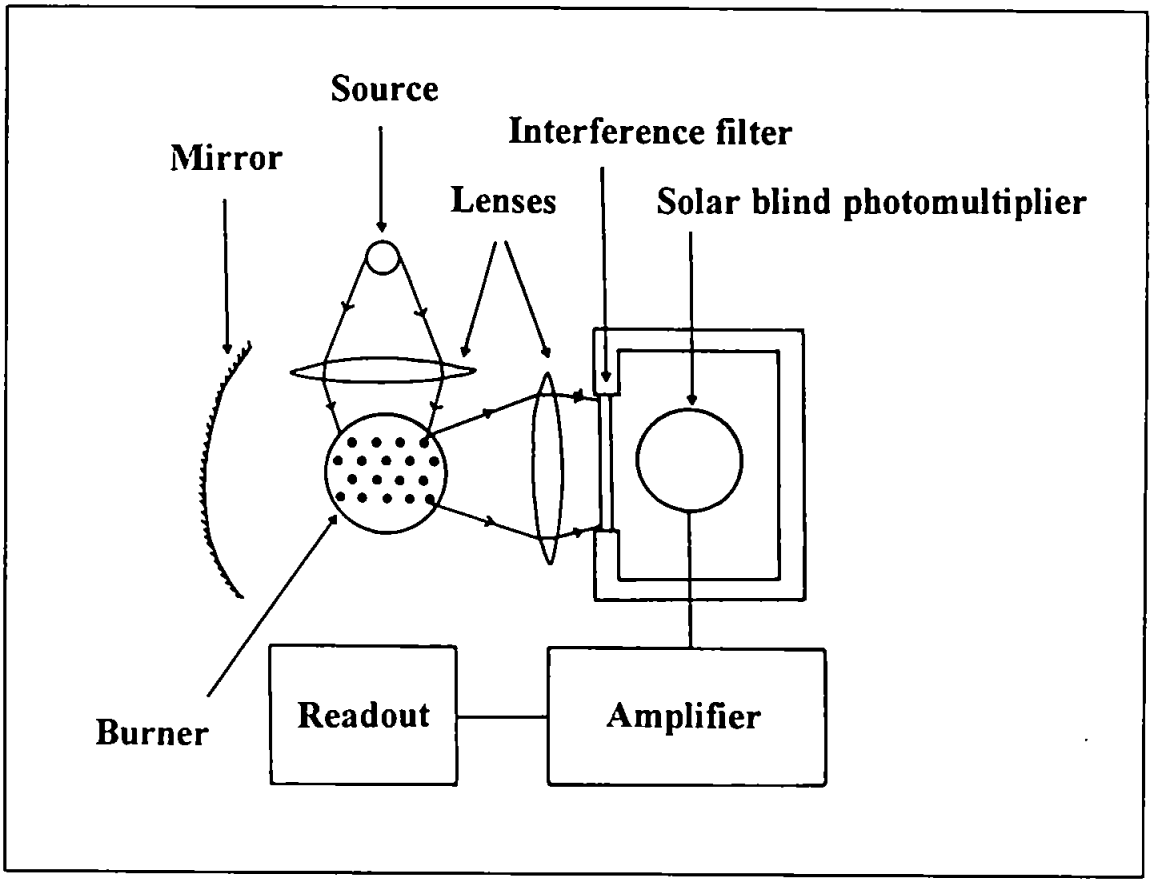
The analytical potential of AFS was not realised until 1962, being suggested by Alkemade and further demonstrated by Winefordner in 1964[186]. In contrast to AAS the fluorescence signal is proportional to the intensity of the excitation source and thus a high intensity, stable line excitation source is required to achieve sufficient sensitivity. It was this requirement which initially hindered the development of the technique. Continuum sources do not provide sufficient intensity[164], particularly in the UV region of the electromagnetic spectrum; xenon arc lamps proved problematic due to scatter and vapour discharge lamps although successful are limited in their range. Hollow cathode lamps were also investigated however are insufficiently powerful, unless pulsed[163]. Lasers are very intense, however due to their high cost have not been routinely used for this application[163]. Microwave-excited electrodeless discharge lamps are also very intense (200-2000[163] times more so than hollow cathode lamps) and have been widely used however they often lack stability. Boosted hollow cathode lamps have also been developed[187] which are capable of a high intensity emission but are more expensive than the conventional hollow cathode lamps. Generally the latter two, electrodeless discharge lamps and boosted hollow cathode lamps, are most commonly used as excitation sources.

Most atomic fluorescence spectrometers are simple and inexpensive and their performance combines good detection limits with high selectivity, few spectral interferences and long linear calibration ranges. However at high analyte concentrations reduction in the fluorescence intensity can occur due to self absorption. The AFS technique has recently found most application in combination with hydride generation techniques (see Section 2.6.1) to give very impressive limits of detection, commonly at the low ng l^{-1} level. Sensitivity is greatest in the far UV region in contrast to techniques such as AAS and AES, however sensitivity is poorer

in the visible region. A disadvantage of the technique is quenching, the term used to describe the process of collisional de-excitation due to collisions of excited atoms with molecules, radicals and elements present in the atomiser, resulting in a reduction in the fluorescence intensity[188].

The design of a typical non-dispersive atomic fluorescence spectrometer is shown in Figure 2.8. The instrument is essentially composed of a line source, the beam of which is focused into the atom cell by a lens. An argon-hydrogen diffusion flame although low-quenching and possessing a low radiative background is too low in temperature to prevent severe chemical interferences occurring[163]. The use of an inert gas sheathed (separated) flame, such as the most commonly used argon separated air-acetylene flame, reduces the flame background emission intensity so that an interference filter may be used instead of a monochromator[164]. A collector lens is positioned perpendicular to the line source to focus the fluorescence signal onto the filter. Light of the desired wavelength is then passed into the detector, commonly a photo-multiplier tube and the output is transferred through an amplifier to the readout device. Due to the very small magnitude of the fluorescence signal the design of the optical assembly is critical in order to efficiently collect all of the light and to avoid degradation of the signal via scatter etc. For dispersive instruments the use of a monochromator is necessary to reduce the amount of flame background emission falling upon the detector which would otherwise give an excessively noisy signal[164].

Figure 2.8: Components of a Non-Dispersive Atomic Fluorescence Spectrometer



2.6 Vapour Generation Techniques

Vapour generation techniques can be used in combination with a number of atomic spectroscopy techniques in order to dramatically improve limits of detection of selected elements (As, Cd, Sb, Se, Te, Bi, Sn, Pb and Ge) by between 10 and 1000 times[188]. This is achieved by merit of more efficient sample introduction, pre-concentration and matrix removal.

2.6.1 Hydride Generation Techniques

In the late 1960s the inherent specificity and sensitivity of the atomic absorption technique made it the technique of choice for many applications. However sensitivity was poor for a number of elements whose optimal analytical absorption lines were located in the far ultraviolet region of the electromagnetic spectrum, such as arsenic and selenium. In 1969 Holak[189] developed a method for the determination of arsenic by AAS after arsine generation by reaction with zinc and HCl. He noted that the potential for spectral interferences were substantially reduced, in comparison to conventional sample analysis, since the analyte is separated from the sample matrix. In addition an inefficient nebulisation step was avoided and transport efficiency was close to 100% efficient. Since then hydride generation has become a well established technique and has been successfully extended to the other hydride forming elements.

In early work the formation of hydrides using HCl and zinc or some other metal was hindered by the long reaction time, in excess of 20 minutes[190], resulting in broad analyte response peaks. In addition only As, Se and Sb hydrides could be formed and there seemed little possibility for automation of the technique. The use of sodium borohydride ($NaBH_4$) as a reductant was introduced by Braman *et al.*[191] in 1972 and solved many of the earlier problems. In acidified solution hydrides are generated according to Reaction 2.1[190].

$$NaBH_4 + 3H_2O + HCl \xrightarrow{E^{m+}} H_3BO_3 + NaCl + 8H\cdot \rightarrow EH_n + H_2 \text{ (excess)} \quad \textbf{(Reaction 2.1)}$$

where E is the element of interest and m may or may not equal n.

The use of sodium borohydride has now virtually replaced the metal-HCl reaction, having a number of distinct advantages. Hydride generation is possible for all the hydride forming elements. Reaction times are faster (generally 10 to 30s[190]) thus avoiding the need for a collection reservoir and providing potential for automation of the system[192,193]. For this purpose instead of dropping sodium borohydride tablets into the reaction vessel aqueous solutions can be prepared. The acidified sample and sodium borohydride can then be pumped into a reaction vessel, usually a gas-liquid separator, and the volatile hydrides formed swept from the aqueous phase into a suitable detector by means of a carrier stream of inert gas such as argon, helium or nitrogen. A number of detectors such as AAS[194] (with flame or electrically heated atomisation), AFS[195], direct current plasma-atomic emission spectrometry (DCP-AES)[191], microwave induced plasma (MIP)[196], ICP-AES[197] and ICP-MS have been coupled with the hydride generation technique although AAS remains the most commonly used approach to date[177]. In this case the hydrides are often swept into a quartz T-tube mounted in the flame where dissociation to their constituent atoms is achieved.

One problem of both the metal/acid and sodium borohydride/acid methods is interferences from a number of cations and anions. Spectral interferences are rarely encountered as the elements of interest are separated from the matrix constituents. However chemical interferences are more problematic. Sodium borohydride is a strong reducing agent which is capable of reducing a number of transition metals e.g. Cu^{2+}, Co^{2+}, Ni^{2+} to the free elements. These species may interfere with the hydride generation process by a number of mechanisms including consumption of the reducing agent, formation of metals which react with the hydride, adsorption of hydrogen and disturbance of hydride transfer to the gas phase. Such problems can be overcome by a number of techniques such as separation by solvent extraction, co-precipitation or ion exchange and the addition of releasing and masking reagents[164].

2.6.2 Determination of Mercury by Cold Vapour Generation Techniques

The determination of mercury by FAAS or ICP-AES is unsuitable for many environmental applications due to low limits of detection, typically in the range 0.1-1 mg l^{-1} [175]. This is because at the most sensitive atomic absorption line (184.9 nm) most flame gases and the atmosphere also absorb strongly, thereby degrading sensitivity. Major improvements to sensitivity can be made by use of the cold vapour (CV) technique, originally developed in 1968 by Hatch and Ott[198]. The technique is based upon the reduction of inorganic mercury to elemental mercury by reaction with a suitable reductant such as tin (II) chloride or sodium borohydride. Elemental mercury has an appreciable vapour pressure at room temperature and exists as a monatomic vapour. Therefore when a carrier gas (usually argon, nitrogen or air) is flushed through the solution the elemental mercury is rapidly volatilised into the gas stream and can be swept into a suitable detector, usually AAS or AFS. Mercury is present in the elemental form and so no heat source is required to atomise the species prior to detection.

For the analysis of mercury by the CV-AAS technique detection limits of 0.2 ng ml^{-1} can be achieved[184] by sweeping the mercury vapour into a long path-length absorption cell, usually a glass tube atom cell. This is commonly positioned on the top of a conventional AAS burner head (without the flame) and a hollow cathode lamp is used as the light source. Sensitivity can be improved to low ng l^{-1} levels by utilising an AFS detector. In this case the mercury vapour can be swept out of an open, circular tube immediately adjacent to a suitable source such as a small mercury vapour lamp. Argon may be utilised to minimise quenching of the fluorescence signal[163], since it has negligible quenching cross-section in comparison to gases such as nitrogen or oxygen. For both AAS and AFS detection a water trap or membrane dryer are commonly employed to avoid problems caused by condensation in the atom cell[199].

3. Batch Microwave Digestion Methods for Botanical Samples

3 BATCH MICROWAVE DIGESTION TECHNIQUES FOR BOTANICAL SAMPLES

3.1 The Determination of Trace Elements in Tea using Microwave Digestion at Atmospheric Pressure

3.1.1 Introduction

In the UK on average 1 litre of tea is consumed per person per day[200]. The accurate determination of the trace element content of tea is thus important for the assessment of potential implications to health. Aluminium is an important element to consider since it is associated with a number of health conditions including neurological and behavioural disorders and is accumulated in high concentrations by the tea bush (*Camellia sinensis*). Concentrations of up to 23,000 μg g^{-1} Al[201] have been reported in tea leaves which is far higher than for other plants which do not normally exceed 200 μg g^{-1} [202]. However tea may also have nutritional qualities, for example it has been suggested that tea provides a valuable source of a number of minerals e.g. calcium, magnesium, manganese, potassium and zinc[200].

Several papers have been published reporting the aluminium content of a variety of tea leaves[201-204], although the results obtained are often in poor agreement. Coriat and Gillard[201] reported dry weight concentrations (by gravimetric analysis) of aluminium in tea leaves from China, India and Russia of 8,700 μg g^{-1} to 23,000 μg g^{-1} which is consistent with the levels of up to 20,000 μg g^{-1} quoted by Talibudeen and Sivasubramania[202]. However conflicting results have since been generated by Koch *et al.*[209] who reported far lower levels of aluminium in a range of teas from India and Africa of between 400 and 1000 μg g^{-1}. This was achieved by a nitric/perchloric acid hotplate digestion followed by ICP-AES analysis. A hotplate digestion with nitric and sulphuric acid and analysis by ICP-AES and AAS was

employed by Wang *et al.*[204] for the digestion of the certified reference material NIES No. 7 'Tea Leaves', however results for a range of elements were generally low. Using this method aluminium levels of between 916 and 1299 μg g^{-1} were determined in samples of Taiwanese tea.

Little work has been published regarding the use of microwave digestion techniques for tea leaves. Kojima *et al.*[205] employed a closed vessel pressurised microwave digestion technique for the analysis of biological and botanical samples, including tea leaves. A number of trace elements were determined in the certified reference material NIES No.7 'Tea Leaves' however aluminium was not included. In addition this procedure employed hydrofluoric acid which may be of restricted use in some laboratories.

Discrepancies also exist in the literature regarding the aluminium levels found in tea liquors. Coriat and Gillard[201] reported levels of 40-100 μg ml^{-1} for tea samples infused for up to 30 minutes, whereas Fairweather and Tait[206] suggested far lower levels of between 2.7 and 4.9 μg ml^{-1} for 1 g of tea infused in 100 ml of water. Koch *et al.*[203] carried out similar analyses with results more in agreement with those of Fairweather and Tait[206], quoting aluminium levels of 0.9 to 3.2 μg ml^{-1} for 0.5 g of tea infused in 45 ml of water for 5 minutes. In addition Takeo[207] undertook trace element determinations of the Japanese Sen-cha and Kamairi-cha teas, prepared by infusing 3 g of tea, in 200 ml of boiling water, twice. They reported lower aluminium levels of 0.8 to 1.41 μg ml^{-1}.

It was thus decided to base the preliminary studies reported in this thesis on a comprehensive study of the trace element content of tea leaves and tea liquors prepared from teas from a range of origins.

3.1.2 Experimental

3.1.2.1 Instrumentation

3.1.2.1.1 Microdigest 301

Microwave digestions were carried out in a single mode reflux microwave system (Microdigest 301, Prolabo, Paris, France) with a maximum power setting of 200 W. This is an open focused microwave system, the principles of which are described in Section 1.3.2.2. Digestions were undertaken in glass vessels with a maximum sample capacity of 2 g for solid samples and 50 ml for liquid samples. A reflux column (Prolabo) was situated above the sample flask to help minimise the loss of volatile elements to the atmosphere. Acid vapours evolved during the course of the digestion were effectively neutralised by use of an Aspivap fume treatment system (Prolabo). For this purpose the suction rate can be adjusted between 0 and 12 l min^{-1} as required. In addition evaporation to dryness can be undertaken in situ by lowering the aspiration head which effectively increases the suction rate of the Aspivap fume treatment system.

The Microdigest 301 was controlled by a microprocessor TX32 programmer (Prolabo), allowing control of the power output (in steps of 10 W) and the heating time (in 1 minute intervals). The TX32 programmer also controls a PS 11 3-way pump (Prolabo) which allows up to 3 reagents to be dispensed into the digestion flask at any stage of the procedure. The programmer allows accurate control of the volume of each reagent dispensed (in increments of 1 ml) and also the speed of addition. A total of 100 programmes, each of which can be comprised of up to 10 successive digestion steps can be stored in the memory.

3.1.2.1.2 Liberty 200 ICP-AES

Analysis of all samples was performed using inductively coupled plasma-atomic emission spectrometry (Liberty 200, Varian Instruments Ltd, Warrington, UK). Samples are introduced by way of a peristaltic pump. After passing through a V-Groove nebuliser (see Section 2.2.1) the aerosol formed enters a Sturman-Masters double-pass spray chamber, made from inert material. The RF generator is a solid-state crystal control locked type with direct serial coupling (DISC). This directly couples the RF tuning circuitry to the load coil and ensures that the tuning circuit responds to load changes in the shortest possible time, thus sustaining high plasma stability. The instrument employs a conventional sequential scanning 0.75 m Czerny-Turner monochromator with holographic gratings (1800 grooves/mm). For detection across the full wavelength range (150 to 900 nm) two photo-multiplier tubes were employed. A solar blind PMT was used for wavelengths below 300 nm and a wide range PMT for higher wavelengths. For determinations below 190 nm the monochromator was evacuated by pumping down to 200 mTorr and purging with argon until a steady vacuum is achieved.

Since the ICP-AES analysis was multielement in nature default conditions were used throughout except for the independent optimisation of the viewing height for each element. The operating conditions employed are given in Table 3.1. For calibration multielement working standards were prepared containing Al, Ba, Ca, Cu, K, Mg, Mn and Zn by diluting high purity 1000 mg l^{-1} stock solutions (Spectrosol, Merck) with deionised water and nitric, perchloric and hydrogen peroxide to obtain the same concentration as in the samples. Cadmium was not included in the study as the concentrations present were below the limit of detection of the ICP-AES technique. For the tea liquor analysis standards were prepared in

deionised water (unacidified) in plastic volumetric flasks. Sample blanks were also routinely prepared and were used to correct for potential contamination of the samples.

Table 3.1: Instrumental Operating Conditions for the Trace Element Analysis of Tea Samples by ICP-AES

RF frequency	40.68 MHz
Operating power	1 kW
Plasma gas flow rate	15 l min^{-1}
Auxiliary gas flow rate	1.5 l min^{-1}
Snout purge	Low
Torch type	Standard one piece quartz torch with 1.4 mm i.d. injector tube
Nebuliser type	V Groove
Nebuliser pressure	150 kPa
Sample flow rate	3 ml min^{-1}
Integration time	3 s
Peak search window width	0.08 nm
Viewing height	Optimised independently for each element
Background correction	Dynamic
PMT voltage	650 V [except for Ca (550 V)]

3.1.2.2 Reagents

Nitric acid (Analar), hydrogen peroxide (Analar) and 70% perchloric acid (Aristar) were obtained from Merck Ltd, Poole, Dorset, UK. Deionised water (18 $M\Omega cm^{-1}$) purified through a Millipore purification system (Millipore-Waters, Bedford, Massachusetts 01730, USA) was used for all dilutions. Digestion vessels, glass and plastic ware were thoroughly cleaned in 2% (v/v) Decon 90 (Merck), acid washed in 5% (v/v) nitric acid (Analar) and rinsed in deionised water prior to use. Samples were filtered where necessary (Whatman No.1 Qualitative filter paper, Merck) and stored in polyethylene bottles (Merck) prior to analysis. The microwave digestion procedure was validated by analysis of the certified reference material NIES No. 7 'Tea Leaves', obtained from the National Institute for Environmental Studies (Japanese Environment Agency). A number of other tea samples, obtained from a local supermarket, were also analysed by the optimised procedure.

3.1.3 Sample Preparation

3.1.3.1 Microwave Digestion Procedures

3.1.3.1.1 Nitric Acid Digestion

Initial experiments were undertaken using concentrated nitric acid as the sole digestion reagent. 0.25 g of Assam tea was weighed into a glass digestion vessel and heated with 5 ml of nitric acid at a power setting of 60 W. The heating time was systematically increased between 5 and 20 minutes. After cooling, samples were quantitatively transferred into 50 ml volumetric flasks and made up to volume with deionised water. The final solution was yellow in colour and contained a substantial portion of insoluble material. The amount of insoluble material decreased with increasing heating time, but after 25 minutes was still very prominent and thus filtration of the samples was necessary before analysis.

3.1.3.1.2 Nitric Acid and Hydrogen Peroxide Digestion

Due to the incompleteness of the nitric acid digestion procedure the addition of hydrogen peroxide to the sample was investigated. After initial heating with nitric acid at a power setting of 60 W, 5 ml of hydrogen peroxide was added and heating continued for a further 5-15 minutes. The resulting solution was paler yellow in colour than before and there was a marked reduction in the amount of insoluble material present in the final solution. However filtration of the samples was again necessary before analysis.

3.1.3.1.3 Results

Following microwave digestion, samples were anlaysed by ICP-AES for Al, Ca, Cu, Mg, Mn and Zn. The trace element content of Assam tea following microwave digestion with nitric acid alone and in combination with hydrogen peroxide at various heating times is given in Table 3.2. For the nitric acid digestions there is a general increase in levels with increasing heating time, however for the nitric acid-hydrogen peroxide digestions the effect of an increase in heating time does little to increase recoveries. Overall the results for the nitric acid-hydrogen peroxide digestion procedure are not significantly higher than those for digestion with nitric acid alone.

To examine whether the presence of undigested material has an effect on the recoveries of the elements under study a nitric and perchloric acid mixture was investigated due to the powerful oxidising properties of the latter in hot and concentrated solution.

3.1.3.1.4 Nitric and Perchloric Acid Digestion

0.25 g of tea leaves were initially digested with 5 ml of nitric acid at a power setting of 60 W for 15 minutes (t_1) in order to totally digest all of the easily oxidised organic matter in the sample. This was necessary before the perchloric acid addition in order to minimise the risk of explosion. 1 ml of 70% perchloric acid was subsequently added and heating continued for a further 5 to 20 minutes (t_2). As heating time increased, the resulting digest became more colourless and contained less insoluble particles. Only the t_2=5 minutes sample needed filtering. A clear solution was obtained after t_2=15 minutes and contained little undissolved matter.

Table 3.2: Comparison of the Trace Element Content of Assam Tea following Microwave Digestion with Nitric Acid, Alone and in Combination with Hydrogen Peroxide

Digestion Procedure	Al ($\mu g\ g^{-1}$)	Ca ($\mu g\ g^{-1}$)	Cu ($\mu g\ g^{-1}$)	Mg ($\mu g\ g^{-1}$)	Mn ($\mu g\ g^{-1}$)	Zn ($\mu g\ g^{-1}$)
HNO_3 (5 min)[1]	622±43[2](3.5)[3]	3825±188(2.5)	24.3±0.8(1.7)	1841±40(1.1)	684±22(1.6)	32.6±1.3(2.0)
HNO_3 (10 min)[1]	629±34(2.7)	3850±194(2.5)	24.5±1.1(2.2)	1865±64(1.7)	680±33(2.5)	37.4±2.4(3.2)
HNO_3 (15 min)[1]	624±37(3.0)	3782±222(2.9)	24.1±1.4(2.8)	1855±52(1.4)	699±32(2.3)	33.9±8.5(12.5)
HNO_3 (20 min)[4]	631±29(2.3)	4091±237(2.9)	23.9±1.2(2.5)	1922±53(1.4)	728±9.3(0.6)	-
HNO_3 (15 min), H_2O_2 (5 min)[1]	616±34(2.8)	3971±211(2.7)	28.8±1.5(2.7)	2075±61(1.5)	697±27(1.9)	30.5±7.1(11.6)
HNO_3 (15 min), H_2O_2 (10 min)[4]	637±45(3.5)	3956±212(2.7)	24.5±0.7(1.3)	1980±162(4.1)	749±40(2.6)	30.3±6.7(11.0)
HNO_3 (15 min), H_2O_2 (15 min)[4]	625±35(2.8)	3900±128(1.6)	24.3±2.3(4.7)	1975±75(1.9)	744±24(1.6)	30.4±5.5(9.1)
HNO_3 (20 min), H_2O_2 (5 min)[1]	636±17(1.3)	3982±34(0.4)	28.4±2.7(4.8)	2045±43(1.1)	718±33(2.3)	30.8±7.2(11.6)

Notes:
1: n=5
2: Uncertainties are calculated as twice the standard deviation
3: Values in parentheses are relative standard deviations (%)
4: n=3

Table 3.3 shows the results obtained for Assam tea following increasing heating time with perchloric acid. In addition results for the most effective nitric acid and nitric acid-hydrogen peroxide digestion procedures are included for comparison. Generally results for the nitric-perchloric acid procedure are higher than those obtained in the earlier procedures and thus this reagent combination was chosen for further investigations.

3.1.3.1.5 Optimisation Studies

Studies were undertaken to optimise the power setting and heating time for the two stages of the nitric-perchloric acid microwave digestion procedure (t_1 and t_2 respectively).

i) Power Setting

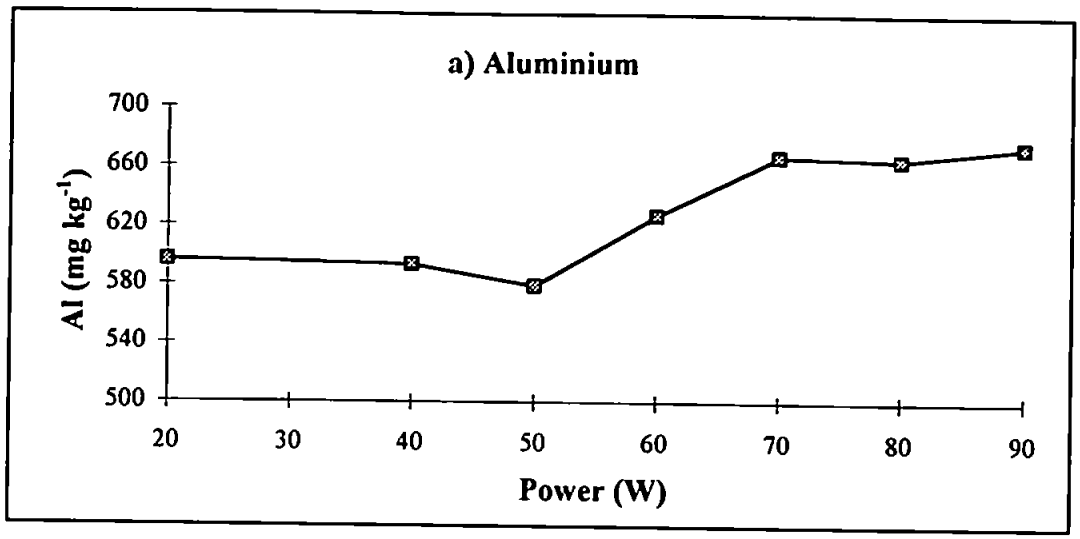
Samples of Assam tea were heated for 15 minutes with nitric acid followed by the addition of perchloric acid and further heating for 20 minutes. To optimise the power setting the power was systematically increased from 20 W to 90 W. The results obtained for Al, Cu, K, Mg, Mn and Zn (n=3) are given in Figure 3.1. Generally results improved with increasing power setting up to a maximum at 70 W power. At higher settings the reaction became very vigorous and the sample tended to boil up into the reflux column above the digestion vessel. It was therefore necessary to add anti-bumping granules to the sample flask. The lower recoveries experienced at ≥ 80W can therefore be explained by loss of sample rather than to incomplete digestion.

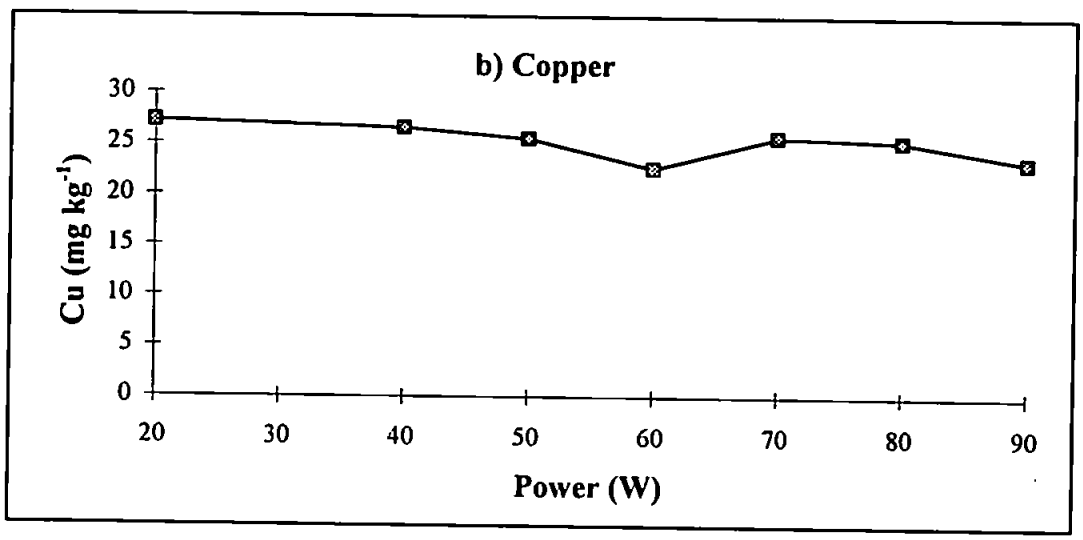
Table 3.3: Comparison of Results for Assam Tea following Microwave Digestion with Nitric Acid, Alone and in Combination with Hydrogen Peroxide and Perchloric Acid

Digestion Procedure	Al ($\mu g\ g^{-1}$)	Ba ($\mu g\ g^{-1}$)	Ca ($\mu g\ g^{-1}$)	Cu ($\mu g\ g^{-1}$)	Mg ($\mu g\ g^{-1}$)	Mn ($\mu g\ g^{-1}$)	Zn ($\mu g\ g^{-1}$)
HNO_3(15 min)	624±37[1](3.0)[2]	-	3782±222(2.9)	24.1±1.4(2.8)	1855±52(1.4)	699±32(2.3)	33.9±8.5(12.5)
HNO_3(15 min), H_2O_2(15 min)	625±35(2.8)	-	3900±128(1.6)	24.3±2.3(4.7)	1975±75(1.9)	744±24(1.6)	30.4±5.5(9.1)
HNO_3(15 min), $HClO_4$(10 min)	640±39(3.1)	32.1±1.4(2.2)	3878±481(6.2)	26.1±2.5(4.8)	1940±65(1.7)	683±88(6.5)	28.1±3.1(5.4)
HNO_3(15 min), $HClO_4$(15 min)	684±69(5.1)	32.9±2.9(4.4)	4014±376(4.7)	26.9±2.8(5.2)	2084±219(5.3)	701±91(6.5)	30.8±6.2(10.0)
HNO_3(15 min), $HClO_4$(20 min)	687±17(1.2)	33.3±3.3(4.9)	4197±238(2.8)	28.0±2.7(4.8)	2171±63(1.4)	734±30(2.0)	31.6±4.5(7.1)

Notes:
1: Uncertainties are calculated as twice the standard deviation
2: Values in parentheses are relative standard deviations (%).

Figure 3.1: Effect of Power on Elemental Recoveries for Assam Tea





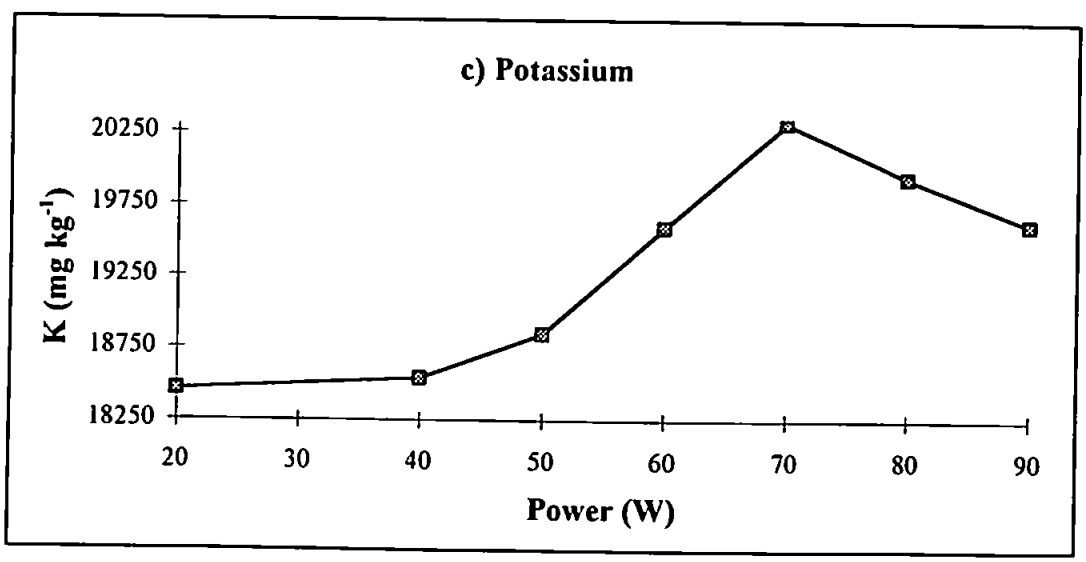
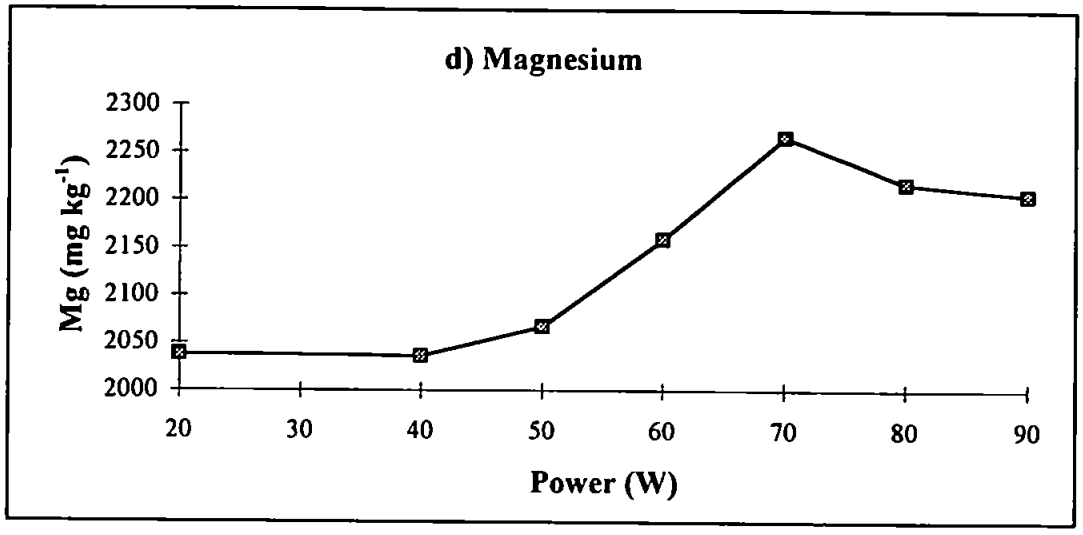
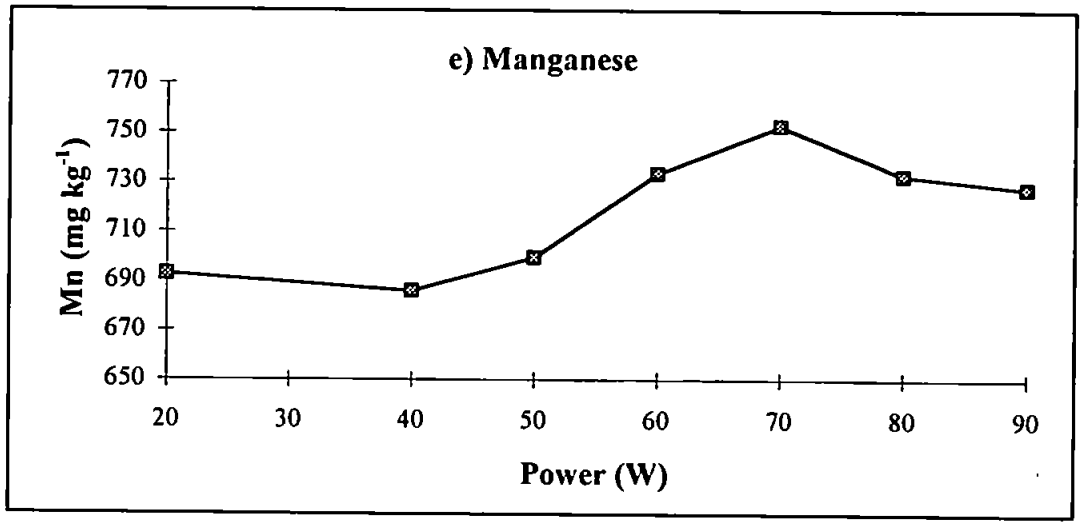
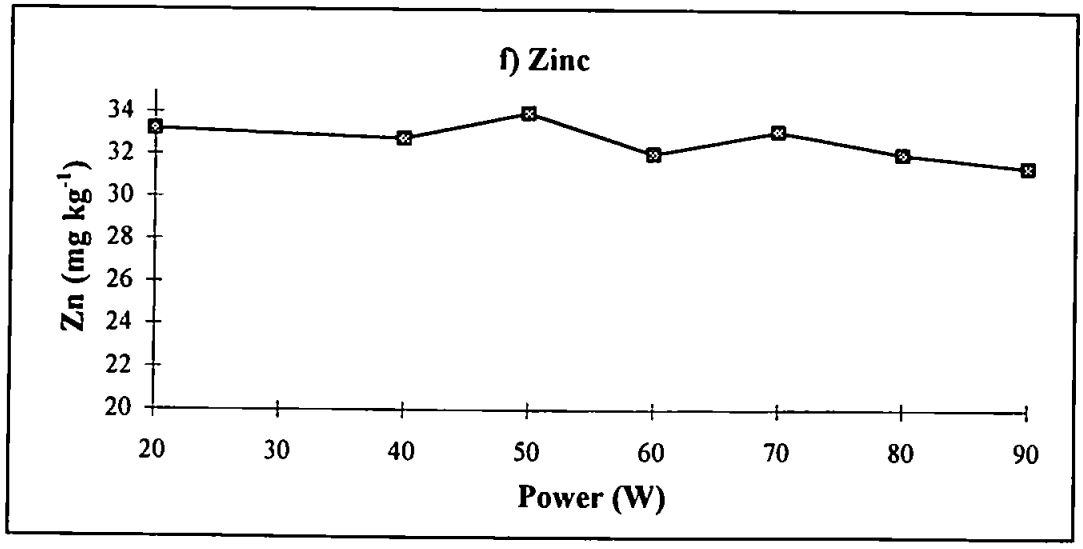


Figure 3.1: Effect of Power on Elemental Recoveries for Assam Tea Continued







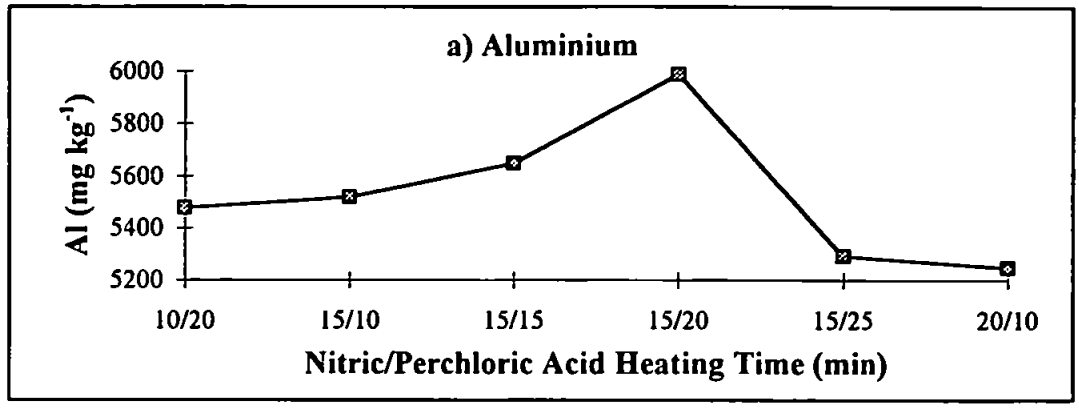
ii) Heating Time

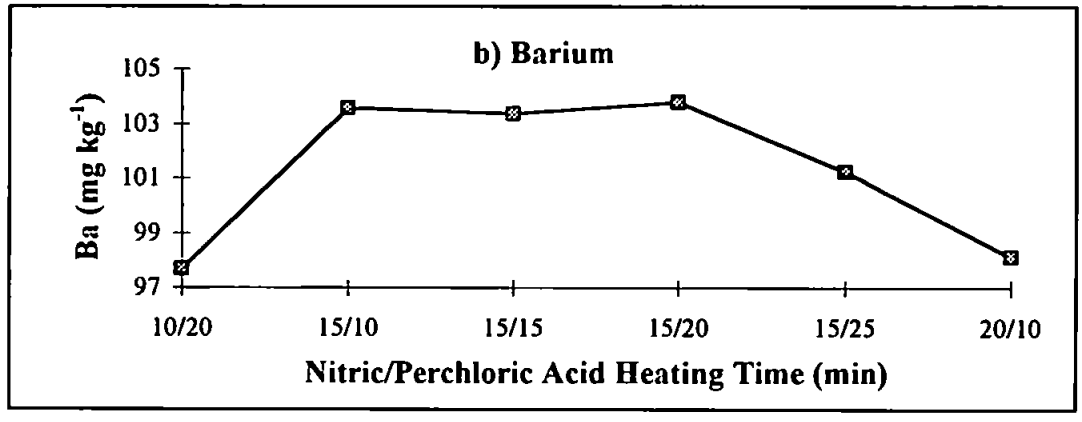
The power setting was then set at 70 W for various combinations of heating times for the two stages of the digestion procedure. Figure 3.2 shows the effect of heating times on the elemental recoveries using this two stage procedure. As can be seen optimum recoveries are generally obtained after 15 minutes heating with nitric acid and 20 minutes with perchloric acid. The lower recoveries for the samples heated for longer periods of time can again be explained by losses due to extensive heating rather than to incomplete digestion. Table 3.4 summarises the details of the optimised digestion procedure which was used throughout the rest of this work.

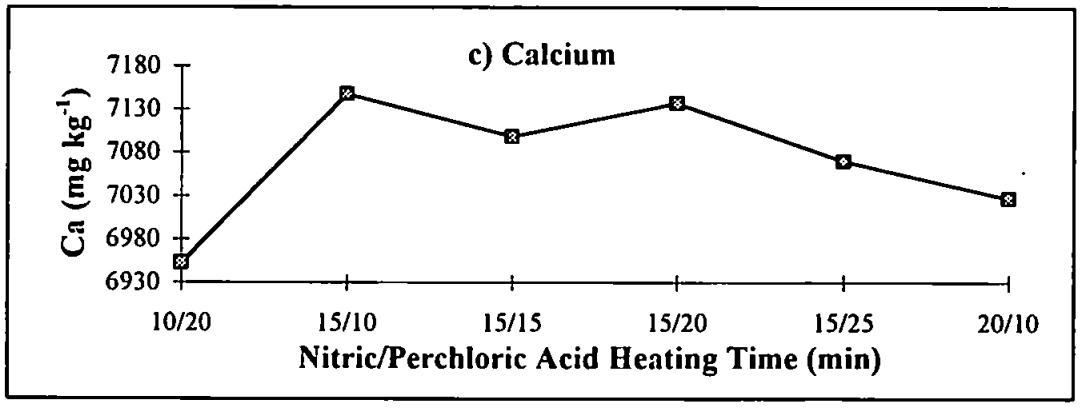
Table 3.4: Conditions for the Optimised Microwave Digestion Procedure

Step	Procedure
1	Heat 0.25 g of tea leaves with 5 ml of nitric acid, Power = 70 W, Time = 10 min.
2	Add 2 ml of deionised water and resume heating, Power = 70 W, Time = 5 min.
3	Add 1 ml of 70% perchloric acid and resume heating, Power = 70 W, Time = 5 min.
4	Add 5 ml of deionised water and resume heating, Power = 70 W, Time = 15 min.
5	Allow digest to cool, Make up to 50 ml with deionised water.

Figure 3.2: Effect of Heating Time on Elemental Recoveries using a Two Stage Digestion Procedure with Nitric and Perchloric Acid







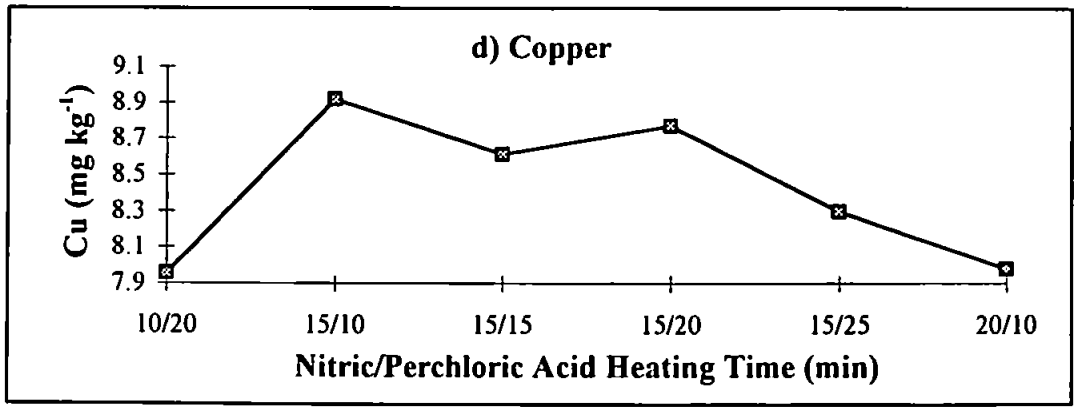
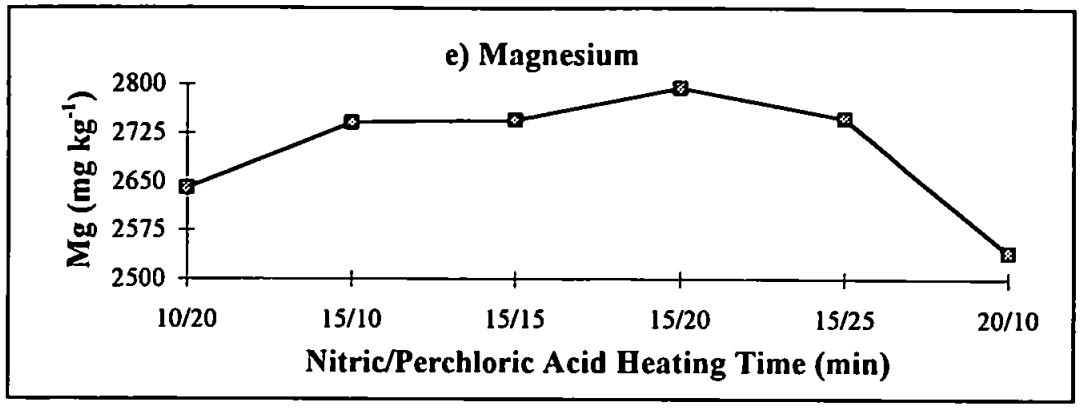
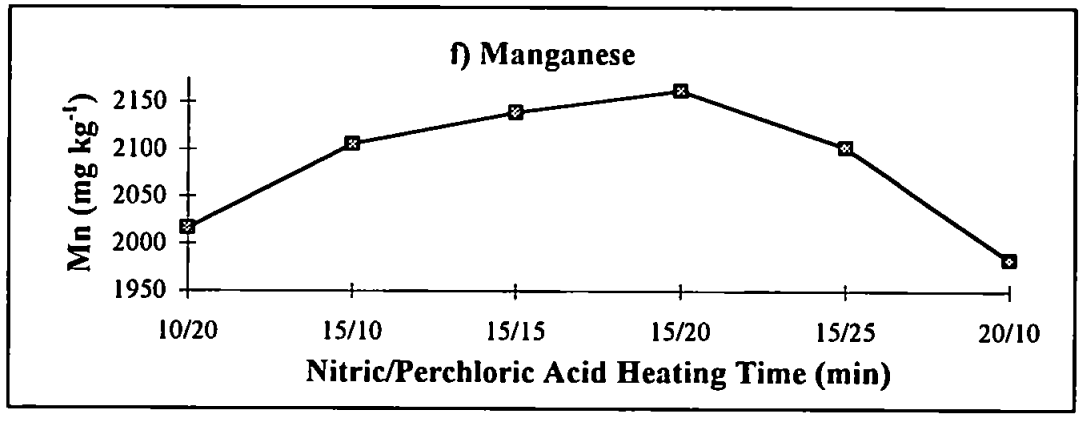
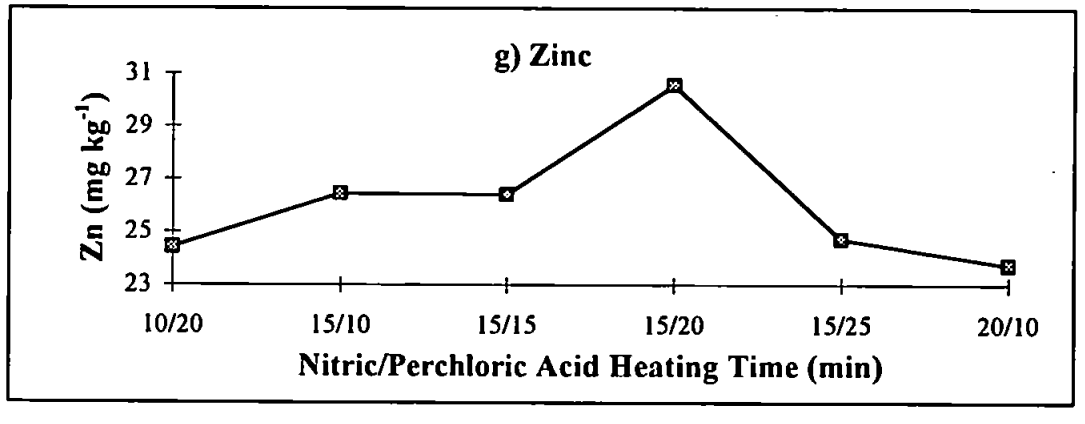


Figure 3.2: Effect of Heating Time on Elemental Recoveries using a Two Stage Digestion Procedure with Nitric and Perchloric Acid Continued







3.1.3.2 Hotplate Digestion Procedure

For comparison, hotplate digestion of the sample was also carried out, the details of which are given in Table 3.5. 0.25 g of tea sample was initially heated with 5 ml of nitric acid in a 100 ml glass beaker on a hotplate. Heat was supplied to maintain gentle boiling of the solution and the beaker was covered with a watch-glass to prevent loss of the sample. After heating for approximately 30 minutes, 5 ml of nitric acid was added and heating continued for a further 30 minutes. 1 ml of 70% perchloric acid was subsequently added and heating continued for 60 minutes until a clear colourless solution was obtained. This procedure was not vigorously optimised although it was found that the sample was not completely digested after only 30 minutes heating with perchloric acid.

Table 3.5: Conditions for the Hotplate Digestion Procedure

Step	Procedure
1	Heat 0.25 g of tea leaves with 5 ml of nitric acid for 30 min. Supply heat to maintain gentle boiling.
2	Add 5 ml of nitric acid and resume heating for 30 min.
3	Add 1 ml of 70% perchloric acid and heat for 60 min until a clear solution is obtained. When necessary add small aliquots of deionised water to the reaction to prevent evaporation to dryness.
4	Cool digest. Quantitatively transfer into a 50 ml volumetric flask and make up to volume with deionised water.

Throughout the digestion procedure it was necessary to add small aliquots of deionised water to the flask to prevent evaporation to dryness and thus reduce the risk of explosion. After cooling the digest was quantitatively transferred into a 50 ml volumetric flask, rinsing with deionised water. Washings from the watch-glass were also added before making the total volume up to 50 ml.

3.1.3.3 Comparison of Results for the Microwave and Hotplate Digestion Procedures

For comparison, results for the microwave and hotplate digestion of Assam and Yunnan teas are shown in Table 3.6. As can be seen results for the two digestion procedures are in good agreement, with neither giving predominantly higher or lower results.

Table 3.6: Comparison of Results for Assam and Yunnan Tea following Microwave and Hotplate Digestion

Element	Assam Tea (μg g^{-1})		Yunnan Tea (μg g^{-1})	
	Microwave Digestion[1]	Hotplate Digestion[2]	Microwave Digestion[3]	Hotplate Digestion[2]
Al	687±17[4](1.2)[5]	690±57(4.3)	1478±137(4.6)	1470±54(1.8)
Ba	33.3±3.3(4.9)	34.6±2.5(3.7)	25.9±2.2(4.2)	26.2±1.6(3.1)
Ca	4197±238(2.8)	4212±550(6.5)	5089±110(1.1)	4956±379(3.8)
Cu	28.0±2.7(4.8)	26.3±3.7(7.0)	22.0±0.6(1.3)	22.7±1.9(4.1)
Mg	2171±63(1.4)	2343±182(3.9)	2823±35(0.6)	2751±140(2.5)
Mn	734±30(2.0)	765±69(4.5)	891±23(1.3)	804±41(2.6)

Notes:
1: n=5; 2: n=4; 3: n=3
4: Uncertainties for the experimental results are calculated as twice the standard deviation.
5: Values in parentheses are relative standard deviations (%).

3.1.3.4 Tea Liquor Preparation Procedure

Experiments were carried out to examine the effect of the degree of agitation and the brewing time on the 'strength' of the tea liquor. It was found that constant stirring throughout a 5 minute brewing process produced an increase in the concentration of certain elements by as much as 250% when compared to samples prepared without agitation. Further experiments showed that samples brewed for 15 minutes (with continuous stirring) contained between 0% and 56% higher concentrations of certain elements than samples brewed for only 5 minutes. It was therefore important to ensure that when preparing the tea liquors that the degree of agitation and the brewing time were identical for each sample.

The procedure adopted for the preparation of the tea liquors is given in Table 3.7. 0.5 g of tea was infused in 50 ml of boiling deionised water for 5 minutes. The mixture was not agitated until 10 s before the end of the brewing time. The flask was then gently swirled before filtering and storing the liquor in a plastic bottle. Samples were analysed immediately by ICP-AES, to minimise the potential loss of trace elements by absorption onto the container walls.

Table 3.7: Tea Liquor Preparation Procedure

Step	Procedure
1	Infuse 0.5 g of tea in 50 ml of boiling water for 5 min.
2	Apply no agitation until 10 s before the end of the brewing time and then gently swirl the contents of the flask.
3	Filter the tea liquor and store in plastic bottle until analysis.

3.1.4 Results

3.1.4.1 Validation of the Optimised Microwave Digestion Procedure

The certified reference material NIES No. 7 'Tea Leaves' was digested using the optimised microwave digestion procedure (see Table 3.4) and also by the hotplate method (see Table 3.5). A clear, colourless solution was obtained in each case and thus filtration of the sample was not required. The results of the subsequent ICP-AES analysis are shown in Table 3.8. It can be seen that the microwave digestion results are in good agreement with the certified values. The precision of the method was also good with typical relative standard deviations of less than 5%. For the hotplate digestion results are also in good agreement with the certified values with the exception of slightly high zinc recoveries and slightly low results for aluminium. In addition the precision of the method for copper was poor.

Table 3.8: Comparison of Certified and Experimental Values for NIES No. 7 'Tea Leaves'

Element	Certified Value ($\mu g\ g^{-1}$)	Microwave Digestion ($\mu g\ g^{-1}$)	Hotplate Digestion ($\mu g\ g^{-1}$)
Al	775 ± 20	766 ± 23[1] (1.5)[2]	754 ± 22 (1.5)
Ba	5.7[3]	5.4 ± 0.04 (0.7)	5.7 ± 0.2 (1.7)
Ca	3200 ± 120	3213 ± 129 (2.0)	3139 ± 63 (1.0)
Cu	7.0 ± 0.3	7.0 ± 0.4 (2.7)	7.1 ± 3.1 (22)
K	18600 ± 700	18800 ± 1051 (2.8)	18776 ± 1109 (3.0)
Mg	1530 ± 60	1498 ± 70 (2.3)	1496 ± 74 (2.5)
Mn	700 ± 25	697 ± 3 (0.2)	689 ± 28 (2.0)
Zn	33 ± 3	35 ± 5 (6.4)	37 ± 2 (3.2)

Notes:
1: Uncertainties for the experimental results are calculated as twice the standard deviation.
2: Values in parentheses are relative standard deviations (%) and n=5 for all digestions.
3: Reference value.

3.1.4.2 Trace Element Analysis of Tea Samples from Different Origins

The optimised microwave digestion method (see Table 3.4) was finally employed to digest a range of tea samples originating from China, Japanese, Africa India and Sri Lanka. In addition a number of blends and a herbal tea were analysed. Prior to digestion coarse samples were ground in a pestle and mortar to aid digestion. Tea liquors were also prepared (see Table 3.7) and both digests and liquors analysed by ICP-AES for Al, Ba, Ca, Cu, Mg, Mn and Zn. The results are shown in Tables 3.9 and 3.10 respectively.

3.1.5 Discussion

3.1.5.1 Microwave Digestion of Tea Leaves

The results of the study highlighted the variability of trace metal concentrations in tea leaves from different origins. For the tea samples analysed it was found that generally aluminium levels fell between 500 and 1000 $\mu g\ g^{-1}$, although two samples contained higher concentrations i.e. Yunnan 1517 $\mu g\ g^{-1}$ and Bancha 3566 $\mu g\ g^{-1}$. Results are thus in agreement with those of Koch *et al.*[203] who quote aluminium in tea leaves at a concentration of between 400 and 1000 $\mu g\ g^{-1}$, and much lower than earlier workers who report concentrations of 8,700 - 23,000 $\mu g\ g^{-1}$ [201,202].

Camomile tea, the herbal tea, contained the lowest concentration of aluminium (542 $\mu g\ g^{-1}$) and manganese (101 $\mu g\ g^{-1}$). Ba, Cu and Zn levels were also generally low but the calcium and magnesium levels far higher than in the other teas analysed.

Table 3.9: Trace Element Content of a Variety of Tea Samples

Sample	Origin	Al ($\mu g\ g^{-1}$)	Ba ($\mu g\ g^{-1}$)	Ca ($\mu g\ g^{-1}$)	Cu ($\mu g\ g^{-1}$)	Mg ($\mu g\ g^{-1}$)	Mn ($\mu g\ g^{-1}$)	Zn ($\mu g\ g^{-1}$)
Assam	India	687 (1.1)[1]	33.3 (5.0)	4249 (0.7)	28.4 (3.4)	2171 (1.5)	734 (2.0)	31.3 (4.3)
Darjeeling	Himalayas (India)	619 (2.2)	35.1 (2.9)	4486 (7.5)	21.0 (5.7)	2433 (1.2)	551 (2.1)	32.8 (1.7)
Yunnan Pu-erh	China	1517 (1.8)	26.4 (2.0)	5089 (1.1)	22.0 (1.4)	2823 (0.6)	799 (1.9)	47.8 (0.2)
Gunpowder (green tea)	China	939 (1.4)	32.6 (5.7)	3782 (4.2)	13.2 (4.2)	2247 (1.2)	881 (3.3)	27.9 (3.0)
Earl Grey (blend)	China	917 (1.2)	22.9 (1.1)	3915 (2.0)	24.9 (0.3)	2330 (0.04)	873 (1.9)	33.9 (2.8)
China (blend)	China	861 (0.0)	24.0 (5.7)	3769 (1.6)	25.0 (2.8)	2623 (2.7)	731 (0.4)	44.2 (2.6)
Ceylon	Sri Lanka	856 (2.7)	14.7 (5.5)	4906 (0.5)	24.6 (1.3)	2203 (0.02)	310 (0.9)	39.5 (0.5)
Kenya	Kenya	631 (0.8)	15.3 (1.6)	3263 (0.9)	11.6 (3.8)	2124 (0.3)	842 (2.1)	26.7 (4.5)
Bancha	Japan	3566 (2.2)	33.3 (4.0)	6261 (0.9)	10.1 (9.7)	1847 (0.9)	2678 (2.6)	31.8 (12.1)
English Breakfast (blend)	Unknown	959 (3.5)	18.7 (3.0)	4741 (0.8)	29.6 (1.5)	2467 (0.8)	453 (1.6)	41.5 (8.7)
"Commercial Blend"	Unknown	781 (2.2)	17.5 (1.9)	3875 (0.1)	12.0 (1.3)	2137 (1.0)	1244 (0.2)	23.2 (1.5)
Camomile	Unknown	542 (4.9)	15.9 (1.8)	14302 (4.9)	12.0 (1.7)	5369 (4.7)	101 (4.8)	31.6 (1.8)

Notes:
1: Values in parentheses are the relative standard deviations (in %). In all cases n=3.

Table 3.10: Trace Element Content of Tea Liquors

Sample	Al (μg ml^{-1})	Ba (μg ml^{-1})	Ca (μg ml^{-1})	Cu (μg ml^{-1})	Mg (μg ml^{-1})	Mn (μg ml^{-1})	Zn (μg ml^{-1})
Assam	3.1 (44.8)[1]	0.0082 (2.5)	3.1 (7.4)	0.068 (24.0)	9.9 (45.4)	2.6 (35.6)	0.14 (45.1)
Darjeeling	1.9 (30.1)	0.0067 (1.9)	2.6 (5.8)	0.058 (27.5)	6.8 (27.9)	1.2 (22.5)	0.13 (40.3)
Gunpowder	1.4 (14.9)	0.0056 (1.7)	2.8 (6.8)	0.050 (37.7)	5.9 (26.4)	1.7 (19.2)	0.10 (36.1)
Yunnan	1.0 (6.5)	0.0078 (3.0)	2.6 (5.2)	0.019 (8.82)	5.1 (17.2)	1.3 (16.4)	0.11 (22.5)
Earl Grey	2.3 (24.8)	0.0061 (2.7)	2.6 (6.9)	0.053 (15.5)	6.6 (28.2)	2.0 (23.1)	0.14 (40.1)
China	1.5 (17.0)	0.0049 (2.0)	2.5 (6.6)	0.046 (18.2)	5.9 (22.4)	1.3 (17.9)	0.14 (31.6)
Ceylon	2.9 (33.7)	0.0040 (2.7)	3.2 (6.5)	0.073 (29.6)	7.3 (33.0)	0.8 (26.2)	0.16 (40.3)
Kenya	1.8 (28.4)	0.0031 (2.0)	2.7 (8.4)	0.035 (30.0)	8.5 (40.2)	3.1 (36.6)	0.15 (54.5)
Bancha	8.4 (23.4)	0.0319 (9.6)	4.1 (6.6)	0.017 (17.3)	4.9 (26.7)	4.7 (17.6)	0.11 (34.6)
English Breakfast	3.1 (32.5)	0.0067 (3.6)	2.9 (6.0)	0.076 (25.6)	7.5 (30.2)	1.1 (24.1)	0.15 (37.0)
"Commercial Blend"	3.0 (38.9)	0.0069 (4.0)	3.1 (6.8)	0.055 (46.1)	9.6 (44.9)	3.9 (31.2)	0.14 (60.7)
Camomile	0.03 (0.6)	0.0214 (13.4)	45.6 (31.9)	0.043 (36.1)	32.8 (61.1)	0.3 (25.1)	0.09 (28.5)

Notes:
1: Values in parentheses are the percentage of the element leached into solution during the brewing process and n=5 for all samples.

Excluding the herbal tea, a number of trends can be seen. The African tea (Kenya) contained the lowest aluminium levels (631 μg g^{-1}) (and the lowest calcium levels). The Indian teas (Assam and Darjeeling) contain the next highest levels of aluminium, followed by Ceylon tea and then the Chinese teas. The Japanese tea (Bancha) contains by far the highest concentration of aluminium (and calcium and manganese) but in contrast has the lowest copper concentration. The commercial blends of tea contain aluminium in the middle of the range, as may be expected.

3.1.5.2 Analysis of Tea Liquors

The majority of the tea liquors contained aluminium in the range 1.0 to 3.1 μg ml^{-1}. The exceptions were the Japanese tea, Bancha (8.4 μg ml^{-1}), and Camomile tea (0.03 μg ml^{-1}). Results are thus in good agreement with those of Fairweather and Tait[206] and Koch *et al.*[203] but are much lower than those of Coriat and Gillard[201]. Such comparisons must however be treated with care since the preparation of the samples varies greatly. For example Coriat and Gillard[201] do not state the amount of tea and water infused, they use local tap water and liquors are brewed for a much longer time of 30 minutes. Also infusions are carried out in a teapot which could be a significant source of contamination.

With regard to calcium, Camomile tea contained by far the highest concentration of 45.6 μg ml^{-1}. For the other teas, levels fell between 2.50 and 4.11 μg ml^{-1} and thus are higher than the results of Takeo[207] (1.55 to 1.74 μg ml^{-1}), but much lower than the 13-110 μg ml^{-1} reported by Gurses and Artik[208] in Turkish black tea liquors.

For Camomile tea magnesium levels were determined at 45.6 μg ml^{-1} and so are again far higher than the 4.9-9.9 μg ml^{-1} determined in the other tea liquors. Barium levels were also high but in contrast the concentration of manganese and zinc were the lowest of all the tea liquors analysed.

It is interesting to note the percentage of each element leached out of the tea leaves into the liquor during the brewing process. These values are displayed in parentheses in Table 3.10. It can be seen that although the percentage of each element leached into the liquor varies with the type of tea, certain elements do seem to be more readily leached than others. Predictably barium and calcium appear to be the least soluble elements (although high levels are leached for Camomile tea) and zinc the most soluble. The percentage of calcium leached into solution was determined at 31.9% for Camomile tea and between 5.2% and 8.4% for the other samples, thus is lower than the 10-12% reported by Jackson and Lee[209]. In contrast the percentage of aluminium leached into the liquor in this study is generally higher than the 15% recorded by Koch *et al.*[203]. Camomile tea is again the exception with less than 1% being leached out of the leaves into the liquor during the brewing process.

3.1.6 Conclusions

To conclude, the batch microwave digestion procedure developed in this study offers an accurate and precise method for the digestion of tea leaves. This is reflected by the good recoveries obtained for the determination of Al, Ba, Ca, Cu, K, Mg, Mn and Zn in the certified reference material NIES No. 7 'Tea Leaves'. Digestions were complete within 35 minutes, a factor of at least 3.5 times faster than for the conventional hotplate digestion. The use of a fully automated microwave system which has the ability to run a number of samples in succession would therefore result in substantial time saving on the conventional techniques. The analysis of a number of different tea samples from a range of origins highlighted the variability in their trace metal contents.

The tea liquor analyses demonstrated the need to prepare samples by the same method if comparisons are to be made with past work. This should include details of the weight and physical nature of the tea used, volume of water used, infusion time and the degree of agitation applied to the sample. Our study showed that the latter point, which has often been overlooked in previous work, together with the brewing time, have a vital effect on determining the strength of the tea liquor.

3.2 Development of an Open Focused Microwave Digestion Method for the Determination of Trace Elements in Seaweed

3.2.1 Introduction

Seaweed is routinely used in the food, cosmetics and pharmaceutical industries by virtue of the richness and diversity of it's trace element content[210]. As a result, accurate analytical techniques are often required to determine both the nutritional and the toxic elements present in the sample. There is however considerable controversy in the literature over the efficacy of different mineralisation methods for the determination of trace elements in higher plants. Dry ashing techniques have been reported to lead to inaccurate results due to volatilisation or insolubilisation[211], whereas contamination problems can occur during conventional wet digestion techniques.

Fleurence *et al.*[210,212] compared the results obtained for Ca, Cd, Co, Cu, Fe, Mg, Mn, Pb and Zn following the digestion of the brown seaweed *Undaria pinnatifida* by eight different digestion procedures. Included were four dry ashing procedures and nitric-perchloric acid and nitric-sulphuric acid hotplate digestions (in both open and closed vessels). Following wet digestion with sulphuric acid, low Ca results were obtained due to the formation of insoluble complexes with calcium sulphate and thus the nitric-perchloric acid digestion was adopted for routine analysis. However for the determination of Pb and Cd low results were obtained for the latter. No significant differences were observed however between the open and closed digestions, suggesting that losses through volatilisation were not occurring. In addition Zarcinas *et al.*[213] obtained low results for K in NBS Orchard Leaves following a nitric-perchloric acid hotplate digestion due to the low solubility of potassium perchlorate. No significant differences however were observed by Isaac *et al.*[214] and Giron[215] for Ca, Cu, Fe, K, Mg, Mn and Zn in plant samples following dry ashing and nitric-perchloric acid hotplate

digestion techniques. Brix *et al.*[216] have undertaken an interlaboratory calibration exercise to evaluate the reproducibility of results for the determination of Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn in marine plants following a number of different mineralisation techniques. Significant variations in the heavy metal concentrations were observed especially for Cd, Cr, Ni and Pb, however there were too many variables to make any firm conclusions as to which procedures were generally the most suitable.

To date no microwave digestion techniques have been reported in the literature for seaweed samples. This chapter reports on the development of an open focused microwave digestion method for the determination of trace elements in seaweed following digestion with a combination of different acids and reagents. The efficiency of the digestion procedures were assessed by analysis of the certified reference seaweed sample NIES No. 9 'Sargasso'.

3.2.2 Experimental

3.2.2.1 Instrumentation and Reagents

Microwave digestions were carried out in a single mode reflux microwave system (Microdigest 301, Prolabo Ltd, Paris, France) as described in Section 3.1.2.1. Nitric acid (Analar), hydrogen peroxide (Analar) and 70% perchloric acid (Aristar) were obtained from Merck Ltd (Poole, Dorset, UK). Deionised water (18 $M\Omega cm^{-1}$) purified through a Millipore purification system (Millipore-Waters, Bedford, Massachusetts 01730, USA) was used for all dilutions. Digestion vessels, glass and plastic ware were thoroughly cleaned in 2% (v/v) Decon 90 (Merck), acid washed in 10% (v/v) nitric acid (Analar) and rinsed in deionised water prior to use. Samples were stored in polyethylene bottles (Merck Ltd) before analysis.

Analysis of samples was performed using inductively coupled plasma-atomic emission spectrometry (Liberty 200, Varian Instruments Ltd, Warrington, UK) as described in Section 3.1.2.1. Since the analysis was multielement in nature default operating conditions were used throughout, except for the independent optimisation of the viewing height for each element. For calibration of the instrument multielement working standards were prepared by diluting high purity 1000 mg l^{-1} stock solutions (Spectrosol, Merck) with deionised water and nitric acid, perchloric acid and hydrogen peroxide to obtain the same concentration as in the samples. This ensured complete matrix matching (in terms of the acid concentration) between samples and standards.

The developed procedure was validated by analysis of the certified reference seaweed NIES No. 9 'Sargasso', obtained from the National Institute for Environmental Studies (Japanese Environment Agency).

3.2.2.2 Microwave Digestion Procedures

Three different microwave digestion procedures were investigated to examine the efficiency of the digestion for NIES No. 9 'Sargasso' CRM.

i) Nitric Acid Digestion

0.5 g of sample was digested with 10 ml of nitric acid for 30 minutes (t=30) at a power setting of 60 W.

ii) Nitric Acid and Hydrogen Peroxide Digestion

Samples were initially digested with 10 ml of nitric acid at a power setting of 60 W for 30 minutes (t_1=30). 5 ml of hydrogen peroxide was then added drop wise and heating continued at 60 W for a further 10 minutes (t_2=10).

iii) Nitric Acid and Perchloric Acid Digestion

Samples were initially digested with 10 ml of nitric acid at a power setting of 60 W for between 20 and 30 minutes (t_1=20, 25, 30). 2 ml of perchloric acid was subsequently added and heating continued for a further 10 minutes (t_2=10).

After cooling, samples were quantitatively transferred into 50 ml volumetric flasks and made up to volume with deionised water. Sample blanks were also prepared and used to correct for potential contamination of the samples. Prior to analysis by ICP-AES, samples were stored in polyethylene bottles. In Table 3.11 the results obtained for As, Ca, Cu, Fe, K, Mg, Mn and Zn are compared with the certified values for NIES No. 9 'Sargasso'.

Table 3.11: Results for the Microwave Digestion of Certified Reference Material NIES No. 9 'Sargasso'

	As ($\mu g\ g^{-1}$)	Ca ($\mu g\ g^{-1}$)	Cu ($\mu g\ g^{-1}$)	Fe ($\mu g\ g^{-1}$)	K ($\mu g\ g^{-1}$)	Mg ($\mu g\ g^{-1}$)	Mn ($\mu g\ g^{-1}$)	Zn ($\mu g\ g^{-1}$)
Certified Value	**115±9**	**13400±500**	**4.9±0.2**	**187±6**	**61000±2000**	**6500±300**	**21.2±1.0**	**15.6±1.2**
HNO_3 (30 min)	123±10[2] (4.0)[3]	13222±447 (1.7)	5.1±0.3 (2.7)	152±10 (3.2)	60986±1502 (1.2)	6179±92 (0.7)	20.0±1.3 (3.3)	14.3±1.0 (3.4)
HNO_3 (30 min), H_2O_2 (10 min)	120±9 (3.7)	14065±158 (0.6)	5.0±0.4 (4.6)	157±5 (1.6)	62487±2143 (1.7)	6374±169 (1.3)	20.3±0.8 (4.0)	15.4±2.4 (8.1)
HNO_3 (20 min), $HClO_4$ (10 min)	115	14305	4.2	151	64622	6459	19.1	13.0
HNO_3 (25 min), $HClO_4$ (10 min)	125	14244	4.2	176	64838	6523	21.3	15.9
HNO_3 (30 min), $HClO_4$ (20 min)	128	14258	4.5	169	61360	6413	21.4	16.7

Notes:
1: n=4.
2: Uncertainty values are calculated as twice the standard deviation of the mean.
3: Values in parentheses are relative standard deviations (%).
4: n=1

3.2.3 Conclusions

For the nitric acid digestion good results were obtained for NIES No. 9 'Sargasso' for As, Ca, Cu and K, however Fe, Mn and Zn levels were slightly low. Better results were obtained after digestion with nitric acid in combination with hydrogen peroxide, giving good results for As, Cu, K, Mg, Mn and Zn. Results for Ca were just above the upper range of certified value and again Fe results were low (84% of the certified value). The efficiency of the digestion with nitric and perchloric acid was generally poor with only good results obtained for Mg, Mn and Zn. As, Ca and K results were generally too high and Cu and Fe results too low. However Fe results were higher than experienced in the earlier digestions (90%).

The nitric acid and hydrogen peroxide digestion was chosen as the most suitable procedure for the digestion of NIES No.9 'Sargasso', giving good results for As, Cu, K, Mg, Mn and Zn. Although Fe results were slightly low, recoveries may be improved by the addition of hydrofluoric acid to the sample to decompose any siliceous material present.

The developed microwave digestion method for the trace metal analysis of seaweed samples offers a number of advantages over conventional digestion techniques. For the latter, procedures are generally lengthy, requiring digestion times often in excess of 3 hours[210,214]. In fact a digestion time of 5 days was advocated by one laboratory in a reproducibility study reported by Brix *et al.*[216]. In addition many dry ashing techniques require multi-step procedures of ashing followed by boiling of the residues in acid and filtration of the samples before analysis[210,214]. In comparison the microwave digestion technique is far simpler. Although this method requires the addition of hydrogen peroxide part way through the procedure, this operation is performed automatically thereby keeping operator attention to a minimum. Conventional wet digestion

techniques also require extensive operator attention to ensure the sample does not reach dryness during heating, especially if perchloric acid is employed, as is often the case for the effective digestion of seaweed samples. This problem is exacerbated by the fact that the amount of energy delivered to the sample is generally difficult to control due to inconsistencies in the intensity of heat produced across the hotplate surface. In contrast the microwave power output is far more reproducible and thus following optimisation of the method the procedure can be repeatedly undertaken without requiring constant monitoring.

4. Batch Microwave Digestion and Extraction Techniques for Sediment Samples

4 BATCH MICROWAVE DIGESTION AND EXTRACTION TECHNIQUES FOR SEDIMENT SAMPLES

4.1 Development of an Open Focused Microwave Digestion Technique for the Trace Element Analysis of Sediment Samples

4.1.1 Introduction

Recent improvements to analytical techniques have left the initial stage of sample digestion as the most time consuming step. This is especially true for the digestion of sediments for trace metal analysis. Sediment samples are one of the most difficult sample matrices to digest, consisting of a combination of different materials such as clay, organic matter and various minerals including siliceous minerals. There are a number of sample preparation techniques available to the analyst although there is considerable controversy over the efficacy of each. As discussed in Chapter 1 conventional wet digestions can prove lengthy, especially if open beakers are used. Fusion techniques also have a number of drawbacks including incomplete dissolution of some minerals and losses of volatile elements. They also necessitate large dilution factors to reduce the salt content of the final solution to an acceptable level to avoid matrix interferences and problems of sample introduction during analysis. As a result no one technique has been uniformly adopted for routine analysis.

For geological samples many workers have demonstrated that digestions using microwaves are much faster and less prone to contamination problems than conventional methods[30,33,35,42,44,55,62,65,66,73,80-83,91,95,104,109,118-147,178,217-224]. The majority of microwave digestion techniques for geological samples have been undertaken in closed Teflon bombs[44,73,81,82,95,104,122-143,145-147,217,219,223,224]. Although this method is far quicker than

many conventional techniques, considerable care must be taken to avoid explosions due to build up of excess pressure. This can be particularly hazardous when using reagents such as hydrofluoric and perchloric acids, and these are often necessary for the complete digestion of geological samples.

Little work has been undertaken on the development of open microwave digestion techniques[33,35,109,118,220-222]. Nadkarni[220] investigated the open microwave digestion of a range of materials including sediment samples. Digestions were undertaken in polycarbonate beakers heated in a domestic microwave oven for 3 minutes. However results for NBS Sediment 1645 and IAEA Sediment SL-1 were generally in poor agreement with the certified values, with particularly low Cr, Mg and Ti recoveries. Using an open focused microwave system Kersabiec *et al.*[221] reported good recoveries for Cr, V and Sr for the certified reference river sediment BCR CRM 320. A similar system was utilised by Thomas *et al.*[222] to obtain good Co, Mn, Pb and Zn results for the certified reference sediment sample NRCC MESS-1, although Cr recoveries were again low. However Quevauviller *et al.*[35] reported good results for Cr in BCR Estuarine Sediment following a hydrochloric and nitric acid and hydrogen peroxide open focused digestion.

For the complete digestion of sediments the method of choice must destroy the original crystal structure of minerals to release the associated elements into solution. The most effective mineral acid for the destruction of the strong Si-O bond of aluminosilicate minerals is the weak non oxidising acid hydrofluoric acid. Destruction of the silica matrix results in the formation of SiF_4 which volatilises upon heating and thus is lost during open digestion procedures. Although the determination of Si is obviously not

possible, the resulting solution is more stable because Si in solution tends to hydrolyse and precipitate out of solution. In addition the loss of Si reduces the salt content of the final solution which is beneficial during analysis as previously stated. However, one disadvantage of the method is that the volatile fluorides of As, B, Ti, Nb, Ta, Ge and Sb are formed and thus may also be lost during heating.

Hydrofluoric acid will readily digest carbonates, phosphates, most silicates and moderate amounts of organic matter in the samples. However many oxide minerals, including important ore minerals such as cassiterite, chromite and wolframite are incompletely attacked or remain totally undigested. Generally digestion under pressure is advantageous for the dissolution of a number of minerals such as beryl, chalcopyrite, chromite, columbite, corundum, garnet, ilmenite, kyanite, pyrite, rutile, staurolite, tantalite and tourmaline which are not usually digested in open vessels[225]. Thus in an open vessel digestion even in the presence of HF certain minerals may not taken into solution.

For the digestion of geological samples HF is commonly used in combination with other acids such as perchloric, nitric, hydrochloric, sulphuric and *aqua regia*. Although it has been shown that HF is effective alone, the mixture of acids increases it's capability in decomposing the accompanying non-silicate rock minerals such as sulphides and oxides. Oxidising agents are also required to destroy the organic portion of the sample. For this purpose perchloric acid is often used after an initial heating step with nitric acid, acting as a strong oxidising agent to oxidise any organic matter still present. Heating results in the formation of perchlorates which are generally soluble in water, except for Cs, K and Rb. However at the levels commonly present in most sediments this is not

generally problematic. The presence of any insoluble perchlorates can be minimised by evaporating the sample to dryness to remove any excess perchloric acid. In addition perchloric acid is also effective at decomposing any insoluble fluorides present, such as calcium fluoride which can incorporate a substantial portion of any lead present in the sample.

Prior to analysis it is also recommended to remove any remaining HF due to the safety risks associated with its use and because it readily attacks glass, which may be encountered during analysis e.g. glass nebulisers and spray chambers. In addition as stated previously fluorides may form insoluble precipitates which may incorporate important analytes. Removal can be achieved by either evaporation to dryness or by complexation with boric acid. The latter may not be desirable for a number of analysis techniques as it can lead to a high salt content in the final solution. Evaporating the sample to dryness is therefore usually the preferred option. The efficiency of the removal of fluorides during evaporation to dryness can be improved by heating with a high-boiling mineral acid, for which perchloric acid is particularly suited.

A major disadvantage of the majority of closed microwave digestion systems [save one commercial system (Milestone MLS 1200 MEGA)] is that evaporation to dryness is not possible and thus it is often necessary to undertake this operation by heating on a hotplate[66,131,178,218]. This introduces an extra handling step into the procedure, especially if the sample is transferred into a beaker beforehand. It also increases the likelihood of losses occurring and substantially increases the total sample preparation time.

An advantage of the open focused microwave system used for this study is that evaporation to dryness can be undertaken in situ (see Section 3.1.2.1). In addition reagents can be automatically delivered into the sample flask at any stage of the digestion procedure. This is a distinct advantage over closed systems which require cooling before the digestion vessel can be opened. The automatic addition of reagents also reduces the hazards associated with the handling of HF and minimises the amount of operator attention required.

This chapter describes the development of an open focused microwave digestion method for the complete digestion of sediment samples for subsequent trace element analysis by inductively coupled plasma-atomic emission spectrometry. A number of different digestion reagents including nitric, hydrochloric, perchloric and hydrofluoric acid and different combinations of power settings and heating times were investigated to determine their effect on the trace element recoveries obtained.

4.1.2 Experimental

4.1.2.1 Instrumentation and Reagents

Digestions were carried out in a single mode reflux microwave digestion system (Microdigest 301, Prolabo) as described in Section 3.1.2.1. Evaporation to dryness can be undertaken in situ. For this purpose the suction head is lowered, increasing the effective suction rate of the fume extraction system. The use of HF necessitated strict safety precautions and the use of PTFE digestion vessels and reflux heads (Prolabo).

Analysis of samples was undertaken by ICP-AES (Liberty 200, Varian Instruments, Warrington, UK) as described in Section 3.1.2.1. Since the analysis was multielement in nature default operating conditions were used throughout, except for the independent optimisation of the viewing height for each element. The operating conditions for the Liberty 200 are shown in Table 3.1. For calibration of the instrument multielement calibration standards were prepared by diluting high purity 1000 mg l^{-1} stock solutions (Spectrosol, Merck Ltd, Poole, Dorset, UK) with deionised water and hydrochloric acid to obtain the same concentration as in the samples. This ensured complete matrix matching (in terms of the concentration of acid) between samples and standards.

Deionised water (18 MΩ cm^{-1}) purified through a Millipore purification system (Millipore-waters, Bedford, Massachusetts 01730, USA) was used throughout. Digestion vessels, glass and plastic ware were soaked in 10% (v/v) nitric acid (Analar, Merck Ltd) and 2% Decon 90 (Merck) and thoroughly rinsed with deionised water prior to use. Nitric acid (Aristar), hydrofluoric acid (Aristar) and hydrochloric acid (Aristar) were obtained from Merck Ltd. Samples were filtered where necessary (Whatman No.1 Qualitative filter paper, Merck) and stored in polyethylene bottles (Merck) prior to

analysis. The certified reference material MESS-1 (estuarine sediment) was obtained from the National Research Council of Canada (Ottawa, Canada) and was employed to assess the efficiency of the different digestion procedures studied.

4.1.2.2 Details of Microwave Digestion Procedures

The certified reference material NRCC MESS-1 was digested by 12 different digestion procedures, the details of which are summarised in Table 4.1 and discussed in more detail in the following section.

Procedure 1 Procedure 1 involved heating 0.5 g of sample with nitric and hydrochloric acid for 10 minutes. This was undertaken to decompose and solubilise the organic portion of the sample. Following the addition of HF the sample was heated for a further 10 minutes in order to destroy the siliceous components of the sample. The excess of HF was then removed by evaporation to dryness (20 minutes) and the remaining residue dissolved in HCl and deionised water. After decanting off the sample it was observed that a substantial black rim of organic material remained inside the digestion vessel which was impossible to remove by washing and ultrasonification. Heating with concentrated nitric and perchloric acid for 30 minutes proved successful in removing this residue and was necessary between all samples to avoid the risk of cross contamination. After adjusting the final volume of the sample to 50 ml with deionised water the resulting solution was pale yellow in colour and contained very small white particles (probably siliceous material) and a substantial amount of black particles (probably organic matter) and thus filtration was necessary before analysis.

Table 4.1: Microwave Digestion Procedures for Sediment Analysis

Procedure	Step 1	Step 2	Step 3	Step 4	Step 5	Step 6
1	6 ml HNO_3, P^1=20, t^2=5	5 ml HCl, P=40, t=5	15 ml HF, P=40, t=10	E.D[3] P=170, t=20	3 ml HCl, P=30, t=10	40 ml H_2O, P=70, t=10
2	6 ml HNO_3, P=20, t=5	5 ml HCl, P=40, t=5	15 ml HF, P=40, t=10, P=60, t=10	E.D P=170, t=18	3 ml HCl, P=30, t=10	20 ml H_2O, P=70, t=10
3	3 ml HNO_3, P=40, t=5	9 ml HCl, P=60, t=15	E.D P=180, t=9	15 ml HF, P=40, t=10, P=70, t=10	E.D P=180, t=22	4 ml HCl, P=80, t=20
4	6 ml HNO_3, P=20, t=5	2 ml $HClO_4$, P=40, t=5	15 ml HF, P=40, t=10	E.D P=170, t=20	3 ml HCl, P=30, t=10	40 ml H_2O, P=70, t=10
5	6 ml HNO_3, P=40, t=5	2 ml $HClO_4$, P=60, t=5	15 ml HF, P=40, t=10, P=60, t=5	E.D P=170, t=30	4 ml HCl, P=40, t=10,	20 ml H_2O, P=70, t=15
6	6 ml HNO_3, P=40, t=10	2 ml $HClO_4$, P=60, t=15	15 ml HF, P=40, t=10, P=70, t=10	E.D P=180, t=30	4 ml HCl, P=40, t=3, P=70, t=20	-
7	6 ml HNO_3, P=40, t=5	1 ml $HClO_4$, P=60, t=20	15 ml HF, P=40, t=10, P=70, t=10	E.D P=200, t=25	4 ml HCl, P=40, t=3, P=70, t=20	-
8	6 ml HNO_3, P=40, t=5	1 ml $HClO_4$, P=40, t=10, P=60, t=30, P=80, t=10	15 ml HF, P=40, t=10, P=70, t=10	E.D P=200, t=25	4 ml HCl, P=40, t=3, P=70, t=20	-
9	6 ml HNO_3, P=40, t=5	1 ml $HClO_4$, P=40, t=10, P=60, t=30, P=80, t=10	15 ml HF, P=40, t=10, P=60, t=20, P=80, t=10	E.D P=200, t=25	4 ml HCl, P=40, t=3, P=70, t=20	-
10	6 ml HNO_3, P=40, t=5	1 ml $HClO_4$, P=40, t=10, P=60, t=30, P=80, t=10	15 ml HF, P=40, t=10, P=60, t=40, P=80, t=10	E.D P=200, t=25	8 ml HCl, P=40, t=3, P=70, t=20	Decant off sample. Heat residue with 4 ml of HCl, P=80, t=15
11	6 ml HNO_3, P=40, t=5	1 ml $HClO_4$, P=40, t=10, P=60, t=40, P=80, t=10	15 ml HF, P=40, t=10, P=60, t=40, P=80, t=20	E.D P=200, t=25	4 ml HCl, P=40, t=3, P=70, t=20	Add 4 ml of HCl and 10 ml of H_2O, P=80, t=30
12	6 ml HNO_3, P=40, t=5	1 ml $HClO_4$, P=40, t=10, P=60, t=40, P=80, t=10	15 ml HF, P=40, t=10, P=70, t=10,	E.D P=200, t=25	4 ml HCl, P=40, t=3, P=100, t=30	4 ml HCl, P=100, t=30

Notes:
1: P = power setting (W)
2: t = time (min)
3: E.D = evaporation to dryness

Procedure 2 In an attempt to break down the residual particles, Procedure 1 was repeated but with an additional heating step with HF at the higher power of 60 W. The resulting solution was again pale yellow in colour but contained no visible siliceous particles. However there was still a rim of black material remaining inside the digestion vessel which as before required boiling with nitric and perchloric acid to completely remove.

Procedure 3 In order to more fully decompose the organic portion of the sample it was heated with *aqua regia* (25% nitric, 75% hydrochloric acid). However this produced extensive frothing of the sample into the reflux column. It was more successful to heat the sample with nitric acid before the HCl addition (still in proportions of *aqua regia*). The sample was then evaporated to near dryness, 15 ml of HF added and heating continued for a further 20 minutes. Evaporation to dryness was then undertaken, followed by dissolution of the residue in HCl. The resulting solution contained a substantial undissolved mass and thus filtration was required prior to analysis. In addition a slight black rim was observed inside the tube again.

Procedure 4 An alternative method for the decomposition of the organic portion of the sample was then investigated. Procedure 1 was again repeated, replacing the hydrochloric acid in Step 2 with 2 ml of perchloric acid. The latter is not only useful for it's strong oxidising properties but is beneficial during the evaporation to dryness step as by virtue of it's high boiling point it can drive out hydrofluoric acid from any insoluble fluorides remaining in the residue. Step 4 (evaporation to dryness) was lengthened due to the high boiling point of perchloric acid in comparison to HCl (203°C *cf* 110°C). Procedure 4 resulted in a colourless solution which contained a small number of siliceous

and black organic particles and so again required filtering. In addition a slight black rim remained inside the digestion vessel.

Procedure 5 In order to remove the unoxidised portion of organic matter, the previous procedure was repeated and the power setting for the nitric acid step (Step 1) increased to 40 W. In Step 2, the HCl was again replaced with 2 ml of perchloric acid, the power setting increased to 60 W and an additional heating step with HF at 60 W introduced. After evaporation to dryness, 4 ml of HCl was added and heating continued at the higher power setting of 40 W. 20 ml of deionised water was then added, followed by further heating for 15 minutes. This procedure resulted in a clear solution with only very slight traces of siliceous and organic matter and so filtration was not necessary. In addition the black rim of organic matter observed in earlier procedures was no longer present.

Procedure 6 Further investigations were undertaken using perchloric acid in Step 2, as this reagent was the most effective at decomposing the persistent organic matter in the sample. Procedure 6 was based on that of Procedure 5 except that the mass of sample digested was reduced from 0.5 g to 0.4 g and the heating times throughout were extended. The resulting solution was clear except for the presence of a few very tiny particles of organic matter and thus filtration was not necessary.

Procedure 7 In an attempt to remove the remaining organic particles present after Procedure 6 the weight of sample digested was reduced to 0.3 g and the heating time with perchloric acid increased to 20 minutes. However the resulting solution still contained a number of small particles of organic matter.

Procedure 8 The heating time with perchloric acid was increased to 50 minutes. This resulted in an almost clear solution except for the presence of a small number of very small siliceous particles. No particles of organic matter were observed. The solution was filtered before analysis.

Procedure 9 The heating time with HF was increased to 40 minutes in an attempt to remove the siliceous matter present in Procedure 8. The resulting solution was colourless but still contained a very small number of small siliceous particles.

Procedure 10 The HF heating time was increased to 60 minutes and the volume of HCl added in the final step increased to 8 ml. The resulting solution contained a substantial insoluble portion which after separation from the supernatant was heated with HCl for a further 15 minutes and then recombined with the previous portion. This approach resulted in a clear, colourless solution with very few small particles remaining, the clearest solution of all the digestion procedures investigated.

Procedure 11 The heating times with perchloric acid, HF and the final HCl step were increased by 10 minutes each. The resulting solution contained undissolved siliceous particles. Extra HCl and 10 ml of deionised water were added and the digest reheated for a further 30 minutes. The resulting solution contained a large amount of white, siliceous material (more so than for Procedure 10). However no black particles of organic matter were observed. The solution was filtered prior to analysis.

Procedure 12 The HF heating time was reduced to 20 minutes as for earlier digestions where no siliceous particles remained (e.g. Procedure 6). The power setting was increased in the final redissolution step with HCl to 100 W. At this stage solid material still remained and so extra HCl was added and heating continued for a further 30 minutes. This resulted in a very cloudy solution with a large amount of undigested siliceous material, but no organic matter remained. Filtration was necessary prior to analysis.

Summary Of Digestion Procedures

In summary, Procedure 1 involved heating of the sample with nitric, hydrochloric and hydrofluoric acid, followed by evaporation to dryness and redissolution of the sample in HCl and water. Procedure 2 was carried out as for Procedure 1, except for increasing the power setting and heating time during the HF step. Procedure 3 involved initial heating with nitric acid followed by hydrochloric acid (in *aqua regia* proportions) and evaporation to dryness before the addition of HF. Procedure 4 was identical to Procedure 1 except that the HCl in Step 2 was replaced with perchloric acid. Procedure 5 again employed perchloric acid although the power settings were increased for the nitric, perchloric and HF heating steps. Procedures 6 and above also employed perchloric acid rather than HCl after initial heating with nitric acid. The effects of increasing heating times and power settings for the different steps of the procedure were investigated in an attempt to produce a clear digestion free of siliceous and organic matter.

Following digestion, samples were allowed to cool and quantitatively transferred into a 50 ml volumetric flask before making up to volume with deionised water. Samples were filtered where necessary and stored in polyethylene bottles prior to analysis by ICP-AES. The elements determined were Al, Ca, Cu, Cr, Fe, K, Mg, Mn, Ni, Pb, Ti and Zn. Dilution of samples by a factor of 10 was necessary for the determination of Al, Ca, Fe, K, Mg and Ti so that the final concentration was within the dynamic linear range of the instrument. Sample blanks were also prepared and used to correct for potential contamination of the samples.

4.1.3 Results

The results of the ICP-AES analysis of NRCC MESS-1 following microwave digestion by each of the 12 procedures are given in Table 4.2. As can be seen there is considerable variation in the recoveries obtained for each of the digestion procedures. Generally good recoveries were obtained for Cu, Fe, Mn, Ni and Zn, however Pb recoveries were generally poor, with only 2 samples having recoveries within the uncertainty limits of the certified values (Procedures 4 and 5). Good results were obtained for Ca, K and Mg for only a small number of procedures and Al, Cr and Ti recoveries were consistently low.

For Procedure 1 good recoveries were obtained for all the elements studied except Al, Cr, Ni, Pb and Ti, although Ni and Pb are only just above the certified range. The effect of increasing the heating time and power setting with HF (Procedure 2) did little to increase Al and Ti recoveries and resulted in low K and Pb results, although recoveries for Cr were slightly improved. Procedure 3 (nitric and hydrochloric acid in the proportions of *aqua regia*) proved ineffectual with only Cu and Ni results in agreement

Table 4.2: Comparison of Certified and Experimental Values for NRCC MESS-1 (n=1)

	Cr ($\mu g\ g^{-1}$)	Cu ($\mu g\ g^{-1}$)	Mn ($\mu g\ g^{-1}$)	Ni ($\mu g\ g^{-1}$)	Pb ($\mu g\ g^{-1}$)	Zn ($\mu g\ g^{-1}$)	Al (%)	Ca (%)	Fe (%)	K (%)	Mg (%)	Ti (%)
Certified Value	**71 ± 11**	**25.1 ± 3.8**	**513 ± 25**	**29.5 ± 2.7**	**34.0 ± 6.1**	**191 ± 17**	**5.84 ± 0.20**	**0.482 ± 0.046**	**3.05 ± 0.18**	**1.86 ± 0.03**	**0.868 ± 0.054**	**0.543 ± 0.017**
1	52	21.6	521	32.6	40.7	197	5.56	0.473	2.98	1.84	0.833	0.362
2	59	21.4	501	32.2	18.8	189	5.56	0.462	3.11	1.81	0.823	0.398
3	52	24.1	292	28.7	17.4	158	1.65	0.337	2.69	1.62	0.392	0.408
4	49	24.2	504	28.0	32.4	184	5.59	0.661	2.68	1.85	0.940	0.424
5	53	21.6	508	30.5	33.1	190	5.34	0.480	3.10	1.86	0.845	0.444
6	52	21.3	504	30.6	18.1	194	5.23	0.471	3.04	1.76	0.809	0.420
7	41	20.5	483	27.2	23.4	171	5.29	0.500	2.92	1.79	0.778	0.419
8	55	19.0	524	33.8	40.3	185	3.51	1.053	2.97	2.17	0.807	0.471
9	54	18.3	445	30.6	43.9	187	1.37	0.376	2.73	1.68	0.152	0.438
10	49	19.2	461	23.0	18.9	197	5.61	0.522	3.04	1.97	0.785	0.477
11	51	20.4	498	29.0	40.4	180	6.18	1.785	3.03	1.70	1.542	0.475
12	59	20.2	437	26.7	44.8	181	3.40	1.172	2.77	1.40	1.242	0.426

with the certified values. This was to be expected in light of the large mass of insoluble material remaining at the end of the digestion. Procedure 4 (introduction of perchloric acid) resulted in good recoveries, except for low Al, Cr, Fe and Ti. The effect of increasing power settings and heating times (Procedure 5) improved Fe results to leave only Al, Cr and Ti below the range of the certified values. Procedure 6 (as Procedure 5 but with increasing heating time with nitric, perchloric and hydrofluoric acids) generally gave worse recoveries than Procedure 4 with only Ca, Cu, Fe, Mn, Ni and Zn in agreement with the certified values. Similar findings were also observed for Procedure 7 (as Procedure 6 but with increased heating time with perchloric acid), resulting in generally low recoveries for all the elements studied except Ca and Ni. The presence of siliceous material, which may be associated with elements such as Al, Cr and Ti, was not observed in Procedures 6 and 7 however no increase to the recoveries of these elements was obtained. The effect of a further increase in heating time with perchloric acid (Procedure 8) resulted in better recoveries for Cr and Ti, however recoveries were generally very poor. Increasing the HF heating time (Procedure 9) resulted in very low recoveries for most elements. Procedure 10 (increased heating with HF and further heating of the final residue with HCl) resulted in least residual matter, however only Ca, Fe and Zn results were in good agreement with the certified values. Titanium recoveries were the highest of all the different procedures studied though. Procedure 11 (increased heating time with perchloric and hydrofluoric acids) was similarly inefficient with good results for only Fe, Mn, Ni and Zn. Finally, generally low results were obtained for Procedure 12 which was to be expected as the final solution contained a large mass of undigested siliceous material.

4.1.4 Further Analysis

Further analysis of the MESS-1 certified reference material by the most effective procedures (1 and 5) was then undertaken in triplicate. In addition further digestion of the residue remaining after digestion by Procedure 1 was undertaken with perchloric and hydrofluoric acids (Procedure 13) as described in Table 4.3. For Procedure 13 there was a reduction in the amount of organic matter present, however a substantial portion of large white crystalline particles was present and thus the sample required filtering. Samples were again analysed by ICP-AES for Al, Ca, Cr, Cu, Fe, K, Mg, Mn, Ni, Ti and Zn. In addition the MESS-1 sample is certified for vanadium and thus this was included in the study. The results obtained are compared with the certified values in Table 4.4.

Table 4.3: Details of Further Digestion Procedure for Sediment Samples

Procedure	Step 1	Step 2	Step 3	Step 4	Step 5	Step 6
13	Steps 1-5 of Procedure 1.	20 ml H_2O, P=70, t=15. Decant off clear solution into a 50 ml volumetric flask.	Digest residue with 1 ml $HClO_4$, and 5 ml HF, P=50, t=30.	E.D P=170, t=25.	3 ml HCl, P=30, t=10.	10 ml H_2O, P=70, t=10. Combine with earlier portion.

Notes:
1: P = power setting (W).
2: t = time (min).
3: E.D = evaporation to dryness.

For Procedure 1 good recoveries were obtained for Al, Cu, Ca, K, Mg, Mn, Ni, Pb, V and Zn although Cr and Ti recoveries were low (72% and 69% of the certified values respectively) and Fe was just outside the lower range of the certified value (91%). For Procedure 5 good recoveries were also obtained for Al, Ca, Cu, K, Mg, Mn, Ni, Pb, V and Zn. Again Cr and Ti recoveries were low (both 77%) and Fe just outside the

uncertainty range of the certified value (93% recovery). Procedure 5 is preferable to Procedure 1, resulting in higher recoveries for Cr, Fe and Ti. In addition, as previously mentioned, following digestion by Procedure 1 a persistent residue of organic matter was present inside the digestion vessel which was inherently difficult to remove. Procedure 13 (further heating of the residue with perchloric and hydrofluoric acid) resulted in no improvements to the recoveries of Cr and Fe. In fact Fe recoveries were actually lower (88%), however Ti recoveries were slightly improved (82%).

Table 4.4: Comparison Of Certified and Experimental Values for NRCC MESS-1

Procedure	Certified Value	1[1]	5[1]	13[2]
Cr ($\mu g\ g^{-1}$)	71±11	51±0.1[3] (3.0)[4]	55±1 (1.3)	50
Cu ($\mu g\ g^{-1}$)	25.1±3.8	27.3±7.6 (13.8)	24.1±1.4 (2.8)	-
Mn ($\mu g\ g^{-1}$)	513±25	488±8 (0.8)	489±11 (1.1)	-
Ni ($\mu g\ g^{-1}$)	29.5±2.7	28.7±2.2 (3.9)	27.4±2.0 (3.6)	-
Pb ($\mu g\ g^{-1}$)	34.0±6.1	37.7±9.5 (12.6)	32.6±7.4 (11.4)	
V ($\mu g\ g^{-1}$)	72.4±14	56.8±10.2 (9.0)	58.3±10.6 (9.1)	-
Zn ($\mu g\ g^{-1}$)	191±17	179±14 (3.8)	182±7 (2.0)	-
Al (%)	5.84±0.20	6.00±0.89 (7.4)	5.78±0.56 (4.8)	-
Ca (%)	0.482±0.046	0.485±0.124 (12.8)	0.450±0.000 (0.1)	-
Fe (%)	3.05±0.18	2.77±0.07 (1.2)	2.84±0.09 (1.6)	2.68
K (%)	1.86±0.03	1.87±0.27 (7.2)	1.90±0.49 (13.0)	-
Mg (%)	0.868±0.054	0.862±0.132 (7.6)	0.829±0.003 (0.2)	-
Ti (%)	0.543±0.017	0.368±0.022 (3.0)	0.417±0.008 (0.9)	0.443

Notes:
1: n=3 for Procedures 1 and 5.
2: n=1 for Procedure 13.
3: Uncertainty values for the experimental results are calculated as twice the standard deviation.
4: Figures in parentheses are relative standard deviations (%).

4.1.5 Conclusions

For the chosen digestion procedure (Procedure 5) good recoveries were obtained for Cu, Mn, Ni and Zn in NRCC MESS-1, which may be expected as these elements are in many cases sorbed on to clay minerals or are in other readily-decomposed phases, rather than within resistant framework-lattice silicates. Thus they are generally released without the need for vigorous digestion techniques such as heating with *aqua regia*, nitric/perchloric acids or nitric acid alone. Milward and Kluckner[223] reported the digestion of sediment samples in a closed microwave system with nitric acid and HCl for 15 minutes to release the "environmentally available" elements Cd, Cu, Hg, Pb and Zn. The work also demonstrated that poor recoveries were obtained for Al, Ca, Cr, Fe, Mg, Mn and Ti, warranting the use of more vigorous digestion reagents such as with hydrofluoric acid.

Following Procedure 5 good recoveries were also obtained for Al, Ca, K, Mg, Pb and V, although Cr and Ti results were generally poor and Fe results only just outside the lower limits of the certified value. Low Cr and Ti results are typical of many digestion techniques, including closed microwave digestion methods. For example Lamothe *et al.*[218] reported low Ti recoveries (80%) for NRCC MESS-1, MAG-1 (Marine Sediment, US Geological Survey) and GSD Stream Sediments Nos. 2, 3 and 8 (Institute of Geophysical and Geochemical Exploration, China) after a closed nitric, hydrochloric and hydrofluoric acid digestion. However without the use of HF very low recoveries have been reported in the literature. For example recoveries of only 5% were obtained for NRCC MESS-1 and BCSS-1 after a hydrochloric and nitric acid closed digestion procedure[223].

Poor Cr recoveries are likely because some Cr-bearing minerals (notably chromite) are not readily attacked by the combination of hydrofluoric and perchloric acids, even in closed vessels at elevated pressure[227]. Lamothe *et al.*[218] and Nakashima[219] reported Cr recoveries of 80-89% in NRCC MESS-1 after a closed microwave digestion with HF. Marr *et al.*[126] obtained even lower recoveries of 56% and 57% for NRCC MESS-1 and PACS-1 respectively following a closed microwave digestion with *aqua regia* and hydrofluoric acid. As previously stated Nadkarni[220] and Thomas *et al.*[222] reported low Cr recoveries (70-80%) in certified sediment samples following an open microwave digestion procedure employing HF. However without the use of hydrofluoric acid even lower recoveries (44%) were obtained for Cr in NRCC MESS-1 and BCSS-1 after closed microwave digestion with just hydrochloric and nitric acid[223]. Low Cr results (72%) were also reported in NRCC BCSS-1 following an on-line hydrofluoric, nitric and hydrochloric acid digestion[55]. However in contrast van Delft *et al.*[224] obtained good recoveries for Cr in IAEA CRM SL-1 Lake Sediment without the use of HF, using an *aqua regia* closed microwave digestion method. A recovery of only 35% was obtained for NRCC PACS-1 using the same reagent combination[127]. Good Cr recoveries were also obtained by de Kersabiec *et al.*[221] for the river sediment BCR CRM 320 after an open focused digestion with hydrofluoric and perchloric acids and in BCR Estuarine Sediment after digestion with nitric acid and hydrogen peroxide[35]. These examples serve to highlight the difficulties in obtaining complete recoveries for Cr and Ti even after heating with hydrofluoric acid under pressure. They also illustrate how the effectiveness of the digestion is highly dependent on the exact sample type. Critical factors include the chemical composition, degree of crystallinity of the mineral phases and the proportion of organic matter in the sample.

It has been suggested that multiple addition-evaporation cycles of perchloric and nitric acid to the sample residues following the initial digestion may decompose resistant Cr minerals[227]. However in this work further digestion of the residue did little to improve the recoveries of Cr, Ti and Fe. An alternative reason for low Cr recoveries has been postulated by Nadkarni *et al.*[220] who carried out a volatility study on a number of different elements. It was reported that 26% of Cr, in a matrix of *aqua regia* and hydrofluoric acid, was lost after 5 minutes heating in an open vessel in a microwave oven. However in real samples elements are not present in the pure ionic form but as oxides and organometallic complexes and so will not necessarily behave in the same way.

In this work heating the samples for prolonged periods of time (Procedures 7-12) or further digestion of the residue (Procedure 13) seemed to have little effect on improving the recoveries of the lattice bound elements e.g. Cr and Fe, although Ti recoveries were slightly improved. It may therefore be concluded that heating under pressure is necessary in order to totally digest these minerals, although as previously noted a number of minerals, including chromite, are not always decomposed even at high pressure[226]. Many of the samples heated for long periods of time (e.g. Procedure 11) produced low recoveries, even for those elements which are easily released into solution e.g. Cu, Zn etc. It is unlikely that the low recoveries are due to losses of volatile compounds as all the fluorides of the elements studied have boiling points higher than the temperatures reached in the digestion procedure. It was observed that as the heating time increased that generally the amount of residual siliceous material increased, whereas the presence of organic matter was less apparent. An explanation for the low recoveries experienced in many of the longer digestion procedures could be that prolonged heating results in the

formation of insoluble compounds which can not be dissolved in the final dissolution step with hydrochloric acid. Thus a less vigorous digestion procedure is beneficial.

Many of the digestion procedures, including the two most effective (Procedures 1 and 5), resulted in solutions containing small black particles of organic origin. Their presence however seems to have little effect on the recoveries of most of the elements studied. It was beneficial however to employ perchloric acid to decompose the majority of the organic portion of the sample and thus avoid the formation of a rim of organic matter inside the digestion vessel. As previously stated this residue was impossible to remove without boiling with nitric and perchloric acids for at least 30 minutes.

In conclusion, the open focused microwave digestion system has been successfully applied to the digestion of the NRCC MESS-1 certified sediment sample. Good recoveries were obtained for Al, Ca, Cu, K, Mg, Mn, Ni, V and Zn, however Fe results were just below the certified range. Although the results for Cr and Ti were somewhat low they are not untypical of those reported in the literature for the NRCC MESS-1 sample. Chromium and Ti are often associated with resistant minerals and thus their decomposition may be aided by heating under pressure, however as previously stated this is not always achieved, even in the presence of hydrofluoric acid.

4.2 Evaluation of a Microwave Extraction Procedure for the Speciation of Organotin Compounds in Sediment Samples

4.2.1 Introduction

Tin compounds are used extensively as fungicides, wood preservatives, molluscicides, catalysts, fire retardants, coatings for food cans, heat and light stabilisers in polyvinylchloride and for a number of uses in the glass and plastics industries[228]. Whilst the presence of inorganic tin compounds in the environment pose little concern, organotin compounds are more problematic due to their toxicity to many organisms[229-230]. Of most environmental concern is the use of triorganotin compounds [tributyltin (TBT) and triphenyltin (TPhT)] as biocides in antifouling paints. These formulations are applied to the exterior of marine structures such as submarines, underwater cages, buoys, radar and sonar equipment and to the underside of sea faring vessels[228]. Their principal mode of action is to slowly release toxic organotin compounds into the surrounding waters to control the growth and attachment of marine organisms, especially barnacles and algae, onto these structures. This helps to prevent a number of serious economic problems. For example, the build up of fouling on ships' hulls interferes with streamlining and thus increases drag effects, which in combination with the increased weight leads to elevated fuel costs[228]. Also the cost of cleaning and repainting as well as the loss in revenue from the time spent in dry-dock must also be considered. For delicate underwater equipment such as sonar equipment, fouling may cause physical damage or accelerate corrosion, inhibiting its operation. The presence of fouling is also detrimental to the dip and buoyancy of navigation buoys and moored underwater systems[228].

Although antifouling paints have proved successful at controlling the problem of fouling, their use has resulted in detrimental effects to non-target organisms such as oysters and mussels[231,232]. It has been demonstrated that levels as low as 1 ng ml^{-1} TBT[231] can cause deformation in shellfish, which may affect the fertility, mariculture and population of these organisms in affected areas. The worst incidents of organotin pollution have been observed in harbours and marinas where vessels, especially pleasure craft e.g. yachts and small boats, are stationary for long periods of time, allowing elevated concentrations to build up in the surrounding waters[228].

Although many countries, including France (1982), USA (1986), UK (1987), Canada (1989), New Zealand (1989) and the European Community (1991)[233], have now introduced restrictions on the use of organotins in antifouling paints, levels still exist in the environment, particularly in sediments, the ultimate sink of these compounds in the marine environment. Their occurrence is either via superficial adsorption by organic complexation onto the organic surfaces of the sediment or by incorporation in the biological debris[234]. There is no involvement in mineralogical processes. Although organotins absorb strongly onto sediments, there is an equilibrium relationship between the species absorbed onto the sediments and those dissolved in the water. Thus the accumulated compounds can be released back into the aquatic environment, creating a long-term ecotoxicological risk, long after the anthroprogenic sources have been removed[235,236]. In addition the degradation rates within sediments are comparatively slow (a matter of years)[237] in comparison to those observed within the water column (days or weeks)[238] and thus persistence in the former is more pronounced.

For the above reasons it is clear that accurate and sensitive analytical techniques are required for the determination of toxic trialkyltin compounds, particularly TBT and its less toxic degradation products dibutyltin (DBT) and monobutyltin (MBT) in sediment samples. From such studies the fate, persistence and mobility of these species in the environment can be monitored. Commonly methods capable of determining levels of organotins at the 0.1 ng g^{-1} level are required to provide sufficient sensitivity for the variety of sediment samples routinely encountered[239]. Suitable methods of analysis must however be capable of distinguishing between the different species, rather than just determining the total tin content. A variety of techniques have been developed however determinations are complicated by the fact that organotins, depending on the alkyl chain length and the number of substituents, are polar, hydrophobic and involatile because of their ionic character[240].

Prior to analysis the compounds of interest must be liberated from the sample matrix without promoting changes to their chemical form i.e. speciation. This is commonly undertaken by a number of traditional extraction procedures which can be grouped into four main classes:

i) ***Organic solvent extraction***, commonly in combination with a chelating agent e.g. methanol and tropolone[241]; hexane and tropolone[242].

ii) ***Acid leaching*** e.g. acetic acid[243-248].

iii) ***Alkali leaching*** e.g. sodium hydroxide[249].

iv) ***A combination of these techniques*** e.g. methanol and hydrochloric acid[234,250-252].

Traditional extraction methods however are often time consuming and ineffectual. For example Zhang *et al.*[234] reported that only three out of ten sample preparation methods described in the literature were able to recover more than 90% of TBT from sediments, only

two for DBT and none were able to recover MBT in a reproducible manner. Recently microwave heating has been shown to be an effective tool for the extraction of a number of compounds from sediments and soils e.g. polyaromatic hydrocarbons (PAHs)[253], pesticides[254] and organotin species[255-257]. For the extraction of organotin compounds complete destruction of the sample matrix is not required since the compounds are not involved in mineralogical processes. However the presence of an acid in the extraction step is beneficial to destroy surface carbonates and to aid the penetration of the leaching agent into the sample.

For microwave extractions acetic acid is preferred to HCl (commonly used in combination with methanol) because of the danger of nucleophilic attack on the organotin compounds, resulting in possible cleavage of side groups[255]. However it has been demonstrated that traditional extraction with HCl is more effective than acetic acid or HBr at liberating MBT from the sediment matrix, whereas recoveries for TBT and DBT are not influenced by the type of acid employed[235]. In addition the use of acetic acid has been suggested to prevent resorption of the species onto the sediment following extraction[255], as was reported to occur immediately following a methanolic HCl extraction procedure[252].

Following extraction of the analytes of interest the analytical technique must be capable of quantifying the individual species present in the sample. This is most commonly performed by a chromatographic method, principally high performance liquid chromatography (HPLC)[258-261] or gas chromatography (GC)[250,262,263], coupled to a suitable detector. In the case of GC a number of sample preparation steps are usually involved in the treatment of the sample since being involatile the analytes often have to be derivatised in order to obtain volatile compounds suitable for analysis. HPLC, although less commonly used for organotin speciation, does not require a derivatisation step and thus sample preparation is generally easier and less time

consuming. Recently, inductively coupled plasma-mass spectrometry (ICP-MS) has been widely adopted as a suitable detector for HPLC[240,245,259,264,265], offering considerable benefits in terms of sensitivity and selectivity.

This chapter describes the detailed evaluation of a microwave extraction technique for the determination of TPhT, TBT, DBT and MBT in sediment samples. Initial work was based on the method of Szpunar *et al.*[255] who developed a method for the microwave extraction of TBT, DBT and MBT in sediments and biological materials. The procedure involved acetic acid leaching followed by derivatisation with sodium tetraethylborate, extraction into isooctane and analysis by capillary gas chromatography-microwave induced plasma atomic emission spectrometry (GC-MIP-AES). Triphenyltin, although commonly used as a biocide, was not however included and thus it was decided that the developed technique should be capable of determining this species. In addition the possibility of analysis by HPLC-ICP-MS, rather than GC-MIP-AES, was investigated. This required modifications to the sample preparation method, following microwave extraction, to convert the sample matrix into a form compatible with the HPLC technique. For this purpose the analytes of interest must be isolated so they can be taken up in the HPLC mobile phase (methanol based) prior to analysis. This can be achieved by back extraction into an organic solvent, followed by its removal by rotary evaporation to dryness. Apolar solvents are most popular as they enable easy phase separation from the acid leachate. However the importance of a polar solvent for the quantitative extraction of MBT (due to its high polarity) has been suggested in the literature[235,266]. In addition if the sediment sample is present during the back extraction step a polar solvent is beneficial to prevent resorption of the extracted species onto the sediment[254]. Toluene (intermediate polarity) was therefore chosen, having been used by a number of workers for this purpose[235,245].

4.2.2 Experimental

4.2.2.1 Instrumention

Microwave extractions were carried out in a single mode reflux focused microwave system (Microdigest 301, Prolabo Ltd., Paris, France) fitted with an Aspivap fume treatment system (Prolabo) as described in Section 3.1.2.1. Extractions were undertaken in both glass and PTFE standard digestion vessels (Prolabo).

For the speciation of TBT, DBT, MBT and TPhT samples were analysed by HPLC-ICP-MS following the method of Rivas *et al.*[245]. Chromatographic separation of TBT, TPhT, DBT and MBT was achieved using two 4.6 mm x 25 cm stainless steel HPLC columns packed in house with Partisil-10 SCX ion-exchange resin (Thames Chromatography, Maidenhead, Berkshire, UK). The analytical column was protected by a guard column (2.5 x 4.6 mm) packed with the same material. Sample injections were undertaken by way of a Cheminert Model C1 injection valve (Valco Instruments Co. Inc., Houston, Texas, USA) equipped with a 200 µl sample loop. The mobile phases were pumped at a flow rate of 1 ml min^{-1} by way of an inert gradient HPLC pump (Model 9010, Varian Ltd., Warrington, Cheshire, UK). The chromatographic separation consisted of a step gradient elution with three different mobile phases, the composition of which are shown in Table 4.5. Each mobile phase was buffered with citric acid-ammonium citrate at the pH values given in Table 4.5. This was achieved by preparing solutions in 0.03 M citric acid and adjusting the pH to the desired value by the addition of ammonium citrate solution. The HPLC columns were equilibrated by running mobile phase 1 (70% MeOH, pH 5.8) at a flow rate of 0.1 ml min^{-1} over night. The flow rate was then increased to 1.0 ml min^{-1} and the HPLC separation proceeded by injection of 200 µl of the sample onto the column. Under these conditions TBT and TPhT are eluted although the more polar DBT and MBT species are retained on the

column. After 2 minutes the methanol concentration of the mobile phase was increased to 85% (mobile phase 2) to facilitate the elution of DBT. MBT was finally eluted by switching to mobile phase 3 (pH 3.4) after a further 2.5 minutes. Conditions finally returned to mobile phase 1 after a further 6.5 minutes and the column was then allowed to equilibrate for 10 minutes before injection of the next sample.

Following chromatographic separation of TBT, TPhT, DBT and MBT sample introduction to the ICP-MS was achieved by passage through a Meinhard nebuliser and a cyclone spray chamber[258] cooled to -4°C by way of a recirculating chiller (Endocal RTE-100, Neslab Instruments Inc., New Hampshire, USA) containing 2-propanol. Cooling helps to reduce the amount of methanol reaching the plasma which may lead to extinction of the plasma. The aerosol formed during nebulisation was then sheathed by a flow of argon gas (to help prevent build up of salts in the injector tube of the torch) before passing through a 35 cm transfer line (Nalgene non toxic autoclavable tubing, 1/4" o.d. x 1/8" i.d., Merck) and entering the base of the torch. To avoid salt deposition in the injector tube a demountable torch with a tapered injector (final i.d. of 1.2 mm) was employed.

Detection was performed using a PlasmaQuad 2+ ICP-MS (Fisons Instruments Elemental, Cheshire, UK) under the operating conditions given in Table 4.6. An addition of oxygen (1.4%) was made to the argon nebuliser gas to avoid carbon deposition on the nickel sampler and skimmer cones (FI Elemental).

For the determination of total tin the ICP-MS instrument was operated under the conditions given in Table 4.7. An internal standard of 100 ng g^{-1} indium (Aldrich Chemicals, Dorset, UK) was added to all samples and standards to correct for instrumental drift during analysis.

Table 4.5: Mobile Phase Compositions and Step Gradient Programme for HPLC-ICP-MS Analysis

Mobile Phase	Methanol:Water (v/v)	pH	Time (min)
1	70:30	5.8	2
2	85:15	5.8	2.5
3	85:15	3.4	6.5

Table 4.6: ICP-MS Operating Conditions for HPLC-ICP-MS Determinations

Plasma gas flow rate	15 l min^{-1}
Auxiliary gas flow rate	1.0 l min^{-1}
Nebuliser gas flow rate	0.2 l min^{-1}
Oxygen addition	1.4%
Sheath gas flow rate	0.75 l min^{-1}
Forward power	1500 W

Table 4.7: ICP-MS Operating Conditions for the Determination of Total Tin

Plasma gas flow rate	15 l min^{-1}
Auxiliary gas flow rate	1.0 l min^{-1}
Nebuliser gas flow rate	0.9 l min^{-1}
Forward power	1500 W

4.2.2.2 Reagents

Tributyltin chloride (96%), dibutyltin chloride (96%) and monobutyltin chloride (95%) were obtained from Aldrich Chemicals; whereas triphenyltin was purchased from Fluka Chemie (Buchs, Switzerland). Stock 1000 $\mu g\ g^{-1}$ solutions of each were prepared (by mass) in methanol (HPLC grade, Rathburn Chemicals Ltd., Peebleshire, Scotland) and stored at 4°C in the dark prior to use. Inorganic tin calibration standards were prepared from a stock solution (9990 $\mu g\ ml^{-1}$) obtained from Aldrich Chemical Co. Glacial acetic acid (Aristar) was obtained from Merck Ltd. (Poole, Dorset, UK) and toluene from Rathburn Chemicals. The HPLC mobile phases were buffered with triammonium citrate and citric acid (Fisons Scientific Equipment, Loughborough, Leicestershire, UK).

Deionised water (18 $M\Omega\ cm^{-1}$) was purified through a Millipore purification system (Millipore-Waters, Bedford, MA, USA). All glass ware was soaked in 2% (v/v) Decon and 10% (v/v) nitric acid (Analar, Merck Ltd.) for at least 24 h prior to use. In addition to remove any organic compounds present on the surface all vessels were rinsed with toluene and then deionised water. To assess the extraction efficiency of the developed technique spike recoveries were performed on a 'clean' sediment. This was collected from an unpolluted source, dried at 110°C and then ground to pass through a 710 μm mesh prior to use. For validation of the technique the certified reference material PACS-1 (harbour marine sediment) was obtained from the National Research Council Canada (Ottawa, Ontario, Canada).

4.2.3 Determination of Total Tin by ICP-MS

Initial studies involved evaluation of the method for only TBT. This enabled assessment of the efficiency of the method by determining the total tin content of the sample, rather than requiring detailed speciation analysis as would be necessary if more than one species were present. This approach also permitted greater sample throughput, a necessity in the early stages of the development and optimisation of the technique.

4.2.3.1 Sample Spiking and Microwave Extraction Procedure

Prior to microwave extraction 0.2 g of the 'clean' sediment was weighed into a digestion vessel and spiked with 1200 ng of TBT (as Sn) and 1 g of methanol. After shaking to ensure complete interaction of the spike with the sediment, the sample was left to equilibrate overnight in the dark at 4°C. For microwave extraction, 10 ml of 50% (v/v) acetic acid was added and the flask swirled to ensure complete mixing with the sample. Initially a sample mass of 1 g was employed however it was found that incomplete mixing with the acetic acid resulted during microwave heating as the sample tended to form a dense mass at the bottom of the digestion vessel. To ensure complete interaction with the acetic acid and thus higher extraction efficiency the mass of sample was reduced to 0.2 g. Microwave energy was then applied at a power of 60 W, before cooling and carefully pouring the supernatant into a separating funnel (100 ml). The sediment residue was then washed with a further 5 ml of 50% (v/v) acetic acid which, after allowing to settle, was also added to the separating funnel. After the addition of approximately 10 ml of deionised water the sample was back extracted with 10 ml of toluene (x 3) and the organic phase collected in a round bottom flask. The toluene was then removed by rotary evaporation to near dryness.

Initial studies highlighted the importance of the rotary evaporation step. Care must be taken to ensure that the volatile organotin species are not driven off during the evaporation of the toluene. Losses were investigated by spiking 30 ml of toluene with a known amount of TBT and carrying through the rotary evaporation step before analysis for total tin by ICP-MS. It was found that the temperature of the water used to heat the round bottomed flask was a critical factor. At a temperature of 80°C recoveries for TBT were poor (40%) however at the lower temperature of 35-40°C complete recoveries (93%) were obtained. Thus the temperature of the water was kept as low as possible to avoid losses of the analytes.

Following evaporation to dryness samples were stored in the dark at 4°C prior to analysis for total tin by ICP-MS. For this purpose the extracts were quantitatively transferred from the round bottom flask into a 25 ml volumetric flask, rinsing with 8 g of methanol. The final mass of the sample was made up to 20 g by the addition of 0.52 M [3.33% (v/v)] nitric acid and 0.2 g of a 10 mg l^{-1} indium solution. This produced a final concentration of 40% (v/v) methanol and 0.31 M [2% (v/v)] nitric acid with an internal standard concentration of 100 ng g^{-1} indium. Dissolution of the sample in methanol and then dilution to a concentration of 40% (rather than initial dissolution in 40% methanol) was undertaken as a precaution to ensure complete uptake of the species from the round bottom flask, in light of the low solubility of organotin compounds in aqueous media. For calibration inorganic tin standards in the range 0 to 80 ng g^{-1} were also prepared in 40% (v/v) methanol and 0.31 M nitric acid. Acidification was necessary to prevent inorganic tin from plating out onto the surface of the glass volumetric flasks. To ensure complete matrix matching, samples were also acidified in this way.

4.2.3.2 Optimisation of the Microwave Extraction Time for TBT

The microwave extraction time was optimised by variation of the heating time between 2 and 5 minutes (power = 60 W). The recoveries obtained for TBT are shown in Table 4.8 (n=3). As can be seen the extraction efficiency increases with increasing heating time to reach an optimum at 4 minutes and thus is higher than the 3 minutes heating proposed by Szpunar *et al.*[255] using a similar procedure. With an extraction time of 5 minutes recoveries were substantially reduced, probably as a result of analyte losses due to prolonged heating.

Table 4.8: Optimisation of the Microwave Extraction Time for TBT

Extraction Time[1] (min)	Recovery (%)
2	74 ± 15[2]
3	73 ± 5
4	96 ± 16
5	58[3]

Notes:
1: Microwave extractions undertaken at 60 W power (n=3).
2: Uncertainties are calculated as twice the standard deviation.
3: n=1.

4.2.4 Speciation of Organotins by HPLC-ICP-MS

4.2.4.1 Extraction Efficiency for TBT, TPhT, DBT and MBT

The possibility of extending the method to include the organotin species TPhT, DBT and MBT was then investigated. For this purpose 0.2 g of 'clean' sediment was spiked with 300ng each of TBT, TPhT, DBT and MBT (as Sn) and allowed to equilibrate overnight as before. An extraction time of 4 minutes (power = 60 W) was chosen based on the earlier results (see Table 4.8). In order to assess the individual recoveries for each species, samples were analysed by HPLC-ICP-MS following the method given in Section 4.2.2.1. Following back extraction and rotary evaporation as before, samples were quantitatively transferred into a pre-weighed 8 ml glass vial and made up to a mass of 3 g with mobile phase 1 (see Table 4.5). Mixed calibration standards of the four species in the range 0 to 250 ng g^{-1} (as Sn) were also prepared in the same matrix. Prior to injection onto the HPLC column it was necessary to filter samples through a 4 mm nylon filter (0.2 μm pore size, Phenomenex, Macclesfield, Cheshire, UK) in order to prevent blockages in the injection valve/HPLC column. The recoveries obtained for TBT, TPhT, DBT and MBT following the microwave extraction (4 min) of replicate samples (n=5) are shown in Table 4.9. Good results were obtained for MBT, however those for TPhT and DBT were somewhat low. In contrast TBT recoveries often exceeded 100%, suggesting that decomposition of another species to TBT (or to a species which coelutes with TBT) may be occurring during microwave heating.

Table 4.9: Recoveries of Organotin Species in Spiked Sediment Samples following Microwave Extraction for 4 minutes (60 W Power)

Sample	Recovery (%)			
	TBT	TPhT	DBT	MBT
1	126	39	41	90
2	105	38	50	94
3	131	42	52	94
4	85	36	61	97
5	124	38	24	95
Mean	124 ± 38^{1}	38 ± 4	41 ± 28	94 ± 4

Notes:
1: Uncertainties are calculated as twice the standard deviation.

4.2.4.2 Studies to Investigate Possible Degradation Products formed during the Microwave Extraction Procedure

In order to explain the elevated recoveries previously obtained for TBT, investigations were undertaken to test the hypothesis that degradation of a species to TBT was occurring during microwave extraction. For this purpose the 'clean' sediment was spiked with the organotin species separately and carried through the extraction procedure as before. The results obtained following microwave extraction for 4 minutes are given in Table 4.10 (n=1). From these results there is strong evidence to suggest that degradation of TPhT is occurring during the extraction procedure. The chromatograms obtained for the TPhT and TBT samples are compared with a mixed standard of 100 ng g^{-1} TBT, TPhT and DBT and MBT (as Sn) in Figure 4.1. For the TPhT sample (Figure 4.1b) it can be seen that a species (referred to as 'Peak 1') is eluting just before the TBT peak, probably with the solvent front, rather than coeluting with TBT as was originally thought. Previously, in the presence of TBT, it was impossible to resolve the two species due to their similar retention times, however following the separate spiking of the sediments it is easier to identify the individual species present.

From Figure 4.1b it can also be seen that a large broad peak is obtained for MBT. However it should be noted that a number of other organotin species such as mono and di ethyl and phenyltin species, such as from the degradation of TPhT, may elute with the same retention time as MBT[267].

Table 4.10: Recoveries of Organotin Species after Spiking Separately into Sediment Matrix and Microwave Extraction for 4 minutes (60 W Power)

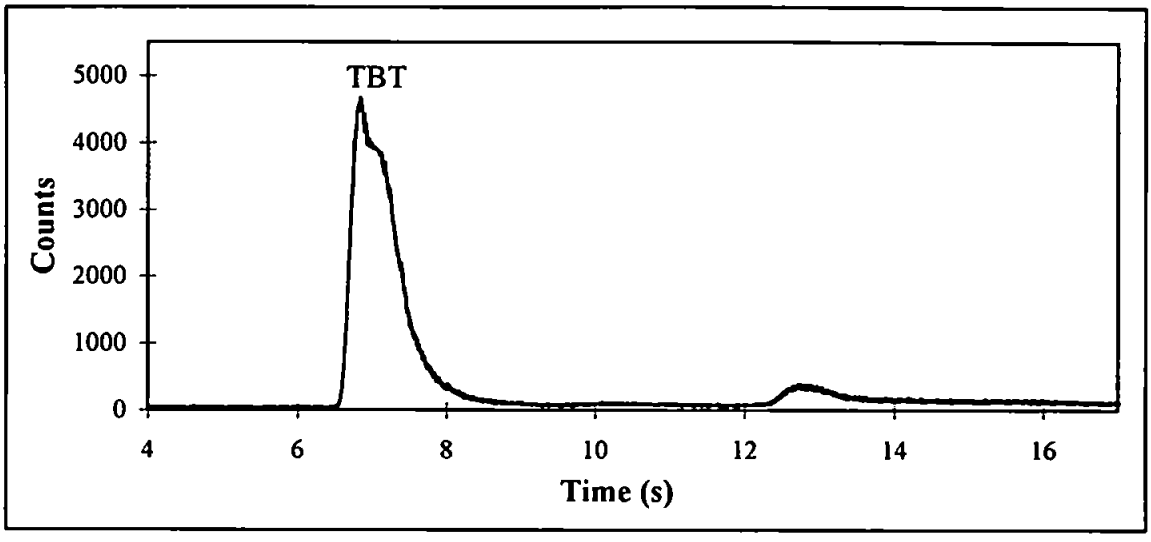
Species Spiked	Recovery (%)				
	'Peak 1' [1]	TBT	TPhT	DBT	MBT
TBT	-	90	-	-	-
TPhT	37	-	17	-	49
DBT	-	-	-	33	-
MBT	-	-	-	-	44

Notes:
1: The unidentified peak is referred to as 'Peak 1'.

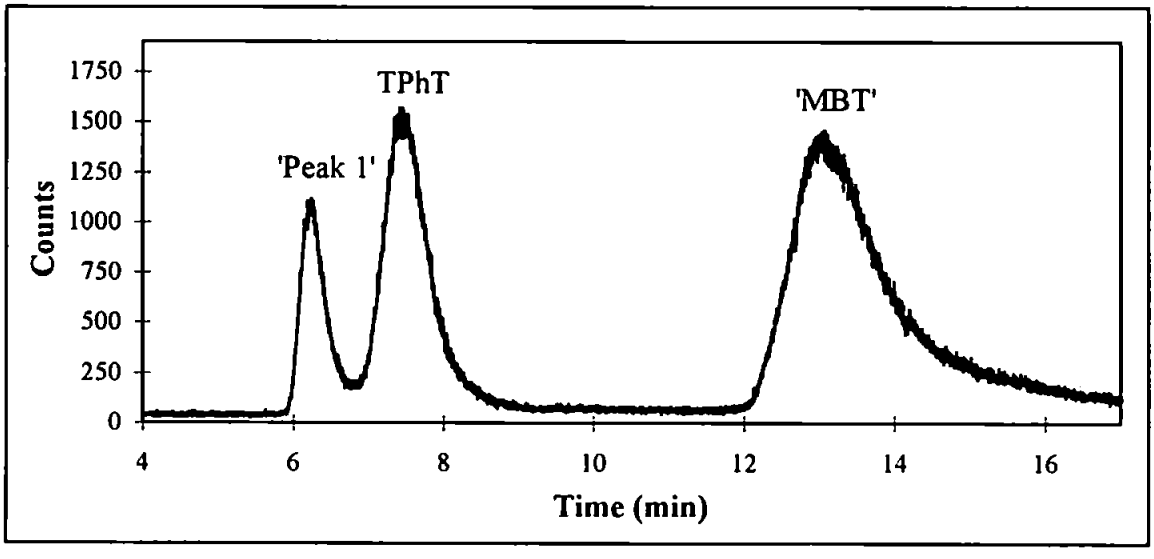
Following separate spiking, the recoveries obtained for each species were in agreement with those obtained for the mixed spiking of the 'clean' sediment (Table 4.9). For example, the sum of the recovery obtained for the species formed from the degradation of TPhT [37% (Peak 1)] and the TBT recovery (90%) (Table 4.10) is similar to the recovery of 124% obtained for TBT in the presence of TPhT (Table 4.9). In addition if the MBT portion originating from the degradation of TPhT (49%) is added to the individual MBT recovery (44%) (Table 4.10) a total of 93% is obtained, similar to the recovery of 94% obtained for MBT in the presence of TPhT (Table 4.9). These results would seem to confirm the hypothesis of degradation and explain the high results obtained for TBT previously.

Figure 4.1: Chromatograms Obtained following Sediment Spiking with a)TBT, b)TPhT and for Comparison c)a Mixed Standard of 100 ng g^{-1} TBT, TPhT, DBT and MBT

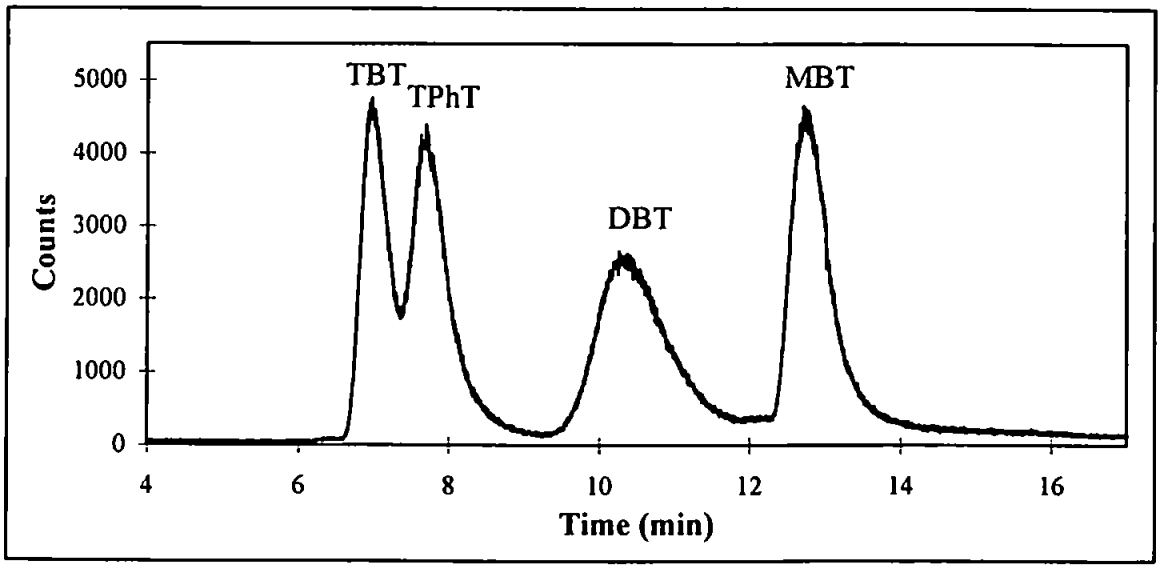
a)



b)



c)



4.2.4.3 Theoretical Degradation Pathway for TPhT

The organometallic tin-carbon bond is usually stable up to a temperature of 200°C[228] and generally requires treatment with strong acids, alkalis, strong oxidising agents, halogens or other nucleophilic agents[259,260] to cleave the organometallic bond. However triaryltin compounds such as triphenyltin chloride or fluoride are more susceptible to acid attack with possible cleavage resulting after treatment with weak acids. Following cleavage, decomposition usually follows the general degradation pathway for organotin compounds i.e. progressive dealkylation to inorganic tin, as shown in Reaction 4.1 below:

$$R_3SnX \rightarrow R_2SnX_2 \rightarrow RSnX_3 \rightarrow SnX_4 \qquad \textbf{(Reaction 4.1)}$$

where R represents an alkyl group and X is an anion or equivalent group e.g. Cl^-, OH^- etc.

From Reaction 4.1 the expected degradation products of TPhT are therefore diphenyltin (DPhT), monophenyltin (MPhT) and inorganic tin. As stated above DPhT and MPhT elute with MBT, thus explaining the presence of the 'MBT' peak obtained. The total recovery for TPhT and its degradation products (103%) (Table 4.10) suggest that no inorganic tin was formed. However the concentration of inorganic tin is unquantifiable due to retention on the HPLC column (verified by injecting a solution of 150 ng g^{-1} inorganic Sn onto the column).

The degradation product of TPhT, 'Peak 1' (Figure 4.1b), elutes with the solvent front and thus can be attributed to a non polar compound. The seemingly only logical explanation is that this peak is tetraphenyltin (TePhT). A plausible explanation for the observed degradation products is therefore that during microwave heating a redistribution reaction takes place to form (TePhT) and (DPhT) and/or (MPhT). However with this chromatographic separation the exact decomposition products of TPhT cannot be identified and thus this hypothesis can not be verified.

These observations thus differ from those of Donard *et al.*[256] who observed degradation products in agreement with the general degradation scheme for organotin compounds (see Equation 4.1). Inorganic tin was reported as the major decomposition product of butyl and phenyltin compounds (including TPhT) following microwave heating in matrices of isoctane, methanol, water and artificial sea water and little to no intermediate species were obtained. However direct comparisons can not be made as the stability of the species in acetic acid were not investigated, nor was TPhT included in the work of Szpunar *et al.*[255] who employed a microwave extraction procedure with acetic acid.

4.2.4.4 Effect of Microwave Heating on the Stability of TBT, TPhT, DBT and MBT in a Matrix of Acetic Acid

To examine further the stability and behaviour of TBT, TPhT, DBT and MBT during microwave heating, standard solutions of the different species [prepared in 50% (v/v) acetic acid] were carried through the whole procedure. The absence of the sediment sample allowed more detailed examination of the processes occurring without being obscured by low results as a consequence of incomplete extraction of the species from the sample matrix. Following preparation of standard solutions (50 ng g^{-1}) of each species in 50% (v/v) acetic acid, 10 ml of each was heated at a power setting of 60 W for 4 minutes (n=1). Samples were also heated for 3 minutes to examine whether decomposition of TPhT also occurred with a shorter heating time.

From the results given in Table 4.11 the decomposition of TPhT is again apparent following microwave heating for 4 minutes. This was also experienced with a lower heating time of 3 minutes, although degradation was not so pronounced, thus indicating the influence of microwave heating on the degradation of TPhT. For both samples the total recovery (84%) however is the same, indicating that losses through volatilisation were not occurring. The

results also suggest that little to no inorganic tin was produced, thus again highlighting the differences of the findings of Donard *et al.*[256] who reported inorganic tin as the major degradation product.

Table 4.11: Recoveries of Organotin Species after Microwave Heating in 50% (v/v) Acetic Acid at 60W Power

Species Spiked	Heating Time (min)	Recovery (%)					
		'Peak 1'	TBT	TPhT	DBT	'MBT'	Total Sn[1]
TBT	3	-	80	-	-	-	80
TBT	4	-	81	-	-	-	81
TPhT	3	19	-	34	-	31	84
TPhT	4	14	-	25	-	45	84
DBT	3	-	-	-	84	-	84
DBT	4	-	-	-	85	-	85
MBT	3	-	-	-	-	64	64
MBT	4	-	-	-	-	66	66

Notes:
1: Total Sn is calculated as the sum of the individual recoveries for 'Peak 1', TBT, TPhT, DBT and 'MBT'.

From Table 4.11 it can also be seen that losses of all species occur during the complete extraction procedure. However the behaviour of the species during microwave heating in a pure solvent (acetic acid only) may well be modified by the presence of the sediment matrix. During heating the release of low polarity compounds from the sediment, e.g. PAHs, may decrease the dielectric constant of the medium, resulting in a smaller energy transfer from the leaching solvent to the analytes. Thus the organotin species may undergo less decomposition in the presence of the sediment than when heated in just the solvent, as was observed by Donard *et al.*[256]. In addition, during the extraction of organotins from a sample the species

will not be in solution for the complete duration of the heating period and thus may not be as susceptible to losses through volatilisation as when heated for the entire time in the pure solvent. Thus the results obtained here serve only as an indication of what may be expected during the analysis of a real sample.

In addition to the losses of analyte which may occur during microwave extraction e.g. decomposition or volatilisation, there are three other potential sources which may be responsible for the low results previously obtained:

i) ***Back extraction step*** i.e. inefficient extraction of species from the acetic acid phase into the toluene phase.

ii) ***Rotary evaporation step*** i.e. volatilisation of species during evaporation of toluene.

iii) ***Redissolution of the sample in the HPLC mobile phase*** i.e. incomplete uptake of the sample from the round bottom flask.

In order to explain the low recoveries previously obtained, investigations were undertaken to examine the losses of analytes occurring at each of the steps listed above.

4.2.4.5 Efficiency of the Rotary Evaporation and Redissolution Stages

To determine potential losses occurring during rotary evaporation and redissolution of the sample in the mobile phase, 30 ml of toluene was spiked with 450 ng of TBT, DBT and MBT (as Sn), rotary evaporated and prepared for analysis as before. The chromatographic separation was modified by increasing the elution time from 2.5 to 4.5 minutes using mobile phase 2 so as to improve the separation between the DBT and MBT species (see Figure 4.1c for previous separation). The effect of this modification is illustrated in Figure 4.2 for a mixed standard of 100 ng g^{-1} TBT, DBT and MBT (as Sn).

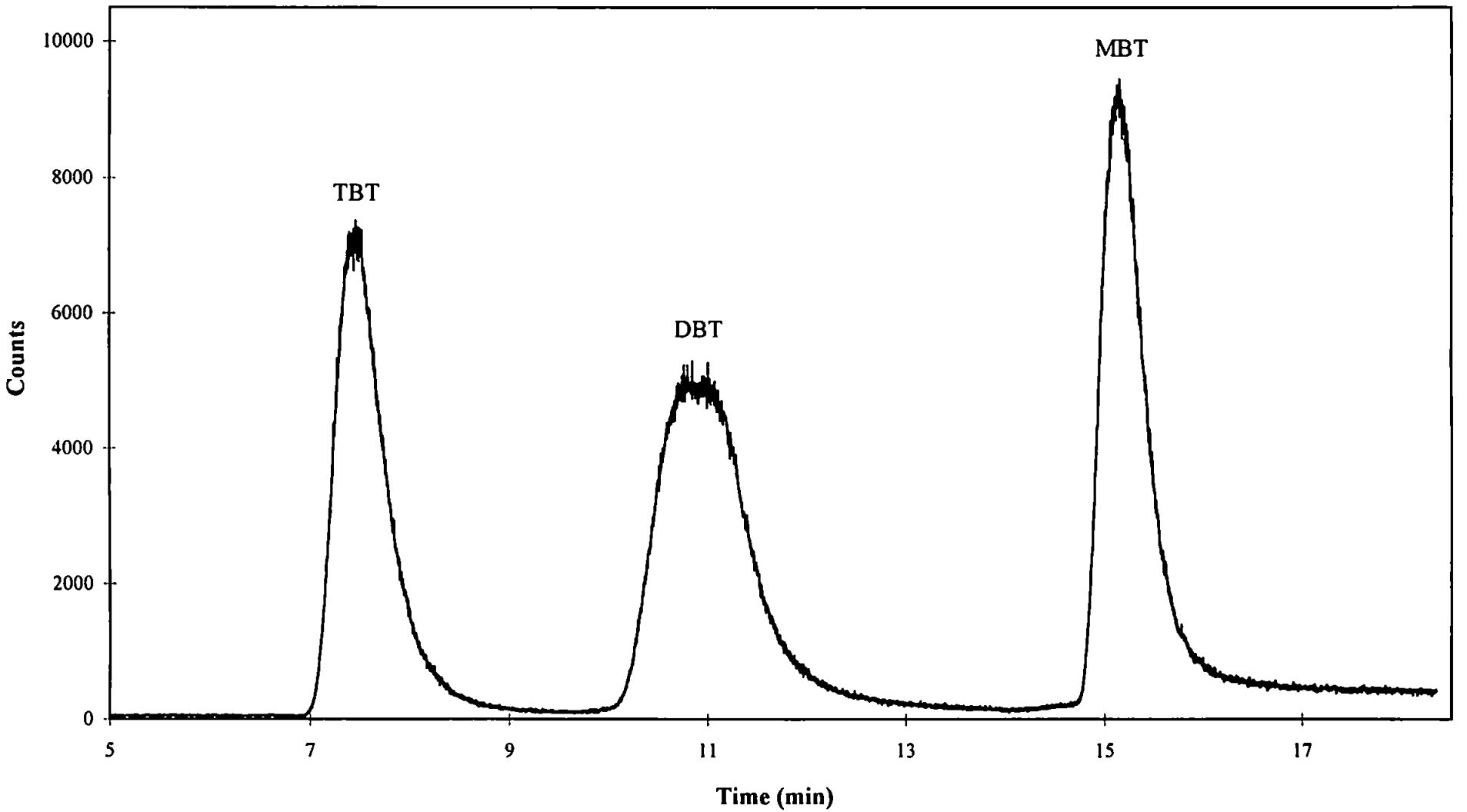
It may be expected that MBT would be the most susceptible to losses through volatilisation during rotary evaporation, having the lowest boiling point of 93°C (for the chloride) compared with 135°C and 172°C for DBT and TBT respectively. However the recoveries in Table 4.12 demonstrate that negligible losses of TBT, DBT or MBT occur during this part of the procedure.

Table 4.12: Efficiency of the Rotary Evaporation and Redissolution Steps for TBT, DBT and MBT

Sample	Recovery (%)		
	TBT	DBT	MBT
1	110	108	96
2	95	96	96
3	104	95	90
Mean	103 ± 15[1]	100 ± 14	94 ± 7

Notes:
1: Uncertainties are calculated as twice the standard deviation.

Figure 4.2: Chromatographic Separation of 100 ng g^{-1} TBT, DBT and MBT



4.2.4.6 Efficiency of the Back Extraction Step

To examine the losses of the different organotin species during the back extraction step, 10 ml of 50% (v/v) acetic acid was spiked with 450 ng of TBT, DBT and MBT (as Sn), back extracted and rotary evaporated as before. From the recoveries obtained for replicate samples (n=4) it can be seen that losses occur for all species (Table 4.13). In addition the results reflect the efficiency of the rotary evaporation and redissolution steps, although as negligible losses were obtained for these stages (see Table 4.12) the low recoveries can in fact be attributed to the back extraction step. This is as a consequence of inefficient extraction of the species from the acetic acid into the toluene phase. As expected the lowest recoveries were obtained for MBT which being the most polar species will have least affinity for the organic phase.

Table 4.13: Efficiency of the Back Extraction Step for TBT, DBT and MBT

Sample	Recovery (%)[1]		
	TBT	DBT	MBT
1	93	90	73
2	83	90	52
3	82	84	67
4	85	77	79
Mean	86 ± 10^{2}	88 ± 12	68 ± 23

Notes:
1: In addition results include the efficiency of the rotary evaporation and redissolution steps.
2: Uncertainties are calculated as twice the standard deviation.

If the results are compared with those obtained following the heating of standard solutions of TBT, DBT and MBT in acetic acid (see Table 4.11) it can be inferred that no losses occur during microwave heating. Losses through volatilisation during heating would not be expected as a temperature of only 86°C was reached during the microwave extraction procedure (at 60W power for 4 min), which is lower than the boiling points of 171-173°C, 135°C and 93°C for TBT, DBT and MBT chloride respectively. The low results in this case (Table 4.11) therefore originate from the inefficient back extraction step rather than to losses during microwave heating.

4.2.4.7 Extraction Efficiency of the Complete Procedure

Further work was undertaken to investigate the extraction efficiency of the complete procedure for TBT, DBT and MBT. TPhT was omitted to avoid obscuring the results obtained for TBT and MBT with the degradation products of this species. The 'clean' sediment sample was spiked with TBT, DBT and MBT and prepared for analysis as before. The results of the HPLC-ICP-MS analysis after microwave extraction for 3 and 4 minutes (at 60 W power) are shown in Table 4.14. As can be seen, in the absence of TPhT reproducibility is greatly improved. The extraction efficiency was greatest with a heating time of 4 minutes and thus these conditions were adopted in further work. Recoveries were also in agreement with earlier work following the separate spiking of the sediment sample with TBT, DBT and MBT (see Table 4.10). The highest results were obtained for TBT which being the least polar species will have least adsorption affinity for the components of the sediment and so will be most easily liberated from the matrix. The results however do not just reflect the efficiency of the microwave extraction step but also represent the losses during the back extraction procedure. If results are corrected for the latter (using results in Table 4.13) the

efficiency of the microwave extraction step can be calculated as 100%, 44% and 76% for TBT, DBT and MBT respectively.

Table 4.14: Extraction Efficiencies for TBT, DBT and MBT following Microwave Extraction at 60 W Power

Extraction Time	TBT	DBT	MBT
3	61	9	49
3	81	11	30
Mean (%)	71	10	40
4	94	43	49
4	83	39	45
4	85	44	59
4	82	32	55
Mean (%)	86 ± 11[1]	39 ± 11	52 ± 12

Notes:
1: Uncertainties are calculated as twice the standard deviation.

4.2.4.8 Validation of the Microwave Extraction Technique by Analysis of the Certified Reference Material NRCC PACS-1

To assess the efficiency of the method for a real sample the certified reference material NRCC PACS-1 was analysed. The results obtained are given in Table 4.15. As can be seen the recoveries for TBT and DBT are poor. For TBT the recoveries are also far lower than the spike recoveries obtained for the 'clean' sediment (Table 4.14). This may be because the PACS-1 sample contains more organic material than the 'clean' sediment (sandy sediment) and thus TBT will be more difficult to extract, having a high affinity for the organic portion of the sample due to its hydrophobic nature. Higher recoveries in the case of the spiked samples may also occur because the spikes are not as strongly bound to the sediment matrix as the species in a real sample.

Table 4.15: Results for the Microwave Extraction of NRCC PACS-1

	TBT ($\mu g\ g^{-1}$)	DBT ($\mu g\ g^{-1}$)	MBT ($\mu g\ g^{-1}$)
Certified Value	1.27 ± 0.22	1.16 ± 0.18	0.28 ± 0.17
Experimental Results[1]	0.39 ± 0.10[2]	0.46 ± 0.05	0.47 ± 0.08
Recovery (%)	29	40	172

Notes:
1: Extractions undertaken for 4 minutes at 60 W power.
2: Uncertainties for the experimental results are calculated as twice the standard deviation.

In contrast to the low results for TBT, MBT results are high, although only just fall outside the uncertainty limits of the certified value. A number of workers have also reported high results for MBT in this sample (see Table 4.16). In fact only three out of the thirteen procedures listed obtained results within the range of the certified value and it has been postulated that the certified value for MBT is too low[255,266]. Although our results are also high for MBT, as previously stated a number of other organotin compounds such as mono and di ethyl and phenyltin species may also elute with the MBT peak and thus the value obtained may not give a true indication of the concentration of MBT actually present. However the possibility that the MBT result may also be elevated by the decomposition products of TPhT is unlikely as these compounds were not used frequently in Canada before the sampling of the PACS-1 CRM sample[268].

Table 4.16: Comparison of Literature Values for MBT in NRCC PACS-1

Procedure	MBT ($\mu g\ g^{-1}$)
Certified Value	**0.28 ± 0.17**
Leaching with concentrated acetic acid	0.55 ± 0.05[246] 0.72 ± 0.16[246] 0.59 ± 0.06[247,248] 0.69 ± 0.18[245]
Extraction with tropolone into hexane	0.36 ± 0.17[242]
Extraction with tropolone into toluene	1.03 ± 0.01[266]
Extraction with aqueous $NaBE_4$ into hexane	0.49 ± 0.09[269] 0.52 ± 0.15[269]
Acidfication with HCl, extraction with tropolone into hexane-ethyl acetate	0.94 ± 0.06[235]
Microwave extraction with 0.5 M acetic acid in methanol	0.37 ± 0.01[256,257]
Microwave extraction with 8.5 M acetic acid	0.76 ± 0.05[255]

4.2.4.9 Spike Recoveries for NRCC PACS-1

Investigations were then undertaken to examine the recoveries obtained following the spiking of NRCC PACS-1 with TBT, DBT and MBT. For this purpose the sample was spiked with these species at similar concentrations to those expected in the original sample i.e. 250, 230 and 60 ng (as Sn) of TBT, DBT and MBT respectively. After allowing to equilibrate overnight samples were extracted and prepared for analysis as before. The spike recoveries were calculated after subtracting the expected concentration of each species extracted from the original sample using the results in Table 4.15. The calculated spike recoveries for TBT, DBT and MBT are shown in Table 4.17.

Table 4.17: Recoveries Obtained Following the Spiking of NRCC PACS-1 with 250, 230 and 60 ng (as Sn) of TBT, DBT and MBT Respectively

Sample[1]	TBT (%)	DBT (%)	MBT (%)
1	82.6	88.7	32.0
2	60.2	85.9	30.6
3	69.2	86.0	47.8
4	71.2	82.8	37.8
5	69.4	82.2	27.1
Mean	**70.5 ± 16.0[2]**	**85.1 ± 5.3**	**35.1 ± 16.2**
RSD (%)	11.3	3.1	23

Notes:
1: Microwave extractions undertaken for 4 minutes at 60 W power.
2: Uncertainties for the experimental results are calculated as twice the standard deviation.

The spike recoveries obtained for TBT and MBT are lower than those obtained previously for the 'clean' sediment (sandy sediment) which may be due to the higher organic content of NRCC PACS-1. Similar observations were made by Ceulemans *et al.*[235] who reported large variations in spike recoveries for MBT (27-93%) in nine different sediment samples. Although no definite correlation could be made between the recoveries and the chemical composition of the sample it was observed that high levels of sulphur and organic matter could negatively affect the efficiency of the extraction for MBT. In contrast however, the spike recoveries for DBT in PACS-1 are far higher than those obtained for the 'clean' sediment sample, the reason for which is uncertain.

The MBT spike recoveries were also lower than the original recoveries for PACS-1 which is not possible unless the spike is more firmly bound to the sediment matrix than the original species, which is very unlikely. It can therefore be concluded that the high results originally obtained for PACS-1 (172%) are either because the certified value is too low or are due to the presence of other compounds eluting with the MBT peak.

4.2.4.10 Recoveries for NRCC PACS-1 after Application of the Correction Factor

It has been demonstrated that spike recoveries can be used as a correction factor to adjust low results for certified reference samples on the understanding that this will take into account any systematic losses throughout the procedure, including low extraction efficiency of the species from the sample matrix[245]. However there is evidence in the literature to suggest that the spike is not recovered in the same way as the original compounds in the sediment[252,266]. If the spiked compounds are more weakly bound to the sediment matrix than the original species (as has been postulated) this will lead to over estimation of the extraction efficiency, thus the correction factor will be too low. Therefore after application of this factor to the

original results, recoveries will still be lower than the true value. Despite this, the original results for PACS-1 were recalculated taking into account the recoveries obtained for the spiked samples. From the results given in Table 4.18 it can be seen that the results for TBT and DBT are still low. MBT was omitted due to the questionable validity of the results in PACS-1, as explained previously.

Table 4.18: Corrected Recoveries for NRCC PACS-1

	TBT ($\mu g\ g^{-1}$)	DBT ($\mu g\ g^{-1}$)
Certified Value	1.27 ± 0.22	1.16 ± 0.18
Experimental Result[1]	0.55 ± 0.14[2]	0.54 ± 0.06
Recovery (%)	43	47

Notes:
1: Results corrected using the recoveries obtained for the spiking of PACS-1 (Table 4.17).
2: Uncertainties are calculated as twice the standard deviation.

4.2.5 Conclusions

The possibility of adapting a microwave extraction-GC-MIP-AES method for the determination of organotin compounds in sediment samples to enable analysis by HPLC-ICP-MS has been investigated. This involved modification of the sample pre-treatment procedure to convert the sample into a form compatible with the HPLC technique. For this purpose following extraction, samples were back extracted into toluene, the toluene removed by rotary evaporation and the sample dissolved in the HPLC mobile phase. Extensive investigations were undertaken to examine the losses of analytes occurring at the different stages of the above procedure. From these studies it can be concluded that negligible losses occurred during microwave heating, rotary evaporation or redissolution of the sample in the HPLC mobile phase. Losses were however identified during the back extraction step into toluene due to inefficient partitioning of the species into the organic phase. As expected this effect was most pronounced for MBT because of its high polarity. A number of workers have suggested that the presence of a complexing reagent, e.g. tropolone, is beneficial to improve the efficiency of the back extraction step, especially for polar compounds. However analysis by ion exchange chromatography is then impossible as the analytes no longer possess a charge.

The possibility of extending the technique to the determination of TPhT was also investigated, however it was observed that degradation of this species occurred during microwave heating. Although the exact degradation products of TPhT can not be identified using the current chromatographic separation there is evidence to suggest that redistribution of TPhT to TePhT and DPhT and/or MPhT is occurring.

For the spiked sediment samples, after correcting results for the inefficient back extraction step, complete recoveries for TBT were achieved, however DBT and MBT results were somewhat lower. The low results for the latter can therefore be attributed to the poor efficiency of the microwave extraction step rather than to any other source of losses. The efficiency of the technique was also assessed by analysis of the certified reference material NRCC PACS-1, however results were disappointingly low for TBT and DBT. Recoveries were also lower than those obtained following spiking of the 'clean' sediment which can be attributed to the higher organic content of PACS-1. The MBT results for PACS-1 were rejected on the basis that with the current chromatographic system their validity could not be guaranteed given that a large number of other organotin species elute with a similar retention time to MBT.

Further work is therefore required to assess the effect of the digestion conditions not only on the behaviour and stability of the various organotin compounds during microwave irradiation but also to improve the extraction efficiency of the species from the sample matrix.

5. Speciation of Arsenic in Biological Samples by High Performance Liquid Chromatography-On-Line Microwave Digestion-Hydride Generation-Atomic Absorption Spectrometry

5 SPECIATION OF ARSENIC IN BIOLOGICAL SAMPLES BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY-ON-LINE MICROWAVE DIGESTION-HYDRIDE GENERATION ATOMIC ABSORPTION SPECTROMETRY

5.1 Introduction

Arsenic is ubiquitous in the environment, being introduced from a number of natural and anthroprogenic sources. As a result of these inputs, high levels are found in many marine animals and plants, typically in the range 1-100 $\mu g\ g^{-1}$ [270]. Due to the toxicity of some forms of arsenic the levels present in mammals and fish are of major international concern and a lively area of research. The toxicity of arsenic decreases in the following order: arsenite [As(III)]>arsenate [As(V)]>monomethylarsonic acid (MMA)>dimethylarsinic acid (DMA)> arsenobetaine (AsBet)[271]. The formulae for the above arsenic species are shown in Table 5.1.

Table 5.1: Selected Arsenic Species

Arsenite	$As(OH)_3$
Arsenate	$AsO(OH)_3$
Arsenobetaine	$(CH_3)_3As^+CH_2COO^-$
Monomethylarsonic acid	$(CH_3)AsO(OH)_2$
Dimethylarsinic acid	$(CH_3)_2AsO(OH)$

The organoarsenic species arsenobetaine, [trimethyl(carboxymethyl)arsonium zwitterion], although yet to be reported in natural waters is the most abundant arsenic species present in marine organisms and has also been detected in human urine after the ingestion of seafood[272]. This species however is considered to be non-toxic[273] and so the determination of the total arsenic content of the sample can give a misleading guide to the actual toxicity. Many analytical techniques such as inductively coupled plasma-atomic emission spectrometry (ICP-

AES), inductively coupled plasma-mass spectrometry (ICP-MS) and atomic absorption spectrometry (AAS) although capable of determining total arsenic levels provide no information on the different species present. Therefore the development of suitable techniques capable of determining species specific information is vital for the accurate assessment of the toxicity of arsenic in biological samples.

Information on arsenic speciation is most commonly obtained by combining the separatory powers of high performance liquid chromatography (HPLC) with a suitable detector such as AAS[274,275], ICP-AES[276,277] or ICP-MS[277-279]. The latter, although the most sensitive technique may not be available in some laboratories, and so AAS or ICP-AES may be employed, but at the expense of a loss in sensitivity. Even for the determination of total arsenic, detection limits for the latter techniques are generally above the levels commonly found in biological samples.

The sensitivity however can be greatly improved by the use of hydride generation (HG) techniques by virtue of more efficient sample introduction and matrix removal. Several arsenic species can be determined if selective reduction is employed by carefully controlling the pH[280] e.g. As(III), DMA, total inorganic As. On reaction with sodium borohydride ($NaBH_4$) reducible arsenic species form arsines (see Section 2.6.1). Arsenite and arsenate are converted to AsH_3, MMA to CH_3AsH_2 and DMA to $(CH_3)_2AsH$[281]. By passage of the sample through a gas/liquid separator the volatile arsines can be swept by a stream of argon gas into a flame heated quartz tube for detection by atomic absorption spectrometry. However a number of important organoarsenicals, for example arsenobetaine, are 'non reducible' i.e. they do not form volatile hydrides and thus are not detected by the HG

approach. Decomposition of these species to a form that is compatible with the hydride generation technique is therefore required prior to analysis.

5.1.1 Decomposition of Organoarsenicals

The decomposition of organoarsenicals can be performed by a number of digestion methods including:

i) ***Dry ashing*** with magnesium nitrate and magnesium oxide[282].

ii) ***UV Photo-oxidation***[283-285].

iii) ***Wet digestion*** with different combinations of acid e.g. nitric, sulphuric and perchloric acid[286]; nitric, perchloric and hydrochloric acid[287,288].

iv) ***Microwave digestion*** with potassium persulfate and sodium hydroxide[289-293].

Microwave digestion with potassium persulfate has been effectively employed for the decomposition of organoarsenic species prior to analysis by HG-AAS. Le *et al.*[289] developed a batch microwave digestion technique for the determination of the total arsenic content of urine. It was found that complete decomposition of AsBet was achieved with a potassium persulfate concentration of 10-16% and a NaOH concentration greater than 1.5 M. In aqueous media the decomposition of AsBet was also achieved using an on-line microwave digestion technique with 4% potassium persulfate and 8% NaOH[289,290]. However incomplete decomposition (65%) of AsBet was reported in urine samples. The effect of lengthening the digestion coil increased noise levels and resulted in poor signal stability. The technique was later adapted for the speciation of arsenic by coupling an HPLC column to the on-line microwave digestion system.

Lopez *et al.*[292]. developed an on-line technique for the HPLC separation of arsenic species in water, sediment and biological samples prior to decomposition of organoarsenic species by microwave digestion (5% potassium persulfate and 2.5% sodium hydroxide) and analysis by HG-AAS. However it was reported that the hydride generation response of the different arsenic species was not uniform, presumably due to incomplete decomposition to the As(V) oxidation state. This is an important pre-requisite if the system is also to be employed for the determination of total arsenic.

5.1.2 Pre-Reduction of Arsenic

The efficiency of arsine formation on reaction with sodium borohydride is pH dependent and is related to the pKa values of the individual arsenic acids. However differences in the analytical response of the different species can also be assigned to differences in reaction kinetics, volatilities and the efficiency of the flame conversion of the corresponding arsines into elemental arsenic[283]. Such differences affect the sensitivity of detection for each species and may introduce bias for some species. However if all the arsenic is present in the same oxidation state prior to analysis, the response of each species is uniform and thus a single species can be used for calibration. For this reason pre-reduction to As(III), the most sensitive oxidation state for hydride generation, is often undertaken prior to analysis. The most widely used pre-reduction method involves reaction with potassium iodide, either alone[294-297] or in combination with ascorbic acid[298]. However a strongly acidic media is necessary and the reaction is reported to take up to 5 hours to complete at room temperature[297].

An alternative pre-reduction method is to pretreat samples with L-cysteine, the benefits of which are well documented in the literature[299-306]. The different arsenic species are converted to their corresponding organo-sulphur-arsenic(III) compounds which form hydrides on reaction with sodium borohydride. The optimum hydride generation conditions for each species are in the same range and in addition give the same response and so a single arsenic species can be used for calibration. It has also been reported that sensitivity is improved by up to 75% under these conditions[299] and that the pre-reduction time is very short e.g. 150 s at 97°C[300]. In addition the concentration of acid needed is generally lower than for the potassium iodide method, typically 0.01-0.5 M[301-303]. This offers benefits in terms of reducing costs, lowering blank levels and is less hazardous. Also less interferences from transition metals and other hydride forming elements such as Cu, Co Ni and Pd are generally experienced[303,304]. Various reaction times and concentrations of L-cysteine and acid have been reported in the literature and are summarised below.

Chen *et al.*[301] reported that heating samples prepared in 0.5% L-cysteine and 0.01 M HCl or HNO_3 in boiling water for 10 minutes was sufficient for the complete reduction of As(V) to As(III). Le *et al.*[302] reported that the reaction was complete after 10-20 minutes at room temperature with a higher concentration of 2% L-cysteine in 0.5 M HCl, whilst Welz *et al.*[300] reported completion of the reaction after 150 s heating at 97°C with 0.6% L-cysteine in 0.5M HCl. Brindle *et al.*[305] developed an on-line technique for the determination of arsenic in water samples. This involved the on-line mixing of the sample with a solution of 5% L-cysteine in 0.02 M HNO_3 (optimum concentration 0.01-0.06 M) before passage through a glass reaction coil placed in a water bath at 95-98°C. This concentration of L-cysteine corresponded to a final concentration of 0.7% in the samples.

5.1.3 Aims and Objectives

This chapter reports the development of an on-line system capable of both the speciation and the determination of total arsenic in biological samples using a combination of microwave digestion and HG-AAS. In order to achieve the latter aim and also to improve sensitivity, the possibility of combining an L-cysteine pre-reduction with the potassium persulfate microwave digestion was investigated. For the speciation of arsenic it was a requirement to develop a system compatible and easily coupled to a suitable HPLC separation. This approach then enabled the determination of AsBet, As(V), DMA, MMA and inorganic arsenic species by HG-AAS, following their decomposition by microwave digestion and L-cysteine pre-reduction to As(III). In addition the possibility of adapting the technique to determine the total 'reducible' arsenic content of the sample (As(III), As(V), DMA and MMA) was investigated.

The presentation of this chapter is split into 5 sections as listed below:

5.3 Development of a batch microwave digestion procedure for the decomposition of arsenobetaine in a standard solution.

5.4 Development of an on-line microwave digestion procedure for the determination of total arsenic in biological samples.

5.5 Development of an on-line HPLC-microwave digestion procedure for the speciation of arsenic in biological samples.

5.6 Development of an on-line technique for the determination of total 'reducible' arsenic in biological samples.

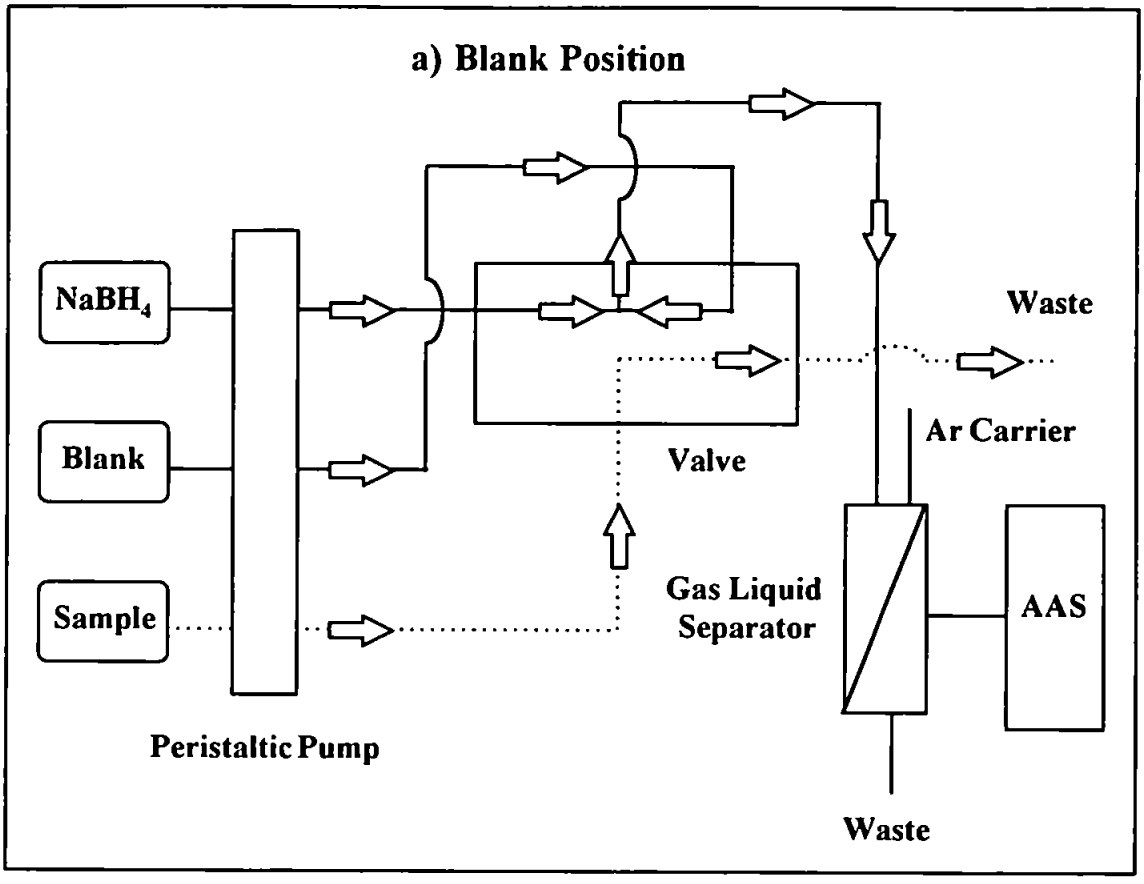
5.7 Figures of merit and validation of the developed techniques by the analysis of certified reference materials.

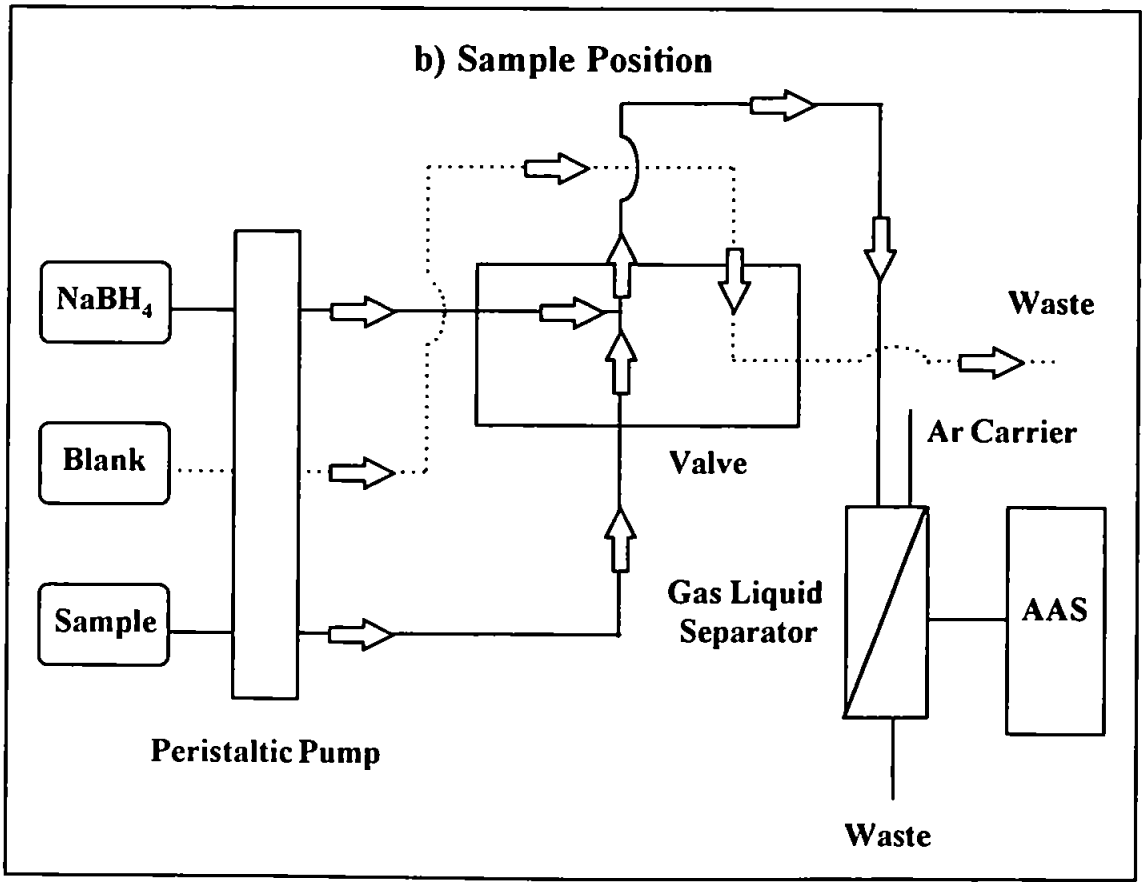
5.2 Experimental

5.2.1 Instrumentation

Sample digestion was carried out in a single mode reflux focused microwave system (Microdigest 301, Prolabo, Paris) as described previously in Section 3.1.2.1. Hydride generation was performed using a commercial hydride generator unit (Model 10.003, P.S. Analytical Ltd, Orpington, Kent, UK). A schematic representation of the system and details of the instrumental parameters are shown in Figure 5.1 and Table 5.2 respectively. The hydride generator was controlled by a computer equipped with Touchstone software (P.S. Analytical Ltd) and consisted of a peristaltic pump delivering three reagents (sodium borohydride, acid blank and sample), a computer controlled switching valve and a gas liquid separator unit (Type 'A', P.S. Analytical Ltd). In the first instance the switching valve was positioned in the 'blank' position (see Figure 5.1a) allowing the acid blank and sodium borohydride solution to continuously mix (sample runs to waste), prior to introduction into the gas-liquid separator. During this time the position of the baseline was established. Sample analysis was undertaken by computer controlled switching of the valve to the 'sample' position (see Figure 5.1b) allowing the sample and sodium borohydride solution to mix (acid blank runs to waste), before passing into the gas liquid separator unit. Here the hydrides once formed were swept, by means of a stream of argon gas bubbled through the solution into the detector. After the analysis period (usually 90 s) the switching valve returned to the 'blank' position, allowing the signal to decay back to the base line. Quantification was undertaken via the Touchstone software by taking peak area measurements from the steady state signals obtained.

Figure 5.1: Schematic Diagram of the HG-AAS System



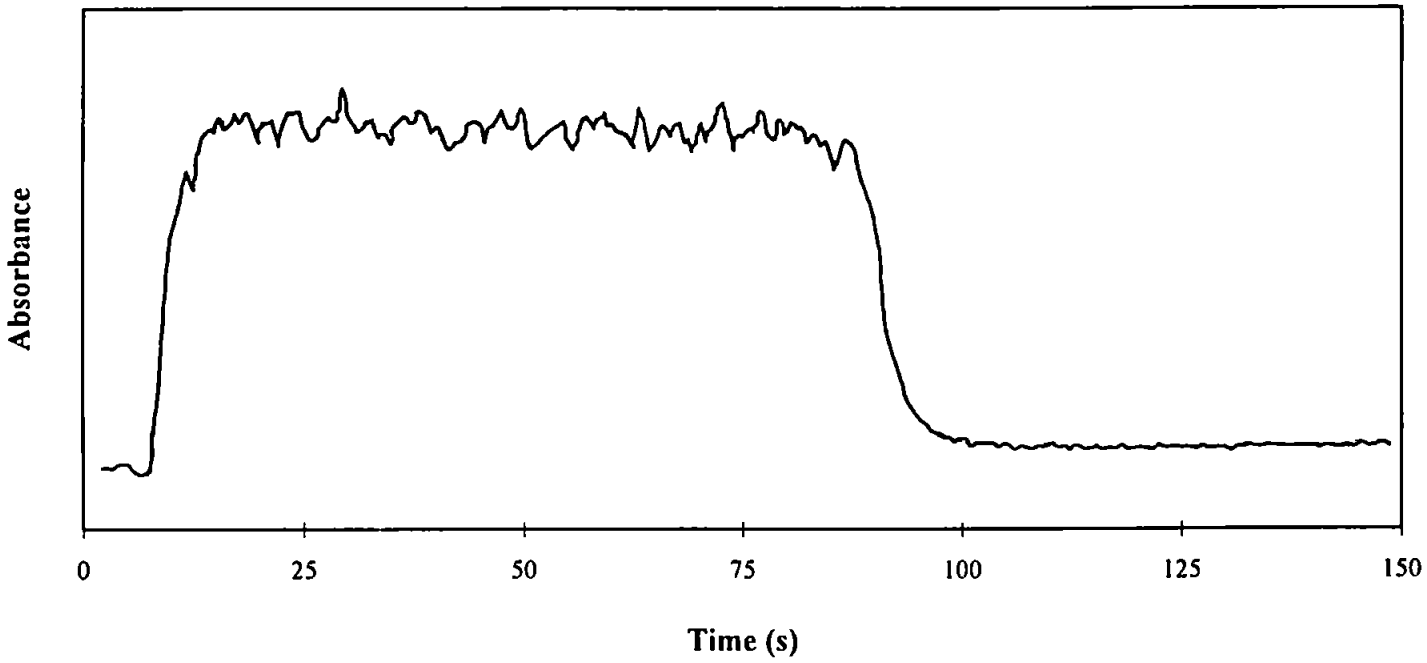


Throughout the course of the work two means of detection were employed, atomic absorption spectrometry and inductively coupled plasma-atomic emission spectrometry. For AAS the hydrides formed were swept into a quartz tube (H. Baumbach and Co. Ltd, Ipswich, Suffolk, UK) situated on the burner head of the AAS detector (Pye Unicam SP9, Cambridge, UK). The exact posistion of the quartz tube was optimised so that the light from the hollow cathode lamp passed exactly through its centre, thereby allowing maximum light to reach the detector. Analysis was undertaken at the 193.7 nm line with an air-acetylene flame and a deuterium lamp was employed for background correction (see Table 5.2). A typical signal obtained for the HG-AAS analysis of a standard solution of 20 ng ml^{-1} As(V) is given in Figure 5.2. For detection by ICP-AES the hydrides formed were swept into the base of the torch of the Liberty 200 inductively coupled plasma-atomic emission spectrometer (Varian Instruments Ltd, Warrington, UK) operated under the conditions described in Section 3.1.2.1.

Table 5.2: Instrumental Parameters for the Determination of Arsenic by HG-AAS

HG Parameters	
Reductant	1% (m/v) $NaBH_4$ in 0.1 M NaOH, 4 ml min^{-1}
Blank	3 M HNO_3, 8 ml min^{-1}
Sample matrix	3 M HNO_3, 8 ml min^{-1}
Argon carrier gas flow rate	300 ml min^{-1}
AAS Parameters	
Flame	Air-Acetylene (lean-blue flame)
Wavelength	193.7 nm
Background correction	Deuterium lamp

Figure 5.2: Typical Response Obtained for the Analysis of 20 ng ml^{-1} As(V) by HG-AAS



5.2.2 Reagents

As(V) standards were prepared from a high purity 996 ± 2 mg l^{-1} stock solution (Spectrosol, Merck Ltd, Poole, Dorset, UK). AsBet, DMA and MMA standard solutions were obtained from the Measurement and Testing Programme (BCR). Solutions of sodium borohydride (98% Aldrich Chemical Co, Gillingham, Dorset, UK), L-cysteine, potassium sulfate and potassium persulfate (BioChemika, Fluka Chemicals, Gillingham, Dorset, UK) were prepared daily. Trypsin powder was obtained from Sigma Chemical Co. (Poole, Dorset, UK), dibutylphthalate from Aldrich Chemicals and nitric acid (Analar), hydrogen peroxide (Analar), sodium hydroxide (Aristar) and ammonium hydrogen carbonate (Analar) from Merck Ltd. All solutions were prepared in deionised water (18 $M\Omega cm^{-1}$) purified through a Millipore purification system (Millipore-Waters, Bedford, MA, USA).

Glassware was thoroughly cleaned in 2% (v/v) Decon 90 (Merck Ltd), acid washed in 10% (v/v) nitric acid (Analar, Merck Ltd) and rinsed in deionised water prior to use. For validation of the techniques the certified reference materials DORM-1 (dogfish muscle tissue) and TORT-1 (lobster hepatopancreas) were obtained from the National Research Council Canada (Ottawa, Canada).

5.3 Development of a Batch Microwave Digestion Method for the Decomposition of Arsenobetaine

Studies in this section involved the development of a batch microwave digestion procedure for the decomposition of a standard solution of arsenobetaine. Analysis was undertaken by HG-AAS, initially from the As(V) oxidation state and later following L-cysteine pre-reduction to As(III) (see Section 5.3.2).

5.3.1 Without L-cysteine Pre-Reduction

Initial investigations involved digestion of samples by the method shown in Table 5.3. A 50 ml aliquot of sample was heated with 7.5 g of potassium persulfate and 5 g of sodium hydroxide for 25 minutes. It was found that at a power setting of ≥ 80 W, boiling of the sample was very vigorous, leading to loss of sample, and thus a setting of 70 W was chosen for future work. Initially the potassium persulfate was present in excess but dissolved on heating. After cooling the sample was transferred to a 100 ml volumetric flask and made up to volume with deionised water, before adjusting the pH of the solution to that of deionised water by the dropwise addition of concentrated nitric acid. Finally the solution was made up to 250 ml with nitric acid and deionised water to obtain a final concentration of 3 M nitric acid.

To assess the efficiency of the batch microwave digestion method standard solutions of 50 ng ml^{-1} AsBet (as As) were prepared in deionised water and digested (n=3) by the method given in Table 5.3. Following pH adjustment samples were analysed by the HG-AAS technique as described in Section 5.2.1. A standard solution of 50 ng ml^{-1} As(V) (n=1) was also digested in order to examine whether any losses of arsenic due to volatilisation occurred during the microwave digestion procedure. In addition blank solutions of deionised water

were digested in triplicate, following the same procedure. After acidification the latter were used to prepare As(V) calibration standards in the range 0-20 ng ml^{-1}. This ensured complete matrix matching between samples and standards, a necessity as the sensitivity of the analysis was far lower in a persulfate matrix than in aqueous solution (see Section 5.4.1.1).

Table 5.3: Batch Microwave Digestion Procedure for the Decomposition of Arsenobetaine

Step	Procedure
1	Heat 50 ml of sample with 7.5 g $K_2S_2O_8$ and 15 g NaOH, Power = 70 W Time = 25 min.
2	Allow sample to cool and make up to 100 ml with deionised water.
3	Acidify with nitric acid to the same pH as deionised water.
4	Make up to 250 ml with nitric acid and deionised water to obtain a final concentration of 3 M nitric acid

Recoveries of 108% for the As(V) sample and 130% for the AsBet samples were obtained. To confirm these results, calibration was repeated using a standard additions approach however recoveries were still very high and unreproducible. The total arsenic content of the AsBet stock solution was verified as 1000 mg l^{-1} by ICP-AES, calibrating with standards of As(V). In addition this solution was analysed for the presence of phosphorus by undertaking a colour test based on the formation of a phosphomolybdate complex. However phosphorus was not detected in the sample (detection limit = 1 mg l^{-1}), thus ruling out its interference during HG-AAS analysis as the cause of the high recoveries. The reason for the elevated results is still uncertain but may be due to the microwave digestion procedure i.e. not all the arsenobetaine was converted to As(V) but to a species which forms hydrides more easily than As(V), such as As(III). This would lead to a greater hydride generation efficiency and therefore higher recoveries.

5.3.2 Pre-Reduction with L-Cysteine

5.3.2.1 Batch Microwave Digestion Procedure

In light of the high recoveries obtained in the previous work, following the microwave digestion procedure an L-cysteine pre-reduction was undertaken. This step ensured that all the arsenic in the samples and standards was converted to the same oxidation state [As(III)] and thus gave the same response during HG-AAS analysis (see Section 5.1.2). The conditions optimised by Brindle et al.[305] i.e. 0.7% (m/v) L-cysteine in 0.05 M nitric acid have been successfully used in this laboratory by a number of workers and thus were chosen for further investigations.

Samples were prepared by the method shown in Table 5.4. After microwave digestion by the previously developed method (see Table 5.3), samples were acidified with nitric acid to pH 1.3 (pH of 0.05 M nitric acid), before the addition of 1.75 g of L-cysteine and making up to 250 ml with 0.05 M nitric acid. Samples were then heated in a water bath at 85°C for 30 minutes to facilitate the pre-reduction of arsenic to As(III). For calibration, As(V) standards (0-20 ng ml^{-1}) were prepared using a blank solution (deionised water) which had previously been digested and pH adjusted in the same way as for the samples. This approach ensured that the samples and calibration standards were again matrix matched. Following preparation standards were also pre-reduced following the method in Table 5.4.

Table 5.4: Batch Digestion Procedure with L-cysteine Pre-Reduction

Step	Procedure
1	As Steps 1 and 2 in Table 5.3.
2	Acidify with nitric acid to pH of 0.05 M nitric acid (pH 1.3).
3	Make up to 250 ml with 0.05 M nitric acid.
4	Add 1.75 g of L-cysteine [0.7% (m/v)] and heat in a water bath at 85°C for 30 min.

Samples (n=5) of 50 ng ml^{-1} AsBet (as As) and 50 ng ml^{-1} As(V) (n=1) were prepared in deionised water and digested following the method in Table 5.4. Analysis by HG-AAS was undertaken as before except for reducing the nitric acid concentration of the acid blank to 0.05 M (*cf* 3 M). Mean recoveries of 95.6% and 99.4% were obtained for the AsBet and As(V) samples respectively. It was thus concluded that the breakdown of arsenobetaine using the developed digestion procedure was complete and that no losses of arsenic due to volatilisation occured during the digestion procedure. In addition the sensitivity of analysis was improved by 40% when compared to analysis without pre-reduction to As(III). This is because as previously stated the rate of hydride formation from As(III) is more efficient than from As(V).

5.3.2.2 Microwave Digestion of NRCC DORM-1 Certified Reference Material

To ascertain whether the developed procedure was effective for the complete breakdown of organoarsenic species in a real sample, the certified reference material NRCC DORM-1 was digested by the procedure given in Table 5.5. 0.25 g of sample (n=5) was heated with 4 ml of nitric acid at a power setting of 60 W for 10 minutes. 2 ml of hydrogen peroxide was subsequently added and heating continued, again at 60 W, for a further 5 minutes. After allowing to cool, samples were quanititatively transferred into 25 ml volumetric flasks and made up to volume with deionised water. Although digestion by such a procedure will not preserve all of the original arsenic species, organoarsenic species such as arsenobetaine are resistant to acid digestion, requiring far stronger oxidising conditions for their decomposition[307].

Table 5.5: Nitric Acid and Hydrogen Peroxide Batch Microwave Digestion Procedure for the Determination of Total Arsenic in Biological Samples

Step	Procedure
1	Heat 0.25 g of biological sample with 4 ml of nitric acid, Power = 60 W, Time = 10 min.
2	Add 2 ml of hydrogen peroxide and resume heating, Power = 60 W, Time = 5 min.
3	Allow digest to cool. Quantitatively transfer into a 25 ml volumetric flask and make up to volume with deionised water.

A 12.5 ml aliquot of each of the digests was diluted to 50 ml with deionised water and subjected to the persulfate digestion and pre-reduction procedure as shown in Table 5.4. This was undertaken to ascertain the efficiency of the persulfate digestion procedure for the breakdown of the non-reducible arsenic species in the sample. Following complete decomposition all species should be converted to a form compatible with the hydride generation technique and thus analysis by HG-AAS should yield results in agreement with the certified value for the total arsenic content of the sample.

Following the initial nitric acid and hydrogen peroxide microwave digestion, samples were analysed for total arsenic by ICP-AES. The results given in Table 5.6 are in good agreement with the certified values, indicating that the procedure enabled the complete liberation of arsenic from the sample matrix. As discussed above samples were then digested further by the persulfate microwave digestion procedure and pre-reduced with L-cysteine before HG-AAS analysis. The results obtained are also given in Table 5.6 and are in good agreement with both the certified value and with those obtained from the ICP-AES analysis. Thus it was concluded that the persulfate microwave digestion procedure resulted in the complete breakdown of the non-reducible arsenic species in a real sample.

Table 5.6: Results for the Microwave Digestion of NRCC DORM-1

Technique	As ($\mu g\ g^{-1}$)
Persulfate Microwave Digestion-HG-AAS[1]	19.0 ± 3.5
ICP-AES[1]	19.5 ± 3.0
Certified Value	**17.7 ± 2.1**

Notes:
1: n=5. Uncertainties for the experimental results are calculated as twice the standard deviation.

5.4 Development of an On-Line Microwave Digestion Technique for the Determination of Total Arsenic in Biological Samples

Further work investigated the development of an on-line microwave digestion technique, initially for the determination of total arsenic in biological samples. During the preliminary stages of the investigation ICP-AES and AAS were employed as suitable detectors following hydride generation.

5.4.1 On-Line Microwave Digestion Technique - Design No. 1

A schematic diagram of the initial design of the on-line system is shown in Figure 5.3. Samples (prepared in deionised water) were pumped (2.2 ml min^{-1}) using a peristaltic pump (Minipuls 2, Gilson, Villiers, Le Bel, France) through Viton tubing (2 mm i.d., Anachem, Luton, Beds, UK) before mixing at a T-junction with the oxidant of 15% (m/v) potassium persulfate in 10% (m/v) NaOH, (2.2 ml min^{-1}) and entering the microwave cavity of the microwave digester. Here the sample passed through two glass digestion coils, one placed inside the other (see Figure 5.4), designed to make use of the total heating capacity of the microwave unit. After digestion at a power setting of 80 W the sample was acidified by a stream of 1.4 M nitric acid and 1.6% (m/v) L-cysteine (3.6 ml min^{-1}) to produce a final concentration of 0.05 M and 0.7% respectively (concentrations used in the batch digestions). The sample was then heated in a PTFE coil (1.5 mm i.d. x 15 m) in a water bath maintained at 85°C to facilitate the pre-reduction of As(V) to As(III). After cooling by passage through a glass coil placed in an ice bath the sample entered the hydride generator unit. Here the arsines formed on reaction with 1% (m/v) sodium borohydride in 0.1 M sodium hydroxide (4 ml min^{-1}) were swept by a stream of argon gas (400 ml min^{-1}) directly into the plasma of the inductively coupled plasma-atomic emission spectrometer. The final concentration of potassium persulfate before HG-ICP-AES analysis was calculated as 4.1%.

Figure 5.3: Schematic Diagram of the On-Line Microwave Digestion System for the Determination of Total Arsenic in Biological Samples - Design No. 1

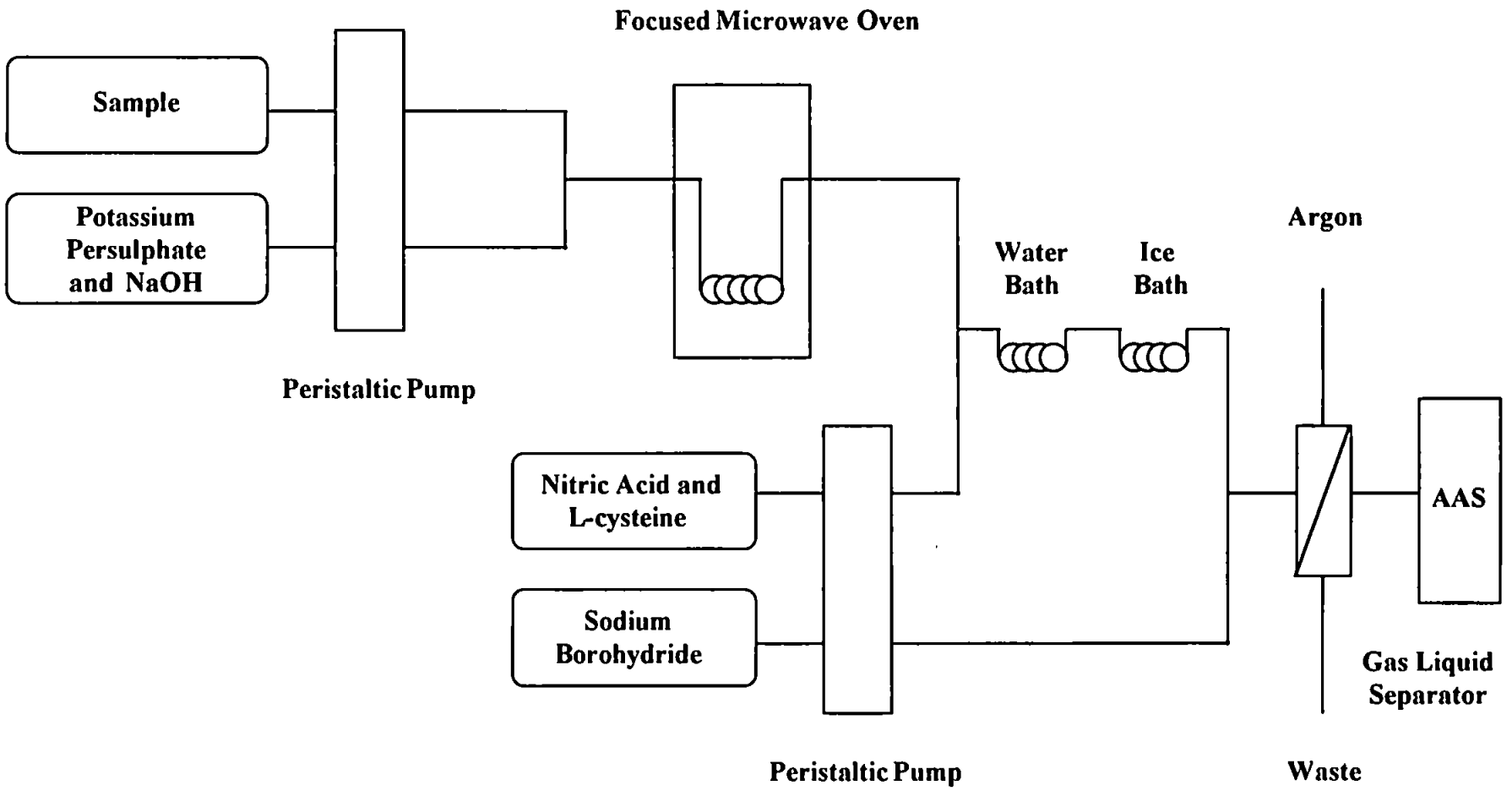
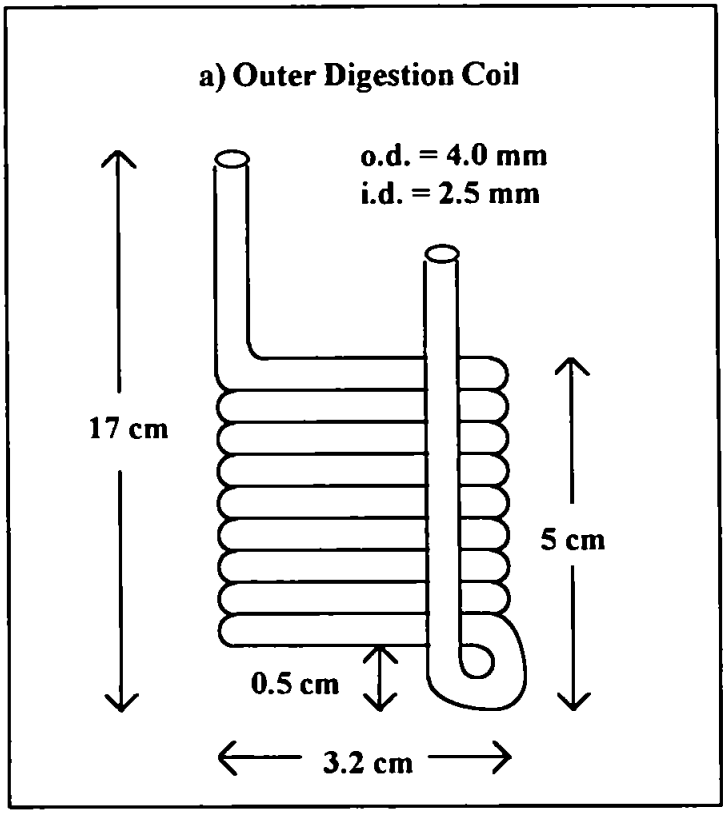
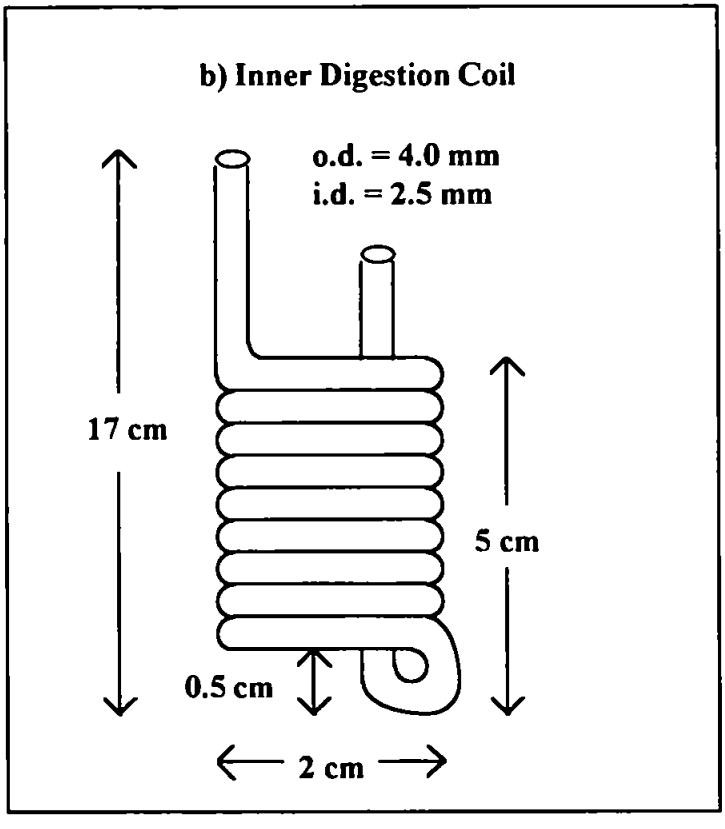


Figure 5.4: Design of the Glass Microwave Digestion Coils





Flow rates were initially chosen to give a final sample flow rate (i.e. sample + potassium persulfate + L-cysteine) of 8 ml min^{-1}, in accordance with the recommended sample flow rate of the P.S Analytical hydride generator unit (see Table 5.2). Variation of the final sample flow rate between 4.8 and 10.0 ml min^{-1} revealed that the signal to background ratio (SBR) was proportional to the flow rate over this range. In the final system in which it was intended to couple a suitable HPLC separation to the on-line microwave technique, the sample flow rate would be dictated by the flow rate of the mobile phase (fixed at approx. 1 ml min^{-1}). Thus any benefit to the sensitivity gained from increasing the final flow rate i.e. increasing the rates of the potassium persulfate and L-cysteine reagents, will be counteracted by increased dilution of the original sample. Thus the original flow rates as shown in Table 5.2 (i.e. final rate of 8 ml min^{-1}) were maintained in further work.

A smooth flow of the sample into the hydride generator could not however be obtained due to boiling of the sample in the digestion coil and the high back pressure in the system. This resulted in continuous pulsing of the sample out of the microwave coil followed by suck back and then further pulsing. As a result a very unstable signal was obtained, rendering on-line analysis impossible. It was observed that passing the sample through the switching valve unit of the P.S Analytical hydride generator (see Section-5.2.1) exacerbated the problem. When the valve was in the 'sample' position the flow from the on-line system into the hydride generator was restricted due to the small internal diameter of the switching valve which severely reduced the flow rate. After analysis, when the valve returned to the 'blank' position the flow speeded up considerably to compensate for the earlier restriction, thus resulting in a very uneven flow rate.

This problem was overcome by bypassing the switching valve and allowing the sample and sodium borohydride to mix continuously at a T-piece before passing into the gas liquid separator as before. Although this approach improved signal stability the resulting signal was still highly unstable, due to pulsing in the system, and thus on-line analysis was again impossible. The oxidation efficiency of the on-line microwave digestion technique was however determined by collection of the sample (after pre-reduction and cooling) in a volumetric flask for off-line analysis by HG-ICP-AES. A recovery of 102% for a sample of 60 ng ml^{-1} AsBet was obtained, indicating that the breakdown of arsenobetaine was complete.

In an attempt to alleviate the problem of high back-pressure, the system was operated at a lower power setting than the 80 W used previously. For As(V) calibration standards, on-line analysis was possible at ≤ 50 W power, resulting in good signal stability and reproducibility and linear calibration over the range 0 to 80 ng ml^{-1}. However no breakdown of arsenobetaine occured and thus it was concluded that the sample must reach boiling point to achieve complete decomposition of this species. At power settings of 60 W and above on-line analysis was not possible due to excessive pressure build up in the system and thus an alternative method of reducing the back-pressure was required.

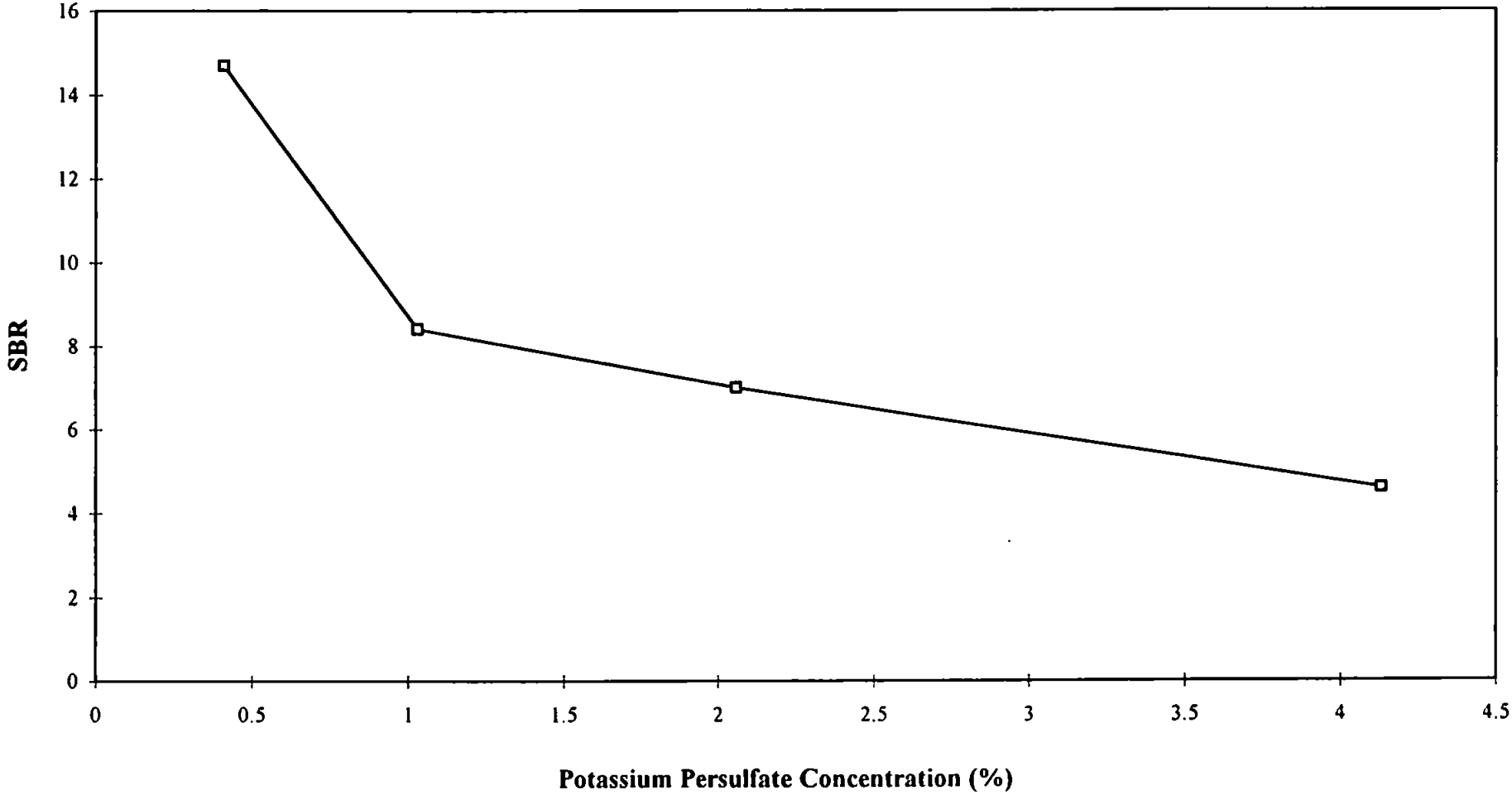
5.4.1.1 Effect of the Potassium Persulfate Concentration on the Sensitivity and Stability of the System.

Initial experiments were aimed at reducing the potassium persulfate concentration from the 15% previously used in order to improve the sensitivity of the analysis and to reduce the amount of oxygen evolved during the digestion, thus reducing pressure build up in the system. An aqueous standard of 20 ng ml^{-1} As(V) was pre-reduced (off-line) with 0.7% (m/v) L-cysteine in 0.05 M nitric acid. Analysis by HG-ICP-AES gave an SBR of 11.6. For comparison, an 80 ng ml^{-1} As(V) standard was digested with 15% (m/v) potassium

persulfate and 10% (m/v) sodium hydroxide, pre-reduced and cooled using the on-line microwave digestion technique before collection in a volumetric flask. The above concentrations were chosen to give a final concentration of 22 ng ml^{-1} As(V) and 4.1% persulfate (as in Section 5.4.1). Analysis by HG-ICP-AES yielded an SBR of only 4.6. This sample was then diluted by a factor of 2, 4 and 10 times. The results of the subsequent HG-ICP-AES analysis are shown in Figure 5.5. As can be seen the corresponding SBR for a standard solution of 22 ng ml^{-1} As(V) is substantially improved by reducing the persulfate concentration, reaching a value of 14.7 at a concentration of 0.4% persulfate.

The on-line microwave digestion procedure was then run with the lower potassium persulfate concentration of 7.5% (m/v) in 5% (m/v) sodium hydroxide, again at a flow rate of 2.2 ml min^{-1}. These concentrations corresponded to a persulfate concentration before HG-AAS analysis of 2.1%. After digestion the sample was mixed with a solution of 1.6% (m/v) L-cysteine and 0.8 M HNO_3 (3.6 ml min^{-1}) to adjust the pH of the solution to that of 0.05 M HNO_3 (pH 1.3). Standard solutions of 0, 20, 40, 60 and 80 ng ml^{-1} As(V) and samples of 40 and 60 ng ml^{-1} AsBet were run through the on-line microwave digestion system. However on-line analysis was again not possible due to excessive pressure build up in the system. Samples were therefore collected, after pre-reduction and cooling, for off-line analysis by HG-ICP-AES. In comparison to analysis with a potassium persulfate concentration of 15% marked improvements in sensitivity and signal and plasma stability resulted. A linear calibration curve (R^2 = 0.9977) and recoveries of 100.3% and 95.3% were obtained for the 40 ng ml^{-1} and 60 ng ml^{-1} AsBet samples respectively.

Figure 5.5: SBR Obtained for the HG-ICP-AES Analysis of 22 ng ml^{-1} As(V) with Different Concentrations of Potassium Persulfate



5.4.1.2 Optimisation of the Sodium Borohydride Concentration for HG Analysis

In order to improve the sensitivity of the analysis experiments were undertaken to optimise the sodium borohydride concentration. Figure 5.6 shows the SBR obtained for a standard solution of 80 ng ml^{-1} As(V) (digested in the on-line system, final concentration approx. 22 ng ml^{-1}) when analysed by HG-ICP-AES with sodium borohydride solutions of 0.6 to 2.5% (m/v) stabilised in 0.1 M NaOH. As can be seen the sensitivity of the hydride generation process increased quite markedly between a $NaBH_4$ concentration of 0.6% and 1.3%, with a more gradual improvement thereafter. This would be expected as a high proportion of the sodium borohydride solution is needed to reduce the excess potassium persulfate in the sample. At higher sodium borohydride concentrations there is more borohydride available to reduce the arsenic present and thus sensitivity is markedly improved. A concentration of 1.5% sodium borohydride was chosen for further work as above 2.5% the stability of the plasma was severely affected.

For comparison, similar investigations were undertaken for a standard solution of 20 ng ml^{-1} As(V). In aqueous media a classic optimisation curve was obtained as shown in Figure 5.7. Again a sharp increase in sensitivity was initially seen but at sodium borohydride concentrations above 1.3% sensitivity gradually decreased due to dilution of the sample by the excess hydrogen produced.

Figure 5.6: Optimisation of the Sodium Borohydride Concentration for the HG-ICP-AES Analysis of 20 ng ml^{-1} As(V) in a Matrix of 4.1% Potassium Persulfate

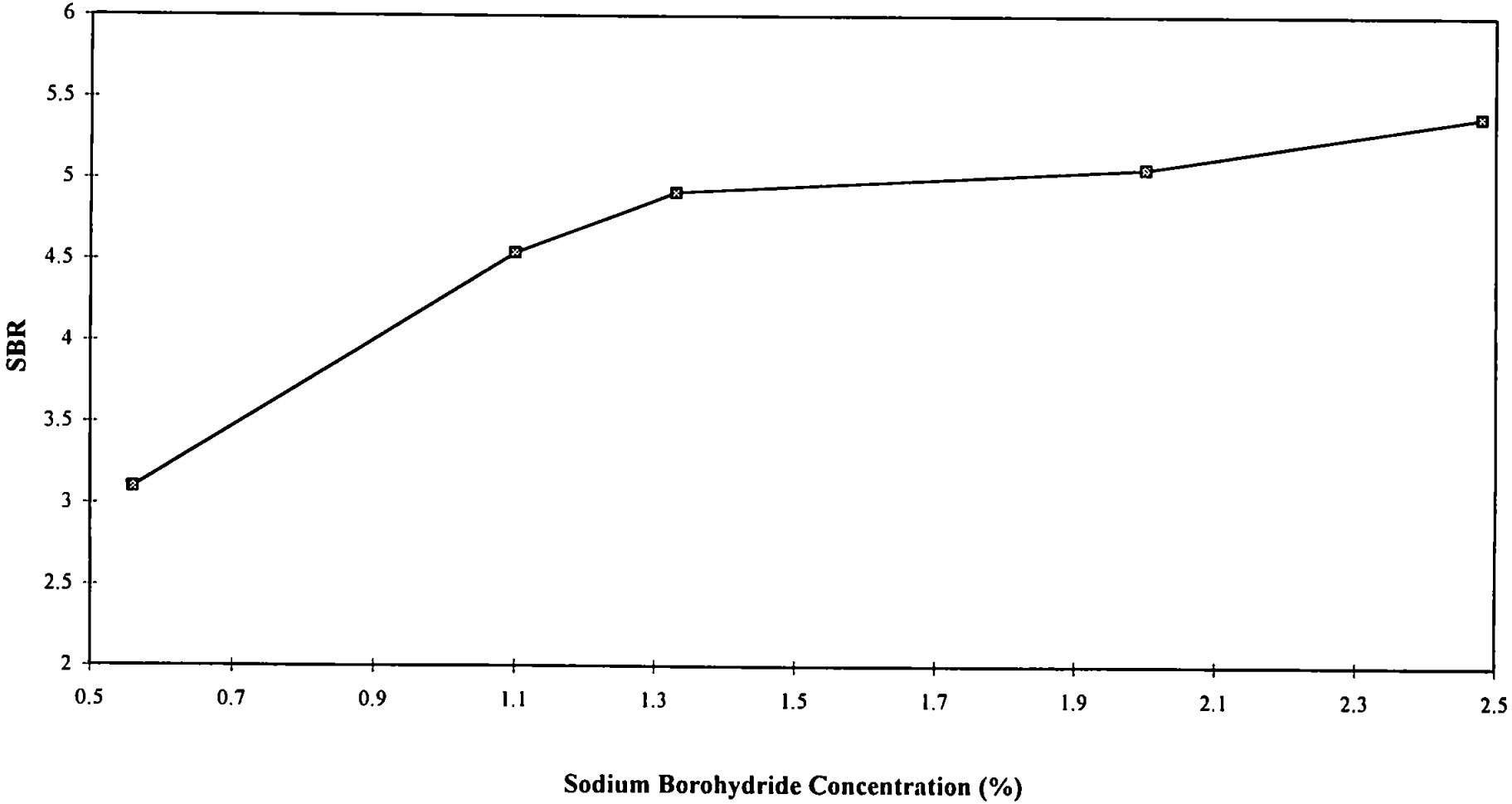
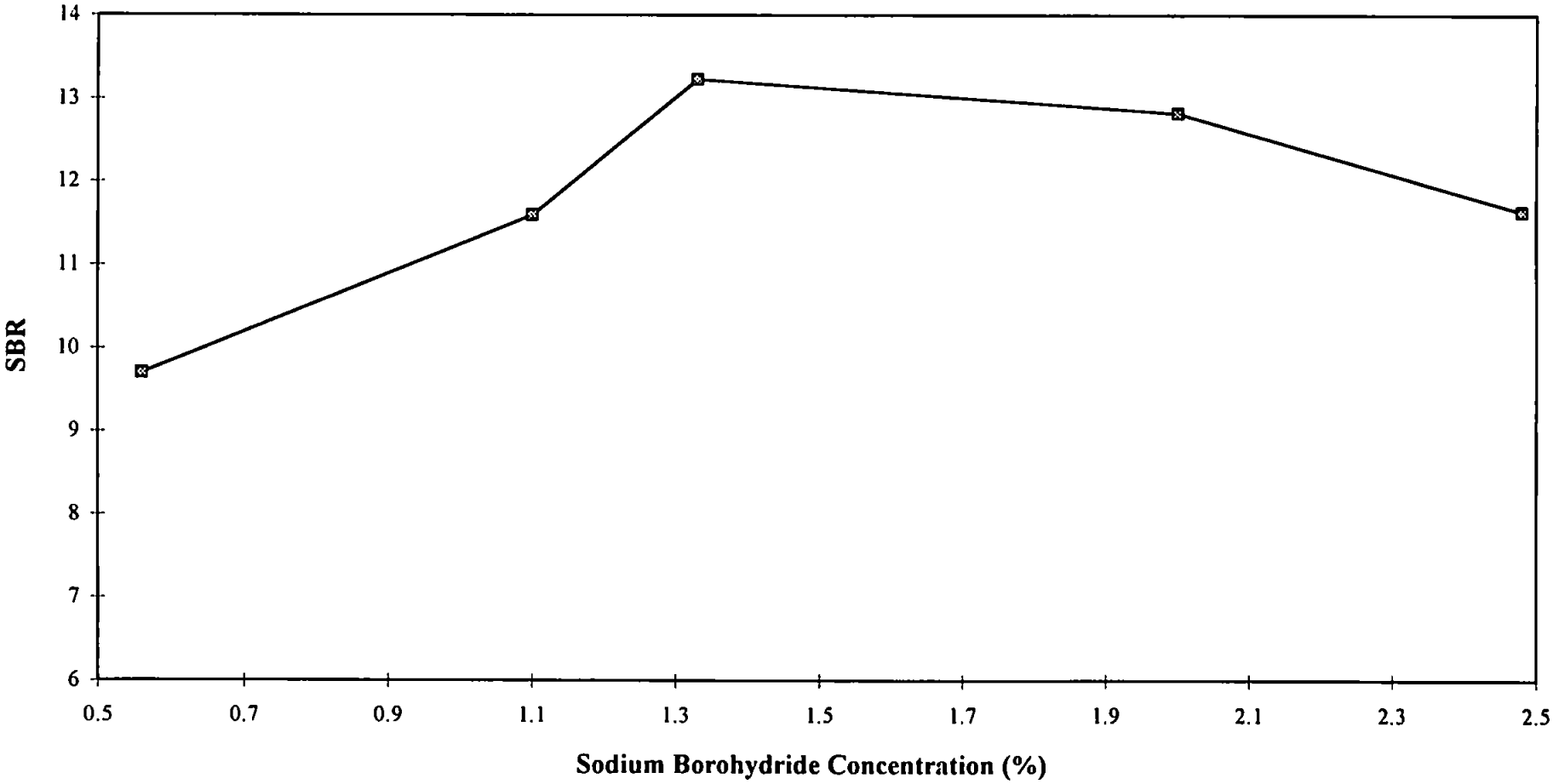


Figure 5.7: Optimisation of the Sodium Borohydride Concentration for the HG-ICP-AES Analysis of 20 ng ml^{-1} As(V) in Aqueous Solution



5.4.1.3 Devices Investigated to Reduce Sample Pulsation and Gas Build up

In light of the difficulties experienced in achieving on-line analysis, investigations were undertaken to directly address the causes of this problem, namely sample pulsation and gas build up in the system. A number of different ideas were evaluated as discussed below.

5.4.1.3.1 Gas Removal

Various devices were evaluated to remove the oxygen evolved during the microwave digestion procedure from the decomposition of the potassium persulfate reagent.

T-Piece Debubbler

This device was designed 'in-house' and consisted of a glass T-piece of which one end was sealed (see Figure 5.8). The pressure in the sealed end is higher than in the open ends and so the solution should not flow into the former. In contrast the gases should come out of solution and be collected in the sealed end. This approach proved unsuccessful because of the large quantities of gas evolved during the reaction. The sealed end quickly became full of gas and thereafter the bubbles remained in solution. A modification of this design in which the sealed end were replaced by a semi-permeable membrane may have proven more successful.

Semi-Permeable Membrane

The solution was passed through a semi-permeable Nafion membrane dryer tube (Perma Pure Products, Monmouth Airport, Farmingdale, NJ, USA) as shown in Figure 5.9. This consisted of two concentric tubes 20 cm in length connected with variable bore T-piece connectors. The outer tube is made from PTFE whereas the inner tube is a hygroscopic Nafion membrane. A stream of argon gas was passed, in the opposite direction to the sample flow, between the outer tube and the membrane to sweep away the gasses diffusing through

Figure 5.8: T-Piece Debubbler

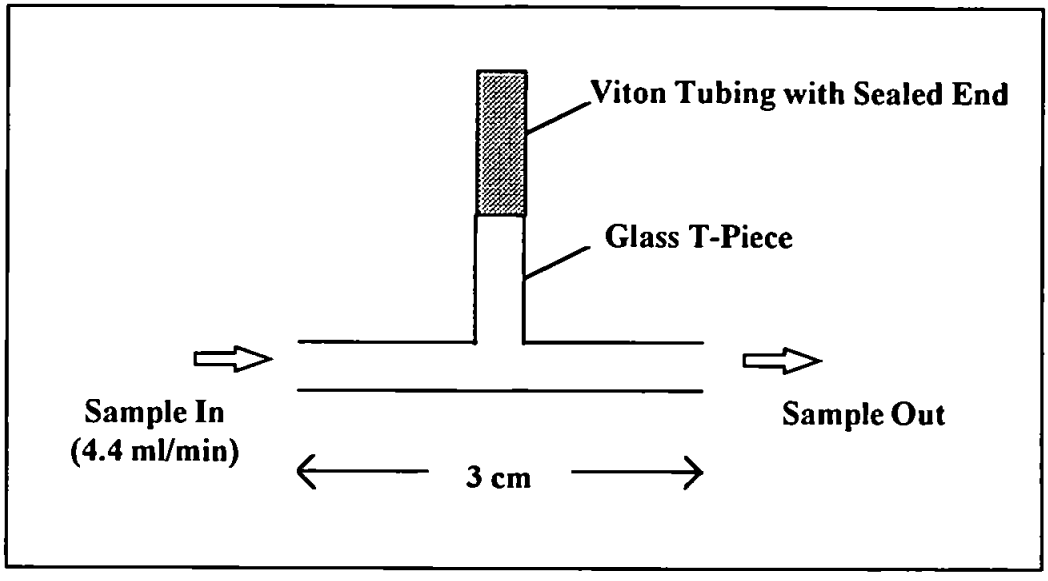
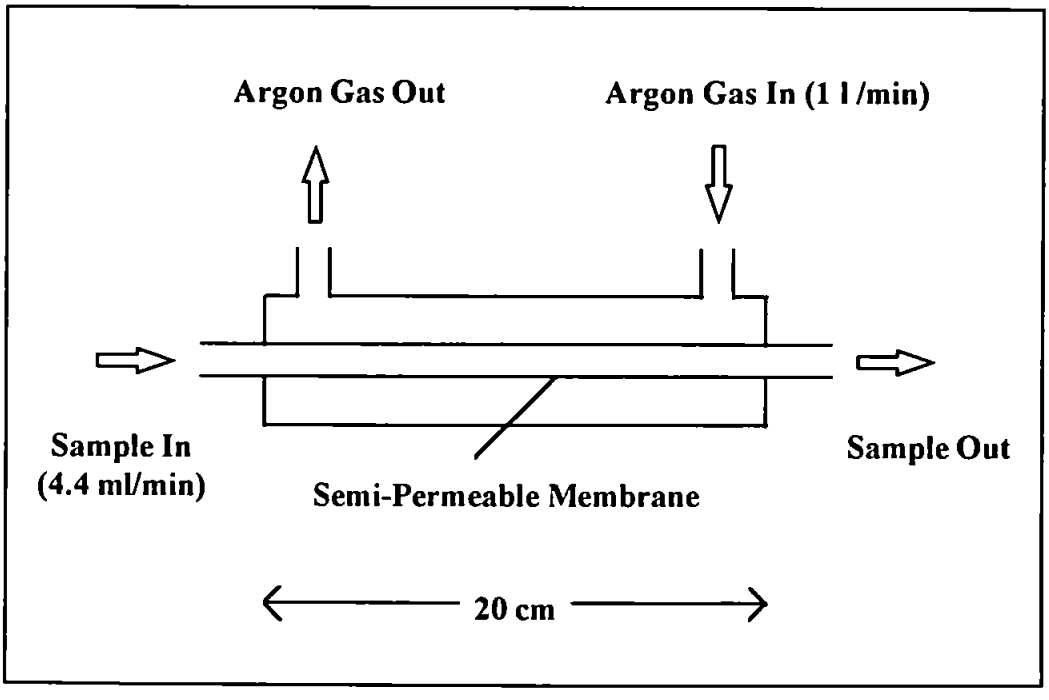


Figure 5.9: Semi-Permeable Membrane

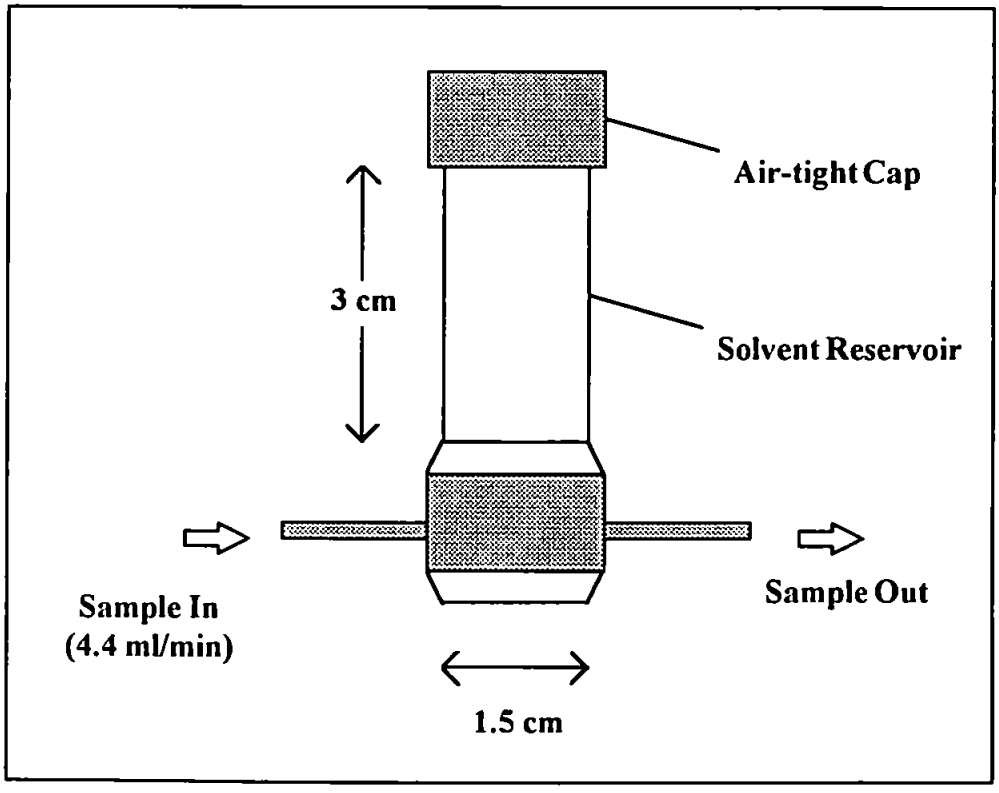


the membrane. This approach however also proved unsuccessful at removing the large quantity of gas evolved during the microwave digestion procedure.

Universal Debubbler

A universal solvent debubbler device (SSI, Anachem), shown in Figure 5.10 was evaluated. The debubbler is in essence a small solvent reservoir with an air-tight cap. Initially the reservoir (1.2 ml capacity) is filled with blank solution. When bubbles are present in solution they float upwards and displace the solution in the reservoir which then enters the sample stream. Periodically the collected gas can be replaced with additional solution by loosening the cap, allowing the reservoir to fill up and the gas to escape. This approach however proved unsuccessful at removing the large quantities of gas evolved during the digestion. A full evaluation of the device is detailed in Section 5.4.2.

Figure 5.10: Design of the Universal Debubbler Device



5.4.1.3.2 Pulse Suppression

Pulse Chamber

A glass pulse chamber was designed 'in house' to the specifications shown in Figure 5.11a. The chamber was placed in-line directly after the microwave cavity, prior to the acidification step. It was found necessary to cool the sample beforehand to stop the sample being squirted up to the top of the chamber, causing excessive dispersion. However the device was ineffectual as the liquid level tended to build up to the top of the output side arm before any of the sample would flow out of the chamber due to pressure differences in the system. At this point a large volume of solution flowed through the outlet tube causing the liquid level in the chamber to drop to the lower level of the outlet tube. This cycle was then repeated.

In order to guarantee a more constant flow it was decided to pump the solution out of the chamber. This was achieved by placing a piece of Viton pump tubing (2 mm i.d.) into the chamber as shown in Figure 5.11b and adjusting the pump rate so that the input flow was equal to the output flow. However maintenance of the exact flow rate to prevent the gradual build up of liquid in the chamber was difficult and required constant monitoring. The resulting analytical signal was however stable although appreciable dispersion of the sample in the chamber resulted. This approach may therefore have been suitable for the determination of total arsenic but for future coupling to an HPLC system the high degree of dispersion would have resulted in substantial dilution and loss of the individual arsenic species. Therefore a system capable of retaining the integrity of the individual species was sought.

Figure 5.11a: Design of the Pulse Chamber

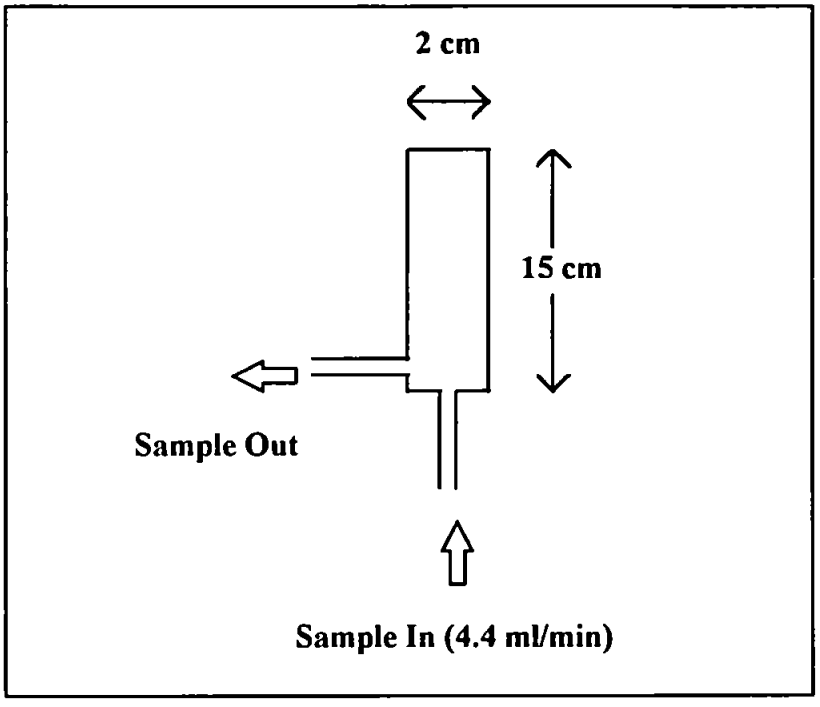
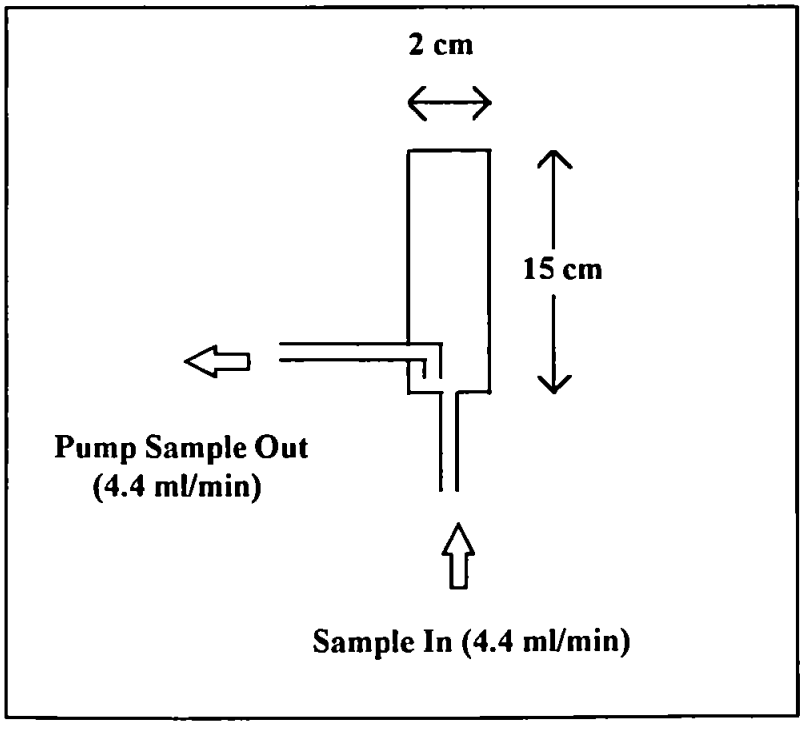


Figure 5.11b: Design of the Modified Pulse Chamber



Use Of A Second Pump

The problem of pulsation was also approached by pumping the solution out of the microwave cavity at the same rate as the inlet flow rate. However this was unsuccessful, resulting in venting of the solution just before the second pump. This was presumably because large pulses of liquid could not leave the microwave cavity, being restricted by the pump heads of the second pump.

Anti-Bumping Granules

Anti-bumping granules were placed in the glass coils in an attempt to dampen out pulsing however this approach also proved ineffectual.

Back-Pressure Regulator

The use of a back pressure regulator device (P736, Anachem) has been reported by Haswell *et al.*[308] for the on-line microwave digestion of slurry samples in order to prevent back flow from occuring. The back-pressure regulator device was placed into the original system following the microwave digestion step. However this device was intended for use with standard 1/16" o.d. PTFE tubing and thus it was impossible to couple to the comparatively wide bore of the original tubing (2 mm i.d.) and glass coils (2.5 mm i.d.). Therefore all of the tubing in the system was replaced with 1/16" o.d. PTFE tubing. Full evaluation of the modified on-line microwave digestion system (Design No. 2) and the back-pressure and universal debubbler devices are detailed in Section 5.4.2.

5.4.2 Evaluation of the Modified On-Line Microwave Digestion Technique - Design No. 2

A schematic diagram of the modified on-line microwave digestion system (Design No. 2) is shown in Figure 5.12. The glass digestion coils were replaced by 9 m of 0.5 mm i.d. x 1/16" o.d. PTFE tubing (Anachem), knitted by the method reported by Selavka *et al.*[309] (see Figure 5.13) around a PTFE backbone (1/4" o.d.). The tubing was twisted into a tight coil before placing into the microwave cavity of the microwave digester. The original peristaltic pump was replaced by a high pressure pump (Waters Associates Chromatography Pump, Milford, MA, USA) and so high pressure fixings were required to couple the pump to the digestion coil. Due to the fact that the pump had only a single channel it was necessary to prepare the samples in potassium persulfate and NaOH rather than mixing on-line as before. This approach was compatible with the ultimate aim of this work i.e. the development of an on-line HPLC-microwave digestion system, since in this case the sample stream will emerge from the output of the HPLC column and so only a single channel pump will be required to deliver the potassium persulfate solution. The use of a chromatography pump required filtration of the potassium persulfate solution through a millipore filtration unit (0.45 μm) before use. This was necessary not only to remove particulate matter but also to degass the solution.

Standards and samples were prepared in 3.7% (m/v) potassium persulfate and 2.5% (m/v) NaOH and passed through the digestion coil at a rate of 4.6 ml min^{-1} (calculated to give a final persulfate concentration of 2.1% as in previous work, see Section 5.4.1.1). This resulted in a residence time of 23 s in the microwave cavity. After microwave digestion the resulting solution flowed through the back-pressure regulator unit and the debubbler device before mixing at a PTFE T-piece (2 mm i.d.) with a solution of 0.9 M nitric acid and 1.6% (m/v) L-cysteine (3.4 ml min^{-1}) as before.

Figure 5.12: Schematic Diagram of the On-Line Microwave Digestion System for the Determination of Total Arsenic in Biological Samples - Design No. 2

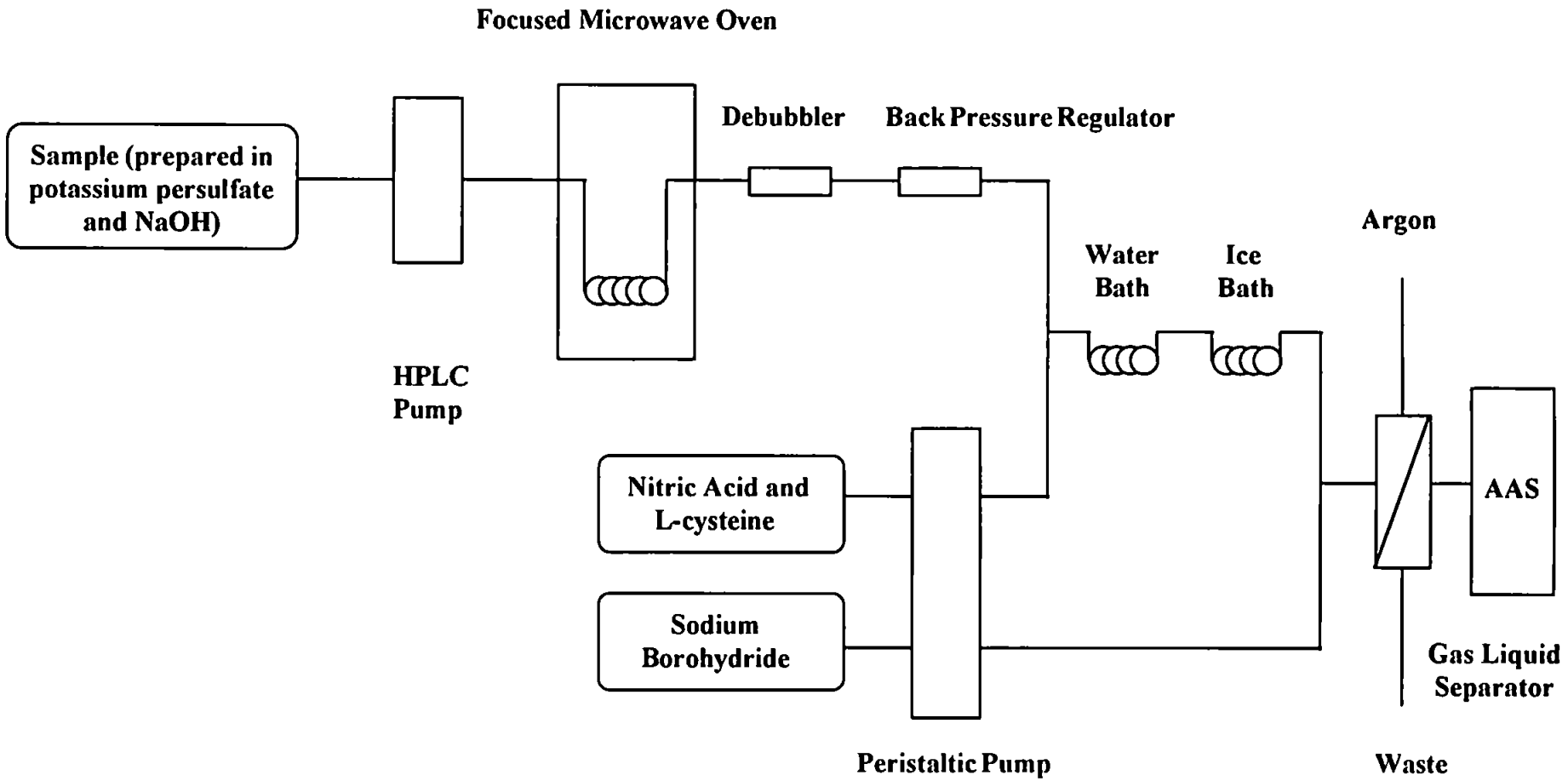
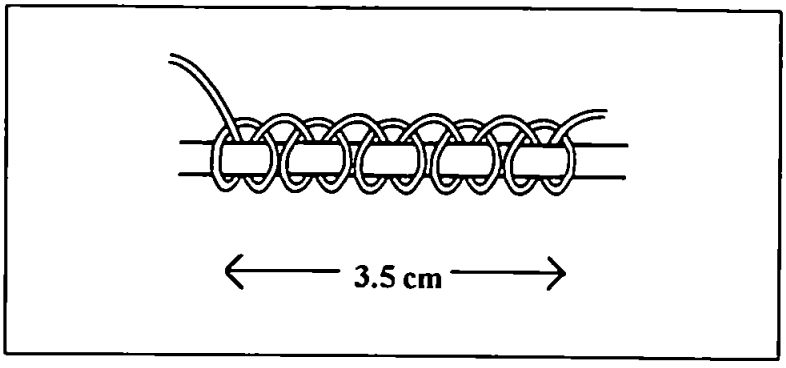


Figure 5.13: Construction of the Knitted PTFE Coil



The debubbler device although successful at gas removal could not remove the large quantities of gas evolved during the reaction. The solvent reservoir was initially filled with blank solution however this was completely displaced by gas after only a few seconds operation and so the reservoir continually required refilling. Obviously this would not be possible in routine use and in addition there is the risk that the important analyte peaks (from the HPLC separation) may be 'lost' in the solvent reservoir, thereby losing the integrity of the separation.

It was observed that the back-pressure regulator effectively stopped pulsing of the solution out of the microwave cavity. However the system could not be run for more than a few minutes without venting just before the sample met the nitric acid and L-cysteine solution. This problem was overcome by cooling the solution prior to the back-pressure regulator in order to condense some of the gases present. For this purpose 9 m of 0.5 mm i.d. x 1/16" o.d. PTFE tubing (knitted as before, see Figure 5.13) was placed in an ice-bath.

After mixing with the L-cysteine reagent the sample was heated by passage through a PTFE coil (1.5 mm i.d. x 15 m) placed in a water bath at 95°C, before cooling in an ice bath (1.5 mm i.d. x 3 m). Hydride generation was then performed by mixing the sample, at a PTFE T-piece (2.5 mm i.d.), with a solution of 1.5% (m/v) $NaBH_4$ in 0.1 M NaOH (2.8 ml min^{-1}). The size of the T-piece was found to be critical. With an internal diameter of 1.6 mm pulsation of the sample into the HG-AAS system occurred, giving a very noisy signal. A larger diameter of 4 mm was also not suitable as this led to appreciable dispersion of the sample, resulting in longer washout times between samples and in addition produced an unstable signal. A T-piece of 2.5 mm internal diameter was finally chosen which gave a far more stable signal without causing excessive dispersion of the sample. It was also found to be beneficial to keep the microwave power on between samples in order to maintain a steady sample flow and in addition to run a blank between each sample in order to correct for baseline drift. Signal output was recorded on a chart recorder (Labdata Instrument Services Ltd., Carshalton, Surrey) and quantification made by taking peak height measurements from the steady state signals obtained.

5.4.2.1 Optimisation Studies

Studies were then undertaken to determine the optimum power setting for the microwave digestion, the argon carrier gas flow rate for HG-AAS analysis and the nitric acid concentration and heating time for the L-cysteine pre-reduction reaction.

5.4.2.1.1 Power Setting

The power setting for the microwave digestion was optimised by systematic variation between 30 W and 80 W. It was observed that at 30 W the digestion was only 4% complete and at 40 W only 21%. However with a power of 50 W a recovery of 103% was obtained for a sample of 30 ng ml^{-1} AsBet, suggesting that the decomposition of AsBet was complete. Higher power settings resulted in poor signal quality, as shown in Figure 5.14, due to pulsation of the sample flow into the detector due to excessive heating of the sample. The optimum setting for further work was therefore chosen as 50 W. A typical response obtained for the digestion of a standard solution of 40 ng ml^{-1} AsBet at 50 W power is shown in Figure 5.15.

5.4.2.1.2 L-cysteine Pre-Reduction Reaction Time

Optimisation of the length of the L-cysteine pre-reduction heating coil was undertaken by pre-reduction of a 40 ng ml^{-1} As(V) standard using heating coils of varying lengths between 0 and 14 m. As can be seen from the results shown in Figure 5.16 a heating coil of at least 5 m was necessary, corresponding to a heating time of 66 s or more in the water bath. However a longer coil of 15 m was needed in order to ensure a smooth sample flow into the hydride generator unit.

Figure 5.14: Typical Signal Obtained for 60 ng ml^{-1} AsBet following On-Line Microwave Digestion at 60 W Power and HG-AAS Analysis

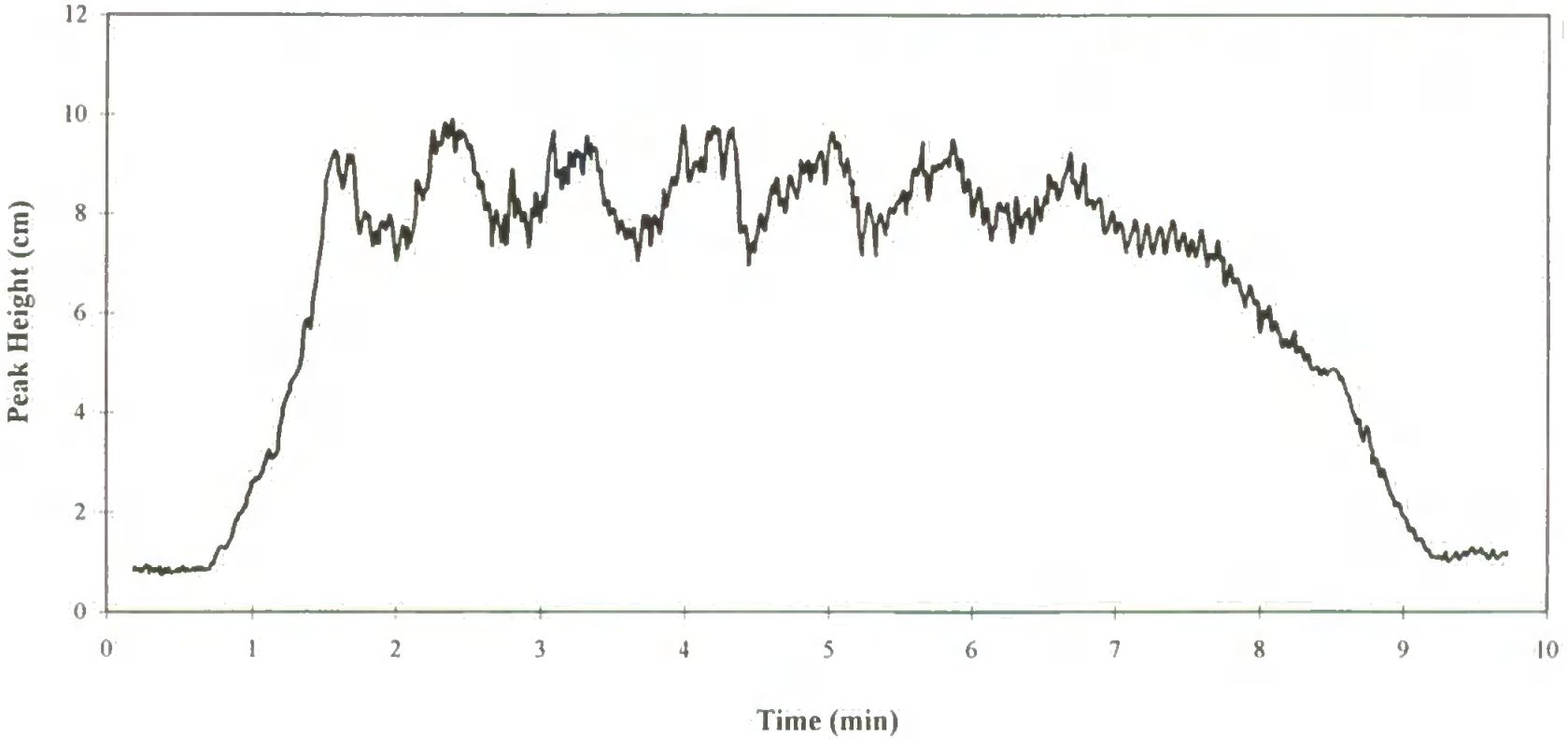


Figure 5.15: Typical Signal Obtained for 40 ng ml^{-1} AsBet following On-Line Microwave Digestion at 50 W Power and HG-AAS Analysis

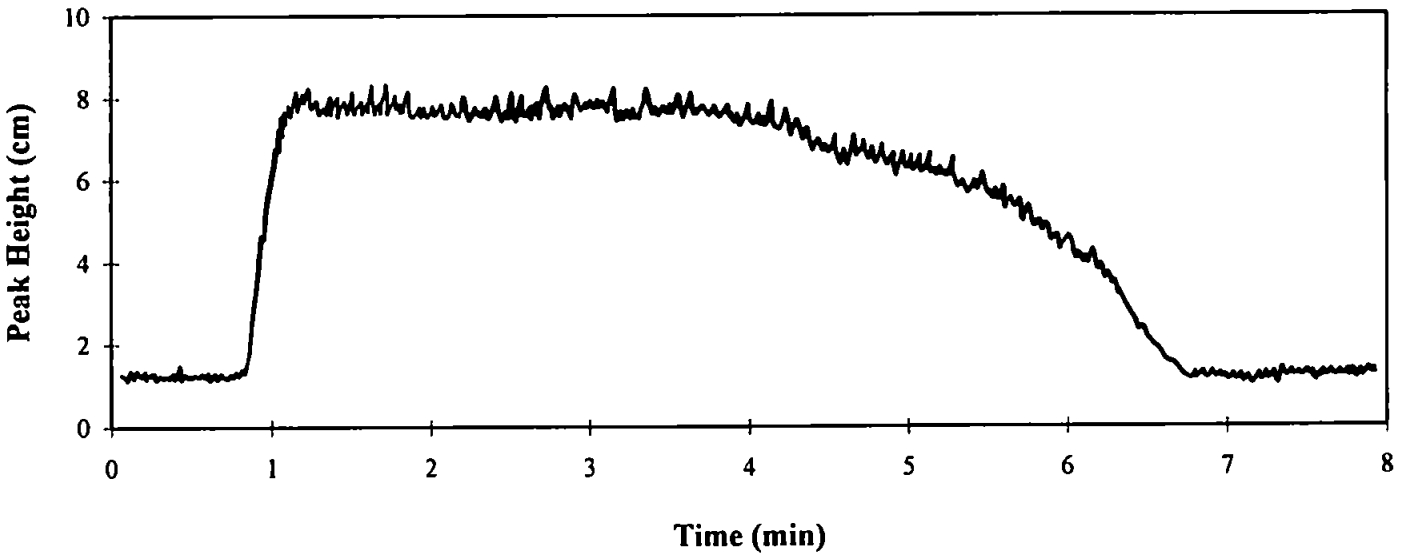
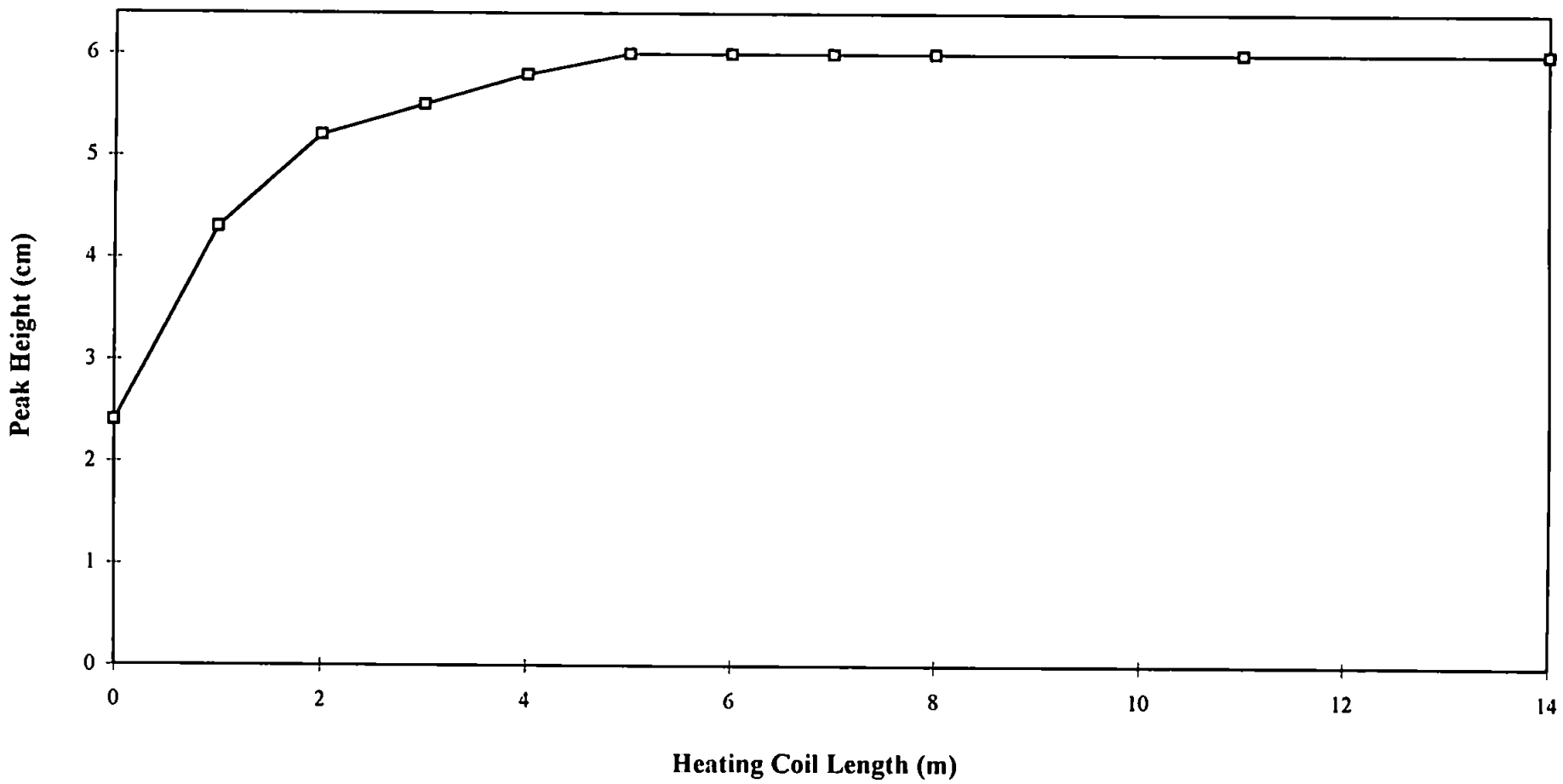


Figure 5.16: Optimisation of the Heating Coil Length for the L-cysteine Pre-Reduction Reaction



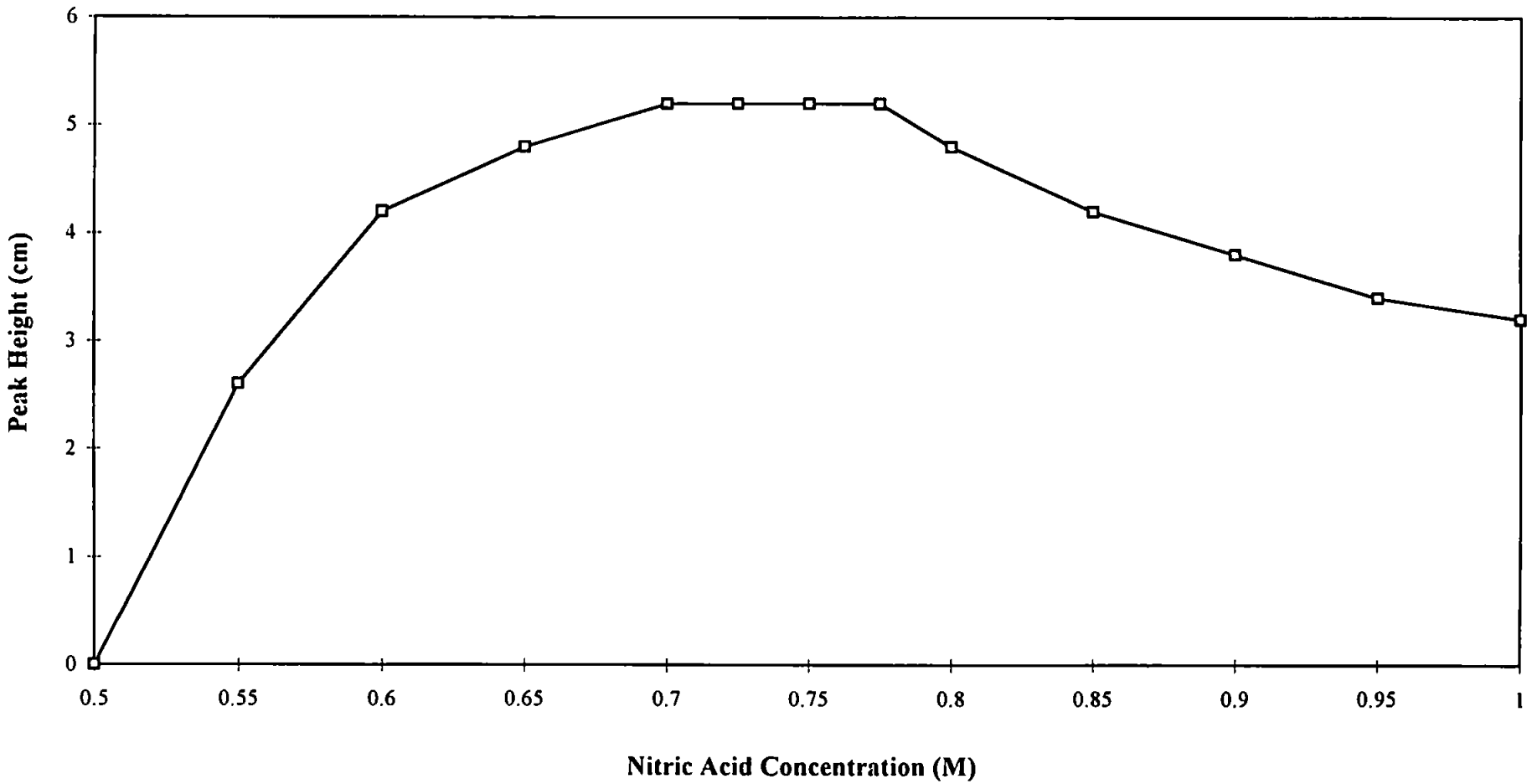
5.4.2.1.3 Nitric Acid Concentration of the L-cysteine Pre-Reduction Reaction

Due to the influence of pH on the efficiency of the L-cysteine pre-reduction reaction and the hydride generation process, the nitric acid concentration was optimised between 0.5 and 1.0 M. From the results shown in Figure 5.17 [again for a standard solution of 40 ng ml^{-1} As(V)] it can be seen that the peak response was obtained at a nitric acid concentration of 0.7-0.775 M. Following mixing with the potassium persulfate/sodium hydroxide reagent stream this corresponded to a pH of between 1.3 and 1.8 for the L-cysteine pre-reduction reaction. A concentration of 0.75 M nitric acid was chosen for further work.

5.4.2.1.4 Argon Carrier Gas Flow Rate

The argon carrier gas flow rate was varied from 200 to 600 ml min^{-1} in order to investigate the effect on sensitivity. It was discovered that the sensitivity of the HG-AAS system was greatly influenced by this parameter. As the argon flow rate was reduced the sensitivity increased due to less dilution of the sample, although the washout time between samples increased. A flow rate of 300 ml min^{-1} was therefore chosen, giving a good signal response whilst keeping washout times to a minimum.

Figure 5.17: Optimisation of the Nitric Acid Concentration of the L-cysteine Pre-Reduction Reaction



5.4.2.2 Validation of the Response of Different Arsenic Species

The L-cysteine pre-reduction reaction should ensure that all arsenic species are present in the same oxidation state, thus giving the same response on hydride generation. This was verified by passing a 40 ng ml^{-1} sample of each arsenic species through the system. AsBet, As(V), As(III), DMA and MMA all gave the same response on analysis by HG-AAS. This finding enabled the system to be employed for the determination of total arsenic.

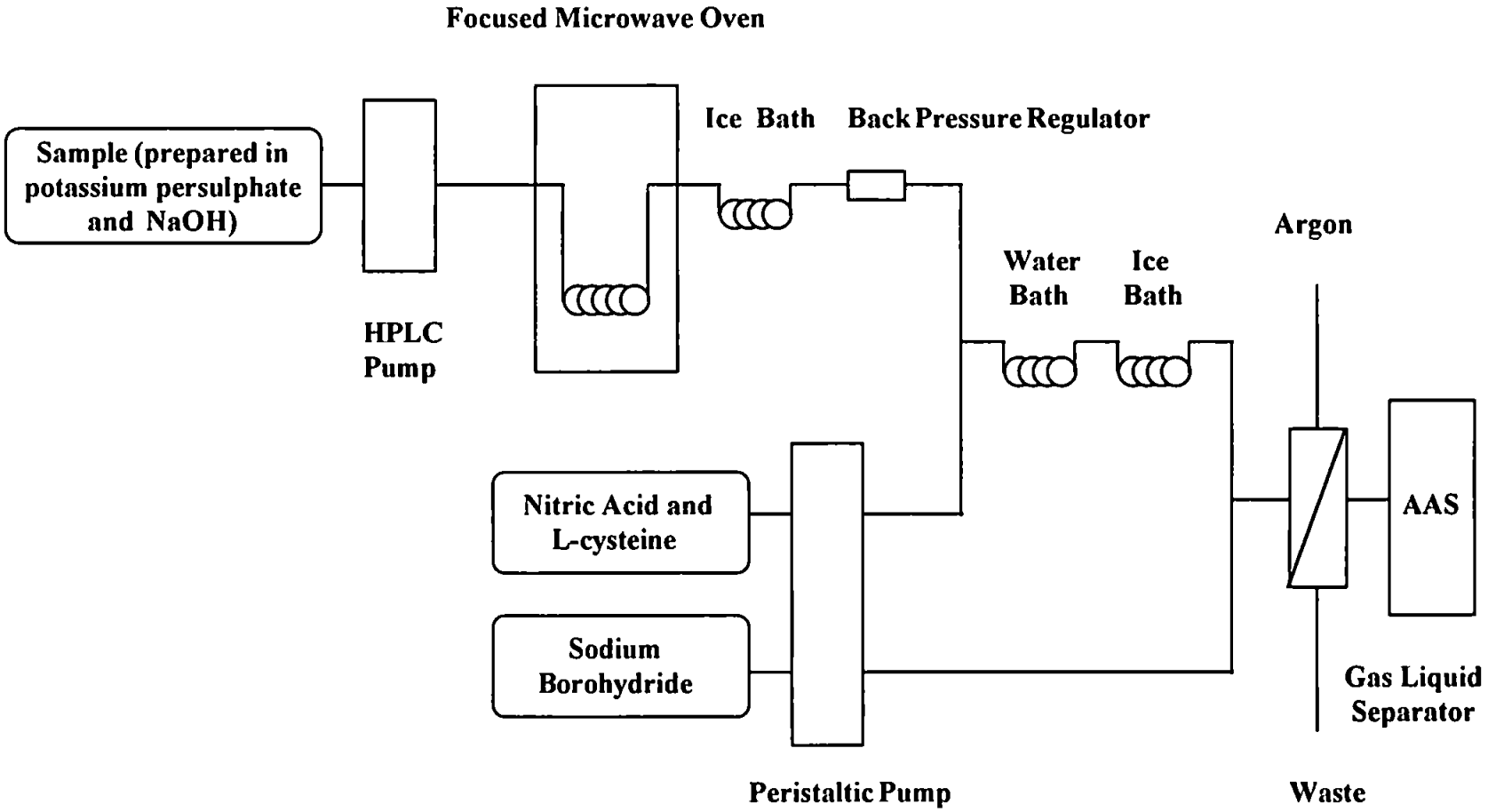
The operating conditions and a schematic diagram of the final design of the on-line microwave digestion system for the determination of total arsenic are shown in Table 5.7 and Figure 5.18 respectively.

Table 5.7: Operating Conditions for the On-Line Microwave Digestion System for the Determination of Total Arsenic

Microwave Digestion		Acidification and Pre-Reduction				Hydride Generation		AAS Detection
Samples/ Standards	Flow Rate[1]	Reducing Reagent	Flow Rate[1]	Heating Coil Length	Sample Flow Rate[1]	Reductant	Flow Rate[1]	Ar Carrier Gas Flow Rate[1]
Prepared in 3.7% $K_2S_2O_8$ and 2.5% NaOH	4.6	1.65% L-cysteine in 0.75 M HNO_3	3.4	15 m	-	1.5% $NaBH_4$ in 0.1 M NaOH	2.8	300

Notes:
1: All flow rates are in ml min^{-1}

Figure 5.18: Schematic Diagram of the On-Line Microwave Digestion System for the Determination of Total Arsenic in Biological Samples - Final Design



5.5 Development of an HPLC-On-Line Microwave Digestion Technique for the Speciation of Arsenic in Biological Samples

For speciation studies the possibility of coupling an HPLC column to the on-line microwave digestion system previously developed was addressed. A schematic design of the system is shown in Figure 5.19. Where applicable the original reagent concentrations and flow rates were retained.

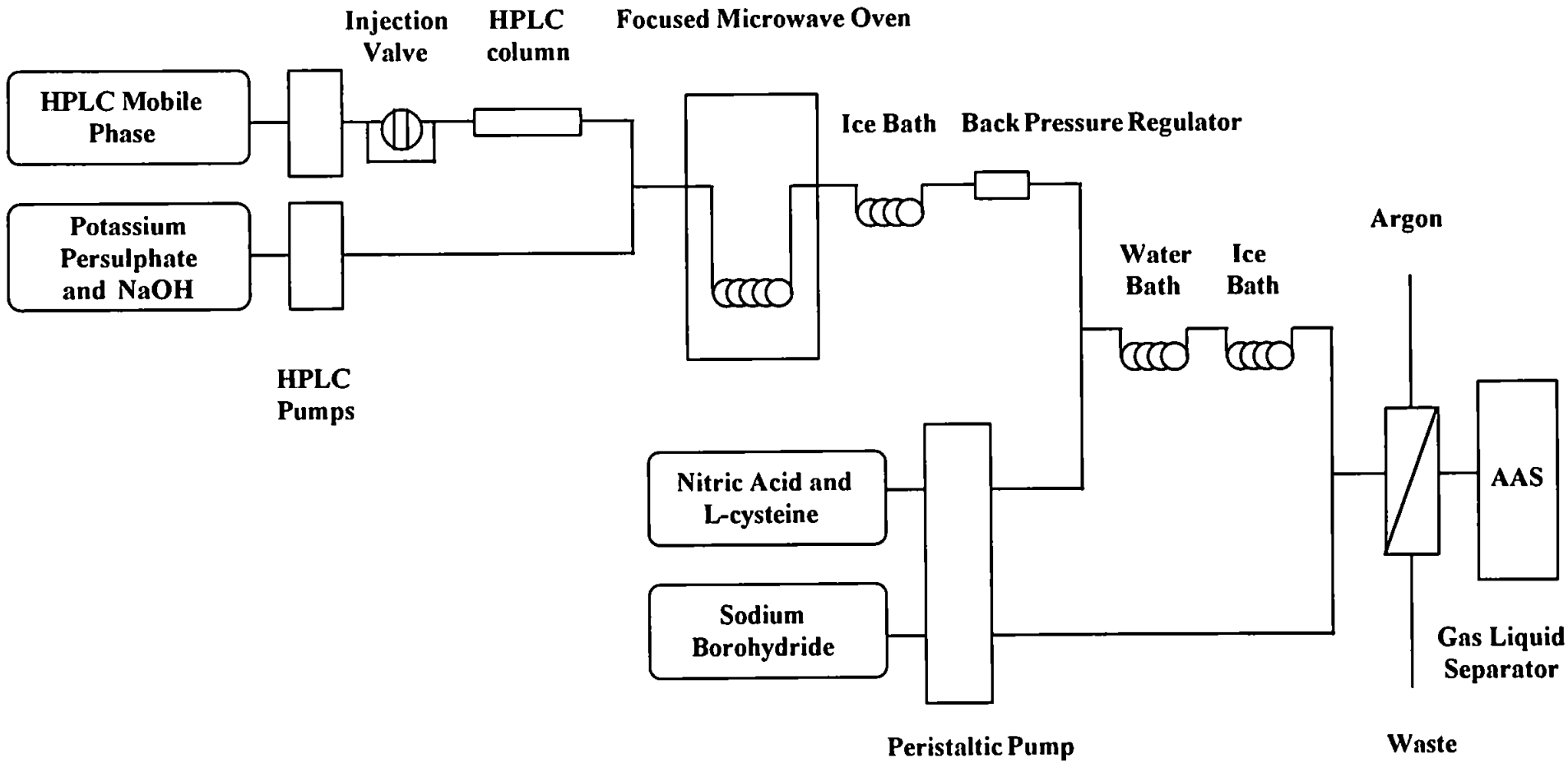
5.5.1 Trypsin Extraction Procedure

Prior to speciation analysis, biological samples were subjected to a trypsin enzymatic extraction procedure[278], the details of which are shown in Table 5.8. 1.0 g of sample was placed in a 50 ml plastic centrifuge and 100 mg of trypsin powder and 20 ml of 0.1 M ammonium hydrogen carbonate solution added. After tightly capping, the tubes were shaken in a water bath at 37°C for 6 hours. Samples were then allowed to cool and quantitatively transferred into 25 ml volumetric flasks, making up to volume with 0.1 M ammonium hydrogen carbonate solution. Samples were stored in the dark at 4°C prior to analysis.

Table 5.8: Enzymatic Extraction Procedure

Step	Procedure
1	Place 1.0 g of sample in a 50 ml plastic centrifuge tube with 100 mg of trypsin and 20 ml of 0.1 M ammonium hydrogen carbonate solution.
2	Cap tubes and shake in a water bath at 37°C for 6 h.
3	Cool samples and quantitatively transfer into 25 ml volumetric flasks. Make up to 25 ml with 0.1 M ammonium hydrogen carbonate solution.
4	Store in the dark at 4°C prior to analysis.

Figure 5.19: Schematic Diagram of the On-Line HPLC-Microwave Digestion System for the Speciation of Arsenic in Biological Samples



5.5.2 HPLC Separation

HPLC separation was achieved using a 4.6 mm x 15 cm stainless steel HPLC column packed with BAX-10 strong anion exchange resin (Benson Corporation, Reno, NV, USA). The analytical column was protected by a 4.6 mm guard column of the same material. The columns were heated at 60°C in a water bath to improve the kinetics of the separation, thereby improving peak shape. Sample injections were performed by way of a Rheodyne 7125 injection valve with a 500 µl sample loop (Rheodyne, Cotati, CA, USA). In order to condition the column a solution of 0.1 mM potassium sulfate (pH 10.4) was run for 15 minutes (0.5 ml min^{-1}) followed by 75 mM for 20 minutes (1.0 ml min^{-1}) and finally with 0.1 mM for 20 minutes (1.0 ml min^{-1}). The separation was achieved by running a mobile phase of 0.1 mM potassium sulfate (pH 10.4) at a flow rate of 1.0 ml min^{-1} for 4 minutes. During this time AsBet and DMA were eluted from the column. The mobile phase was then switched to the higher concentration of 75 mM potassium sulfate in order to elute the more strongly bound MMA and inorganic arsenic species. It was observed that on-column oxidation of As(III) to As(V) occurred thereby allowing quantification of the total inorganic arsenic content of the sample. In real samples there is the possibility that after extraction any As(III) will be oxidised in the environment to As(V) before analysis. Therefore it may well be better to determine the total inorganic arsenic content rather than to quantify As(III) and As(V) separately, since the value obtained may not give a true representation of the original species present.

At a pH above 9.2, DMA, MMA, As(III) and As(V) are ionised and thus have affinity for the column. Arsenobetaine was probably undissociated and thus had no affinity for the column and passed straight through. The order of elution of the remaining species can be in part assigned to their pK_a values (see Table 5.9). DMA is the least acidic (pK_a of 6.27) and thus is eluted before MMA and As(V). Conversely As(V) is the most acidic in character with a pK_a of 2.25 and thus is eluted last[311].

Table 5.9: pKa Values for MMA, DMA and As(V)

Species	pK_a
Arsenic acid (AsV)	2.25, 7.25
Monomethylarsonic acid (MMA)	3.61, 8.25
Dimethylarsinic acid (DMA)	6.27

The HPLC separation of AsBet, DMA, MMA and inorganic arsenic was subsequently investigated using a i) 175 µl, ii) 500 µl and iii) 1000 µl sample loop.

5.5.2.1 175 µl Sample Loop

Baseline separation of the four species was obtained as shown in Figure 5.20, however sensitivity was poor. Limits of detection of 7.1, 24.0, 15.3 and 29.0 ng ml^{-1} were obtained for AsBet, DMA, MMA and inorganic arsenic respectively.

5.5.2.2 1000 µl Sample Loop

In order to gain improvements in sensitivity a 1000 µl sample loop was investigated. As expected peak broadening occured to the extent that the AsBet and DMA, and the MMA and As(V) peaks were not totally resolved from each another (see Figure 5.21).

Figure 5.20: Chromatographic Separation of a 175µl Mixture of 200 ng ml^{-1} AsBet, 200 ng ml^{-1} DMA, 325 ng ml^{-1} MMA and 400 ng ml^{-1} Inorganic Arsenic

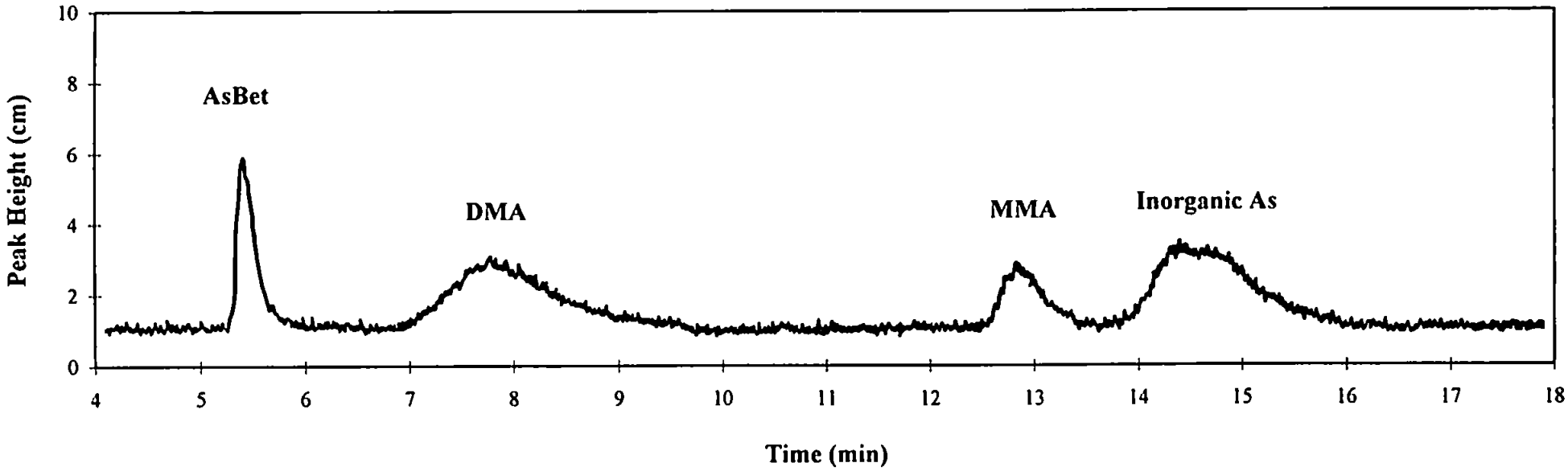
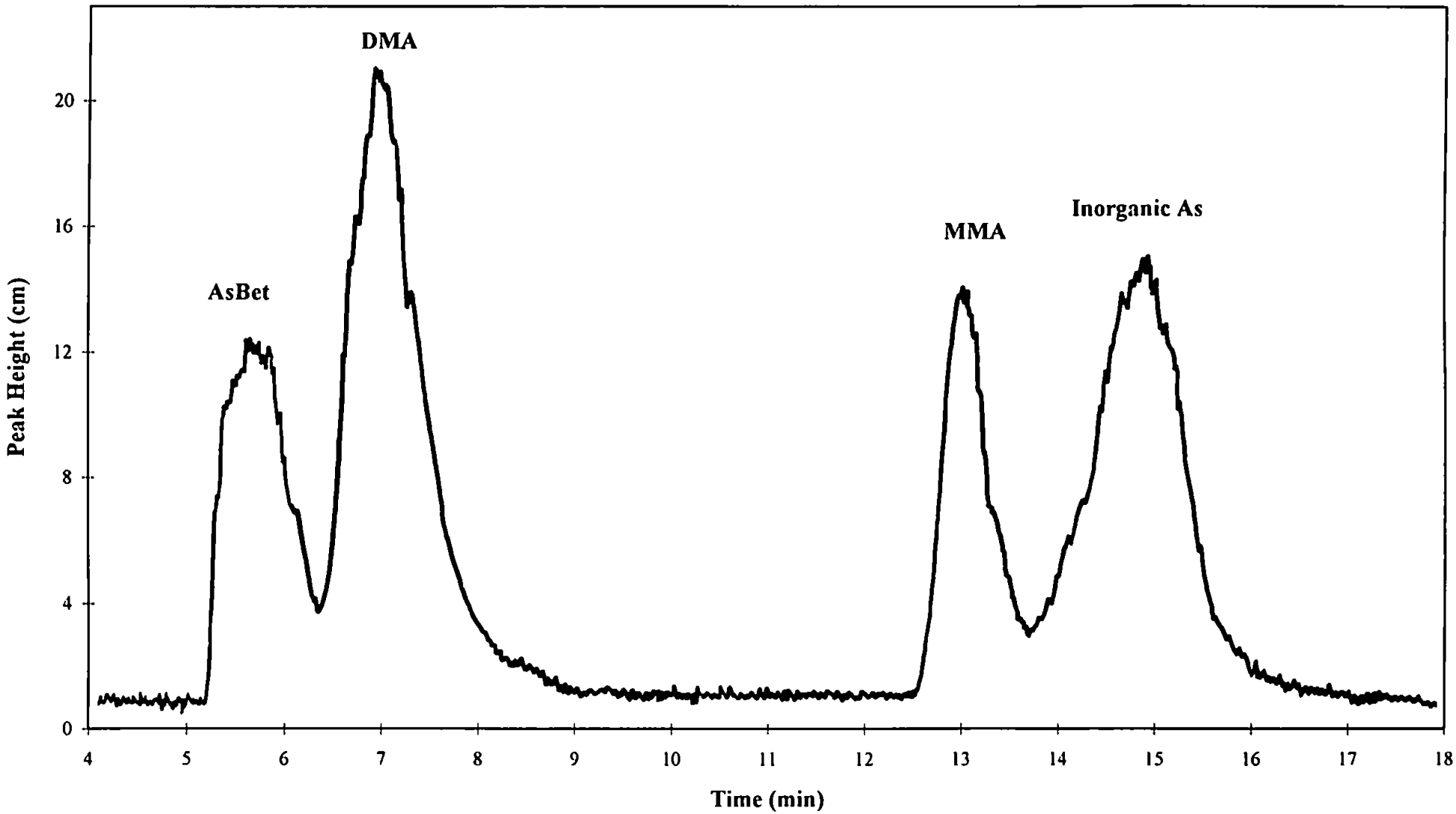


Figure 5.21: Chromatographic Separation of a 1000 µl Mixture of 200 ng ml^{-1} AsBet, 400 ng ml^{-1} DMA, 163 ng ml^{-1} MMA and 200 ng ml^{-1} Inorganic Arsenic



5.5.2.3 500µl Sample Loop

A 500 µl loop was chosen for further work giving complete separation of the four species whilst achieving good sensitivity. A typical chromatogram obtained for the separation of a mixture of 100 ng ml^{-1} AsBet, DMA, MMA and As(V) (prepared in deionised water) is shown in Figure 5.22.

5.5.3 Microwave Digestion, Pre-Reduction and Analysis

The output from the HPLC column (1.0 ml min^{-1}) was mixed at a high pressure T-piece with a solution of 5% (m/v) potassium persulfate in 3.4% (m/v) sodium hydroxide solution (3.4 ml min^{-1}) before entering the microwave digestion coil (dimensions as described in Section 5.4.2). The above concentration of persulfate was chosen to obtain the same final concentration as utlised in the on-line microwave digestion technique for total arsenic (2.1%). Acidification, pre-reduction and HG-AAS analysis were also undertaken under the same conditions as the total arsenic technique. Signal output was recorded on a chart recorder and in the absence of a peak integrator, peak areas were determined by the cut and weigh method.

The optimised conditions and parameters for the on-line HPLC microwave digestion system are presented in Table 5.10.

Figure 5.22: Chromatographic Separation of a 500µl Mixture of 100 ng ml^{-1} AsBet, DMA, MMA and Inorganic Arsenic

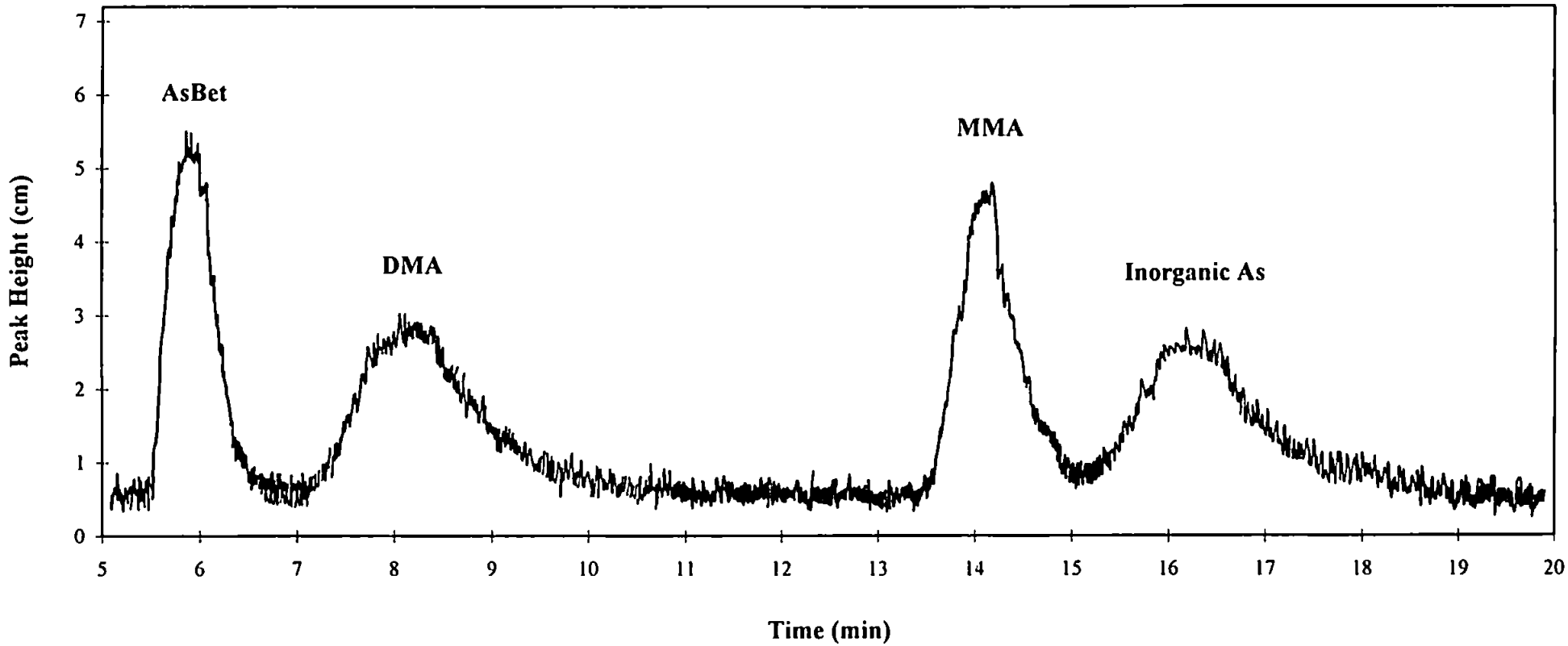


Table 5.10: Operating Conditions for the On-Line HPLC-Microwave Digestion System

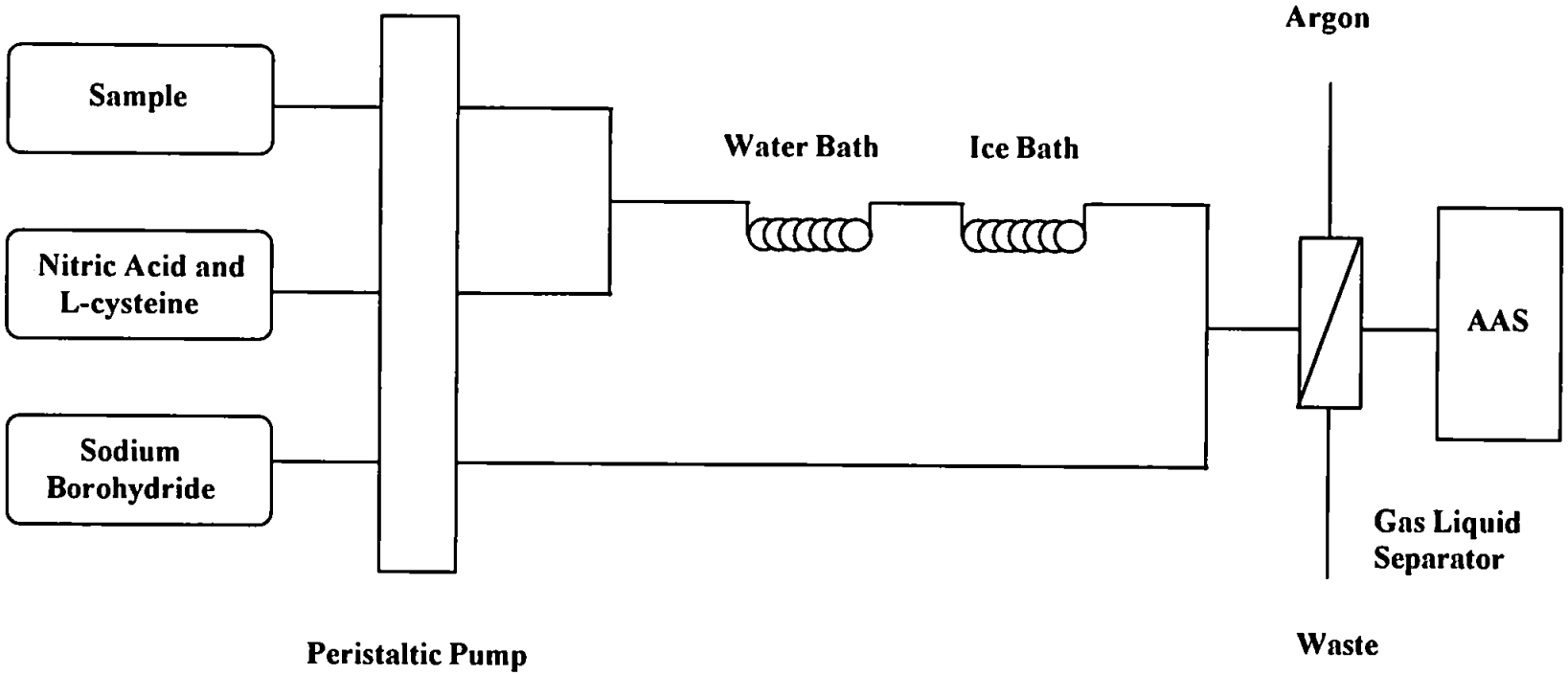
HPLC		Microwave Digestion		Acidification and Pre-Reduction				HG		AAS
Mobile Phase	Flow Rate[1]	Oxidising Reagent	Flow Rate[1]	Reducing Reagent	Flow Rate[1]	Heating Coil Length	Sample Flow Rate[1]	Reductant	Flow Rate[1]	Ar Carrier Flow Rate[1]
0.1 mM K_2SO_4 for 4 min followed by 75 mM K_2SO_4 (pH 10.4)	1.0	5% $K_2S_2O_8$ in 3.4% NaOH	3.4	1.65% L-cysteine in 0.75 M HNO_3	3.4	15 m	-	1.5% $NaBH_4$ in 0.1 M NaOH	2.8	300

Notes:
1: All flow rates are in ml min^{-1}

5.6 Development of an On-Line Pre-Reduction Technique for the Determination of Total 'Reducible' Arsenic in Biological Samples

For the determination of total reducible arsenic (As(III), As(V), DMA and MMA) an on-line L-cysteine pre-reduction system was developed as shown in Figure 5.23. The system comprised of essentially the latter half of the on-line microwave digestion system as described in Section 5.5. Samples and calibration standards were prepared in 0.03 M HNO_3 which corresponded to a pH of 1.5, the optimum pH for the pre-reduction reaction as determined in Section 5.4.2.1.3. The sample (6 ml min^{-1}) and a solution of 2.8% (m/v) L-cysteine in 0.03 M HNO_3 (2 ml min^{-1}), were pumped continuously and mixed at a PTFE T-piece (1/8" i.d.) before passing through a PTFE coil (1.5 mm i.d.) immersed in a water bath maintained at 85°C. Optimisation studies revealed that a heating coil of 5 m was sufficient for the complete reduction of arsenic to As(III). A coil of 7 m was chosen to ensure completion of the reaction, corresponding to a residence time of 92 s in the water bath. Following pre-reduction the sample flow was cooled by passage through an ice-bath (1.5 mm x 1.5 m) before entering the hydride generator unit. Here the sample was mixed with a solution of 1% (m/v) $NaBH_4$ solution in 0.1 M NaOH (2.8 ml min^{-1}) before passing into the gas-liquid separator for subsequent analysis by AAS.

Figure 5.23: Schematic Diagram of the On-Line Pre-Reduction System for the Determination of Total 'Reducible' Arsenic in Biological Samples



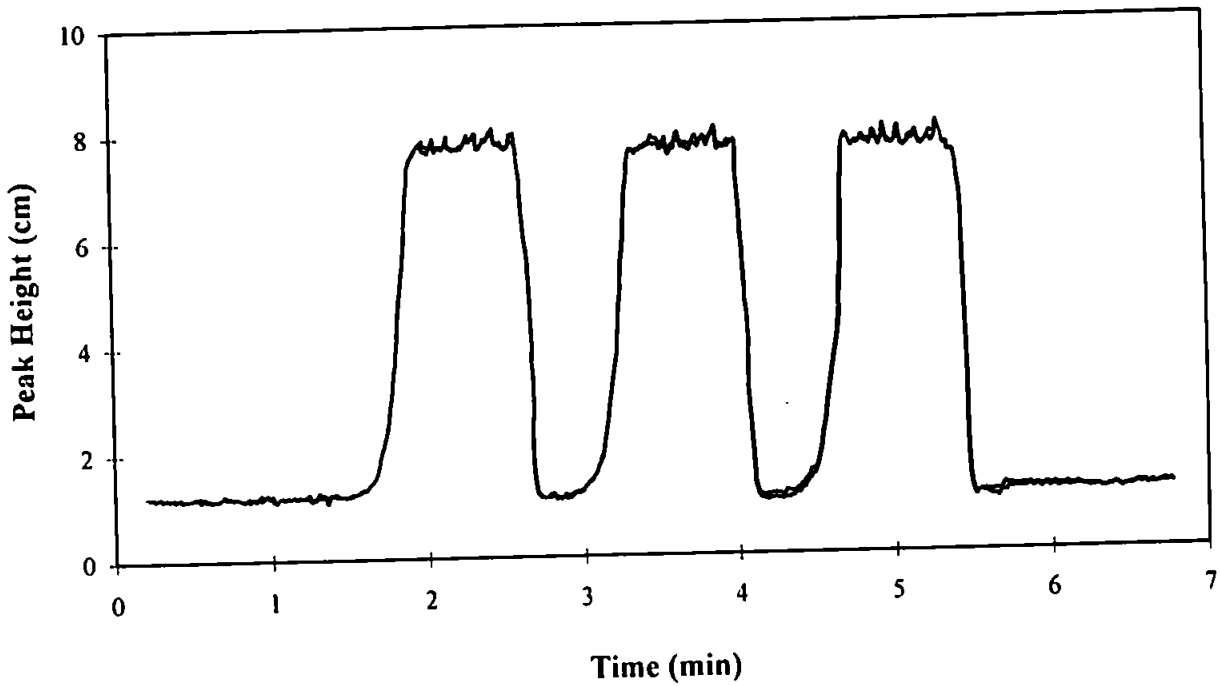
The operating conditions for the on-line system for the determination of total 'reducible' arsenic are given in Table 5.11. Quantification was achieved by taking peak height measurements from the steady state signals obtained. A typical output obtained for a standard solution of 40 ng ml^{-1} As(V) is shown in Figure 5.24.

Table 5.11: Operating Conditions for the On-Line Pre-Reduction System for the Determination of Total 'Reducible' Arsenic in Biological Samples

Acidification and Pre-Reduction				Hydride Generation		AAS Detection
Reducing Reagent	Flow Rate[1]	Heating Coil Length	Sample Flow Rate[1]	Reductant	Flow Rate[1]	Ar Carrier Gas Flow Rate[1]
2.5% L-cysteine in 0.03 M HNO_3	6.0	7 m	2.0	1.0% $NaBH_4$ in 0.1 M NaOH	2.8	300

Notes:
1: All flow rates are in ml min^{-1}

Figure 5.24: Typical Signal Obtained for 40 ng ml^{-1} As(V) following On-Line Pre-Reduction and HG-AAS Analysis



5.7 Results and Discussion

5.7.1 Figures of Merit

A summary of the reagents and conditions employed for the on-line microwave digestion system for total arsenic, the on-line HPLC-microwave digestion system and the on-line pre-reduction system are shown in Table 5.12. Detection limits ($3\sigma_{n-1}$), the dynamic linear range and typical calibration coefficients for each system are shown in Table 5.13. Typical RSDs of 0.5-3% were obtained at a concentration of 50 ng ml^{-1} (n=3) for the on-line HPLC-microwave digestion system.

5.7.2 Validation of the Developed Techniques

For validation of the developed techniques the certified reference materials DORM-1 and TORT-1 (NRCC) were employed. In each case sample blanks were prepared and used to correct for potential contamination of the samples.

Following trypsin enzymatic extraction (see Table 5.8), samples were analysed for total arsenic by the on-line microwave digestion technique and for comparison by ICP-MS (FI Elemental PlasmaQuad, 2+ Turbo, Winsford, Cheshire, UK). For the on-line technique As(V) calibration standards between 0 to 40 ng ml^{-1} were prepared in 3.4% (m/v) potassium persulfate and 2.5% (m/v) sodium hydroxide. Samples were diluted by a factor of 20 so that the concentration of arsenic fell within the calibration range. For analysis by ICP-MS, chloride interferences were overcome by means of nitrogen addition to the argon flow[312]. In order to matrix match, As(V) calibration standards in the range 0 to 100 ng ml^{-1} were prepared in 0.08 M ammonium hydrogen carbonate solution. An internal standard of 100 ng ml^{-1} caesium (Merck Ltd) was also added to both samples and standards to correct for instrumental drift during analysis.

Table 5.12: Summary of Operating Conditions for the Developed Techniques

Technique	HPLC		Microwave Digestion		Acidification and Pre-Reduction				HG		AAS
	Mobile Phase	Flow Rate[1]	Oxidising Reagent	Flow Rate[1]	Reducing Reagent	Flow Rate[1]	Heating Coil Length	Sample Flow Rate[1]	Reductant	Flow Rate[1]	Ar Carrier Flow Rate[1]
On-Line HPLC-MD[2]-AAS	0.1 mM K_2SO_4 for 4 min followed by 75 mM K_2SO_4 (pH 10.4)	1.0	5% $K_2S_2O_8$ in 3.4% NaOH	3.4	1.65% L-cysteine in 0.75M HNO_3	3.4	15 m	-	1.5% $NaBH_4$ in 0.1M NaOH	2.8	300
On-Line MD[2]-AAS (Total As)	-	-	Samples prepared in 3.7% $K_2S_2O_8$/ 2.5% NaOH	4.6	1.65% L-cysteine in 0.75M HNO_3	3.4	15 m	-	1.5% $NaBH_4$ in 0.1M NaOH	2.8	300
On-Line Pre-Reduction	-	-	-	-	2.5% L-cysteine in 0.03M HNO_3	6.0	7 m	2.0	1.0% $NaBH_4$ in 0.1M NaOH	2.8	300

Notes:
1: All flow rates are in ml min^{-1}.
2: MD = microwave digestion.

Table 5.13: Figures of Merit for the Developed Techniques

Technique	Species Determined	Retention Time (min)	Detection Limit[1] ($ng\ ml^{-1}$)	Dynamic Linear Range ($ng\ ml^{-1}$)	Correlation Coefficient[2]
On-Line MD[3]-AAS	Total As	-	0.6	0-45	0.9999
On-Line HPLC-MD[3]-AAS	AsBet	5.5	2.5	0-200	0.9991
	DMA	7.1	5.3	0-200	0.9960
	MMA	13.4	3.3	0-200	0.9988
	Inorganic As	15.1	5.9	0-200	0.9996
On-Line Pre-Reduction-AAS	Reducible As	-	0.5	0-30	0.9998

Notes:
1: Detection limits are calculated as 3 times the SD_{n-1} of 10 replicate measurements of the blank signal divided by the slope of the calibration line.
2: Correlation coefficients are based on calibration with 5 standards.
3: MD = microwave digestion.

As can be seen from the results shown in Table 5.14 recoveries for the total arsenic content of the samples obtained by the on-line microwave digestion technique are in good agreement with the certified values. Extraction efficiencies of 98% and 87% were obtained for TORT-1 and DORM-1 samples respectively. In addition the results for total arsenic compare favourably with those obtained by ICP-MS, demonstrating the efficiency of the on-line microwave digestion procedure for the decomposition of the organoarsenic species in the samples.

For the speciation of arsenic in the certified samples, mixed calibration standards of AsBet, DMA, MMA and As(V) were prepared in the range 0 to 200 ng ml^{-1} (as As) in deionised water. The results of the subsequent analysis using the on-line HPLC-microwave digestion technique show arsenobetaine as the most abundant arsenic species in NRCC TORT-1 and DORM-1, accounting for 93% and 95% of the total arsenic respectively. DMA, MMA and inorganic arsenic were not detected in DORM-1 although in TORT-1 inorganic arsenic was determined at a level of 1.98 $\mu g\ g^{-1}$. In the latter the sum of the detected species (AsBet and inorganic arsenic) agrees well with the total arsenic content of the sample.

The total 'reducible' arsenic species in the extracts were determined following pH adjustment of the samples to the optimum pH for the pre-reduction reaction (pH 1.5), as determined in Section 5.4.2.1.3. This was necessary as the matrix of the samples, 0.08 M ammonium hydrogen carbonate, has a pH of 8. It was also found beneficial to prepare the samples in 0.4% dibutylphthalate (Aldrich Chemicals) in order to control frothing in the gas liquid separator. For both NRCC DORM-1 and TORT-1 the sum of the total 'reducible'

Table 5.14: Results for the Determination of Arsenic in NRCC DORM-1 and TORT-1

Sample	Certified Values ($\mu g\ g^{-1}$)	Extracts[1]							Digestions[2]
		Total As (ICP-MS) ($\mu g\ g^{-1}$)	Total As (On-Line MD[3]-AAS) ($\mu g\ g^{-1}$)	Total Reducible As (On-Line AAS[4]) ($\mu g\ g^{-1}$)	As Speciation (HPLC-On-Line MD-AAS) ($\mu g\ g^{-1}$)				Total As (ICP-MS) ($\mu g\ g^{-1}$)
					AsBet	DMA	MMA	Inorganic As	
TORT-1	24.6±2.2	23.0±1.1[5] (2.3)[6]	24.0±1.1 (2.4)	1.5±0.1 (4.5)	22.3±1.2 (2.6)	n.d[7]	n.d	2.0±0.2 (4.0)	24.7±0.6 (1.3)
DORM-1	17.7±2.1	15.6±1.3 (4.5)	15.4±1.2 (4.0)	0.8±0.2 (10.3)	14.5±1.5 (5.0)	n.d	n.d	n.d	16.5±0.2 (0.6)

Notes:
1: n=3 for extractions.
2: n=4 for digestions.
3: MD = microwave digestion.
4: On-line pre-reduction system.
5: Uncertainties for the experimental results are calculated as twice the standard deviation.
6: Values in parentheses are relative standard deviations (in %).
7: n.d = not detected.

arsenic species and the AsBet results are in good agreement with the total arsenic content of the samples.

The DORM-1 and TORT-1 certified reference materials were also analysed for total arsenic by ICP-MS after batch microwave digestion with nitric acid and hydrogen peroxide (see Table 5.5). For this purpose calibration standards were prepared in 2% (v/v) nitric acid and 1% (v/v) hydrogen peroxide and an internal standard of 100 ng ml^{-1} indium (Aldrich Chemicals) was added to standards and samples to correct for instrumental drift. The results obtained were in good agreement with the certified values for both samples.

5.8 Conclusions

The on-line HPLC-microwave digestion-HG-AAS technique developed in this study has been used successfully for the on-line separation and quantification of AsBet, MMA, DMA and inorganic arsenic in biological samples. Although no species specific certified reference materials are currently available the system has been validated using the certified reference materials NRCC DORM-1 and TORT-1, for which the total arsenic content is known, using a mass balance approach. By removing the HPLC column the system can easily be applied to the determination of total arsenic, giving results in good agreement with those obtained by ICP-MS. In addition the on-line pre-reduction system facilitates the determination of the total 'reducible' arsenic species in the samples.

6. *Development of Batch and On-Line Microwave Digestion Techniques for the Determination of Total Mercury in Environmental Samples*

6 DEVELOPMENT OF BATCH AND ON-LINE MICROWAVE DIGESTION TECHNIQUES FOR THE DETERMINATION OF TOTAL MERCURY IN ENVIRONMENTAL SAMPLES

6.1 Introduction

Considerable interest has been generated in recent years over the levels of mercury present in the environment. Mercury has no known metabolic function in humans and is considered toxic, resulting in often irreversible damage to the sensory part of the nervous system[3]. Over the last hundred years mercury has been used in a number of industrial and agricultural applications such as in dentistry, the chloralkali industry and in the production of electrical equipment, paints and fungicides. As a result of these activities extensive discharges of mercury into the environment have occurred, although since 1974 these have been in steady decline[3]. Although the natural inputs of mercury into the environment far exceed the anthroprogenic contribution they are dispersed on a global scale and so are not generally problematic. The main environmental concern however comes from localised anthroprogenic inputs of mercury, which often lead to extensive contamination of a relatively small area close to the pollution source. For example following the Minimata Bay[313] disaster of 1952 (release of inorganic mercury from a vinyl chloride plant) levels of mercury of up to 2,000 $\mu g\ g^{-1}$ [3] and 178 $\mu g\ g^{-1}$ [314] were reported in sediments and shellfish respectively close to the outlet of the plant[3].

As a result of both anthroprogenic and natural inputs of mercury into the environment mercury is found in soils, waters, minerals and the atmosphere. Inevitably mercury is transported into the food chain and through a very effective mechanism of biomagnification high concentrations are often found in marine organisms in the top trophic levels. For this reason the European Community stipulates a maximum admissible concentration of 1 $\mu g\ g^{-1}$ of total mercury in predator fish and 0.5 $\mu g\ g^{-1}$ in other fish[315] for human consumption. The principal site of mercury accumulation in the marine environment however is in sediments, with typical levels between 0.23 and 3.42 $\mu g\ g^{-1}$ [120].

Rapid, sensitive and accurate techniques are therefore required to study the marine distribution of mercury in the environment. However the low levels of mercury present in many environmental samples often negate the use of a number of analytical techniques such as ICP-AES, FAAS and electrothermal atomisation atomic absorption spectrometry (ET-AAS) due to insufficient sensitivity. However in combination with vapour generation techniques the sensitivity is markedly improved. The most common detectors for cold vapour mercury determinations are AAS and AFS. For the latter, limits of detection at the ng l^{-1} level[120,316,317] are commonly achieved. However an important precursor of the cold vapour generation technique is that mercury is present in the Hg(II) oxidation state so that reduction to elemental mercury [Hg(0)] may be undertaken by a suitable reductant such as tin (II) chloride. Detection can then be performed by sweeping the mercury vapour formed into the AAS or AFS detector.

Organomercury compounds such as methylmercury, the predominant mercury species in marine organisms, are not however reduced to Hg(0) by tin (II) chloride. As a result quantification is impossible unless suitable pre-treatment of the sample is undertaken. For the analysis of environmental samples by CV-AFS or CV-AAS the pre-treatment must achieve two objectives. Firstly the organic matter in the sample must be sufficiently oxidised to liberate the mercury species from the sample matrix, and secondly the liberated mercury must be fully oxidised to Hg(II). The problem of losses of mercury through volatilisation during sample digestion at high temperature precludes the use of fusion and dry ashing procedures. However pre-treatment can be achieved by undertaking a suitable microwave digestion procedure.

For the determination of mercury in biological samples microwave digestion techniques often necessitate the use of oxidising acids and reagents such as hydrogen peroxide, nitric and sulphuric acids[95,97,315,318,319]. Strongly oxidising conditions must be maintained throughout the digestion process so that the oxidation of organomercury compounds to Hg(II) can be achieved. As a consequence of the high boiling points of many of these reagents care must be taken to ensure that mercury is not lost through volatilisation at any stage of the digestion procedure, especially in an open system. In addition, due to the hazardous nature of the reagents safety precautions must be strictly adhered to.

An alternative method for the oxidation of organomercury compounds (after liberation of the mercury species from the matrix) is achieved by reaction with potassium bromide/ bromate[316,317,320-322]. In acidic media bromate oxidises bromide to bromine according to Reaction 6.1:

$$BrO_3^- + 5Br^- + 6H^+ \leftrightarrow 3Br_2 + 3H_2O \qquad \textbf{(Reaction 6.1)}$$

The bromine formed is then capable of rapidly cleaving organomercury bonds and oxidising the inorganic mercury formed to Hg(II).

In addition bromine has the ability to oxidise sulphide ions (interferents in the tin (II) chloride reduction and the subsequent volatilisation of mercury) to sulphate, thereby reducing potential interference. It has been reported that complete oxidation (> 95%) of organomercury compounds to Hg(II) is achieved within 10 to 15 minutes at room temperature[321-323] or in a matter of seconds following heating of the sample in a water bath[317]. However prior to analysis by CV-AFS the excess free bromine formed must be removed to prevent interferences from quenching (non-radiative loss of energy) of the fluorescence signal. This can be undertaken by reduction with hydroxylammonium chloride[324] according to Reaction 6.2:

$$2\,(HO.NH_3.Cl) + Br_2 \leftrightarrow 2HBr + N_2 + 2H_2O + 2HCl \qquad \textbf{(Reaction 6.2)}$$

There has been an increasing number of on-line microwave digestion procedures cited in the literature in recent years (see Section 1.4.2). Morales-Rubio *et al.*[316] developed an on-line nitric acid microwave digestion method for the determination of total mercury in sewage sludge, soil and sediment samples. However the method was not applied to samples containing high levels of organomercury compounds such as biological tissue. On-line methods for the bromide/bromate oxidation of organomercury compounds in water samples have also been developed[152,316,317]. However the possibility of combining a bromide/bromate oxidation reaction with an on-line microwave digestion for solid samples has not be addressed.

This chapter describes the development of both a batch and on-line microwave digestion technique for the determination of total mercury in sediment and biological samples. The batch method was developed to examine whether the determination of mercury was possible following digestion with a mixture of oxidant acids and reagents in an open system. This is of particular interest in light of the strong oxidising conditions needed for the complete oxidation of organomercury compounds which may lead to losses of mercury through volatilisation. For the on-line microwave technique a different approach was investigated whereby a bromide/bromate oxidation reaction was combined with a microwave digestion procedure to achieve complete oxidation of organomercury compounds to Hg(II), prior to analysis by CV-AFS.

6.2 Experimental

6.2.1 Instrumentation

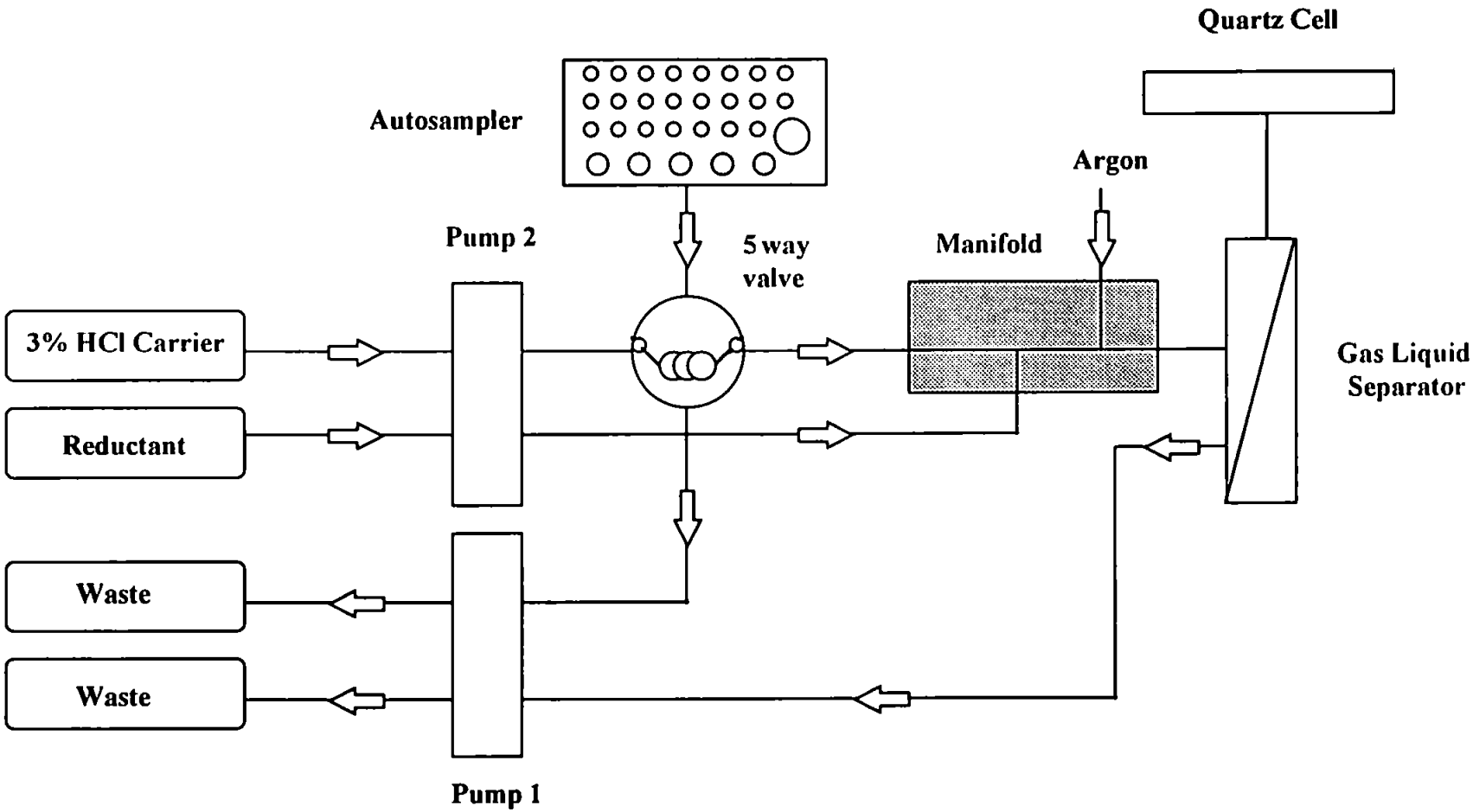
Sample digestion was carried out in a single mode reflux focused microwave system (Microdigest 301, Prolabo Ltd, Paris) as described in Section 3.1.2.1. For the batch microwave digestions the unit was fitted with an Aspivap fume treatment system (Prolabo) which effectively neutralises acid fumes evolved during the digestion.

Samples prepared by the batch microwave digestion method were analysed by flow injection-cold vapour-atomic absorption spectrometry (FI-CV-AAS) following the method of Murphy *et al.*[95]. AAS measurements were undertaken with a Perkin Elmer Model 4100ZL electrothermal atomisation-atomic absorption spectrometer with the furnace head removed and replaced by a quartz cell in an electrically heated mantle. This contained a quartz tube which was maintained at 100°C to prevent condensation of water vapour in the quartz cell. Sample manipulations were undertaken by a Perkin Elmer Flow Injection Analysis System (FIAS 400, Perkin Elmer, Bodenseewerk, Uberlingen, Germany) and an AS-90 autosampler (Perkin Elmer) controlled by a computer equipped with FIAS-Furnace Version 7.21 software. A schematic diagram of the flow injection system is shown in Figure 6.1 and was operated using the sequence conditions in Table 6.1.

Table 6.1: Sequence Conditions for the Analysis of Mercury by FI-CV-AAS

Step	Time (s)	Pump 1 (rpm)	Pump 2 (rpm)	Valve Position
Prefill	20	100	120	Fill
1	10	100	120	Fill
2	20	0	120	Inject

Figure 6.1 : Schematic Diagram of the Flow Injection CV-AAS System for the Analysis of Mercury



The prefill step was used to flush the manifold between samples to avoid cross contamination. In Step 1 with the valve in the 'Fill' position the sample was pumped into the 500 µl sample loop. In Step 2 the valve was switched to the 'Inject' position allowing the carrier [3% (v/v) HCl] to sweep the sample out of the sample loop into the flow injection manifold. Following mixing with the reductant [0.2% (m/v) sodium borohydride in 0.05% (m/v) sodium hydroxide] the sample met the argon carrier stream before entering the gas liquid separator. Here the argon gas (100 ml min^{-1}) effectively purged the mercury vapour from the sample and after passage through a filter membrane (to prevent aerosol droplets from reaching the quartz cell) the vapour entered the quartz cell placed in the path of a mercury hollow cathode lamp. Atomic absorption measurements were then taken under the operating conditions given in Table 6.2.

Temperature measurements of the sample solution were taken throughout the course of the batch microwave digestion procedures by means of a Megal 300 temperature probe (Prolabo). This is in essence an air thermometer with an operating range of 0 to 500°C. The probe was placed directly into the sample and the temperature readings obtained were fed to a computer for digital display and further data treatment.

Table 6.2: Instrumental Operating Conditions for AAS Measurements

Integration time	20 s
Lamp current	6 mA
Slit width	0.7 nm
Wavelength	253.7 nm
Quartz cell dimensions	160 mm x 7.5 mm (i.d.)
Cell temperature	100°C

For the on-line microwave digestion technique reagents were delivered by means of two peristaltic pumps (Minipuls 2, Gilson, Villiers, Le Bel, France) equipped with 1.30 mm i.d. manifold pump tubing (Elkay Laboratory Products (UK) Ltd, Basingstoke, Hampshire). Sample injection was achieved by way of an omnifit rotary injection valve (Anachem Ltd, Luton, Bedfordshire) equipped with a 200 µl PTFE sample loop. Reaction coils were prepared by coiling 0.8 mm i.d. x 1/16" i.d. PTFE tubing (Thames Chromatography, Windsor, Berkshire) and connections made with omnifit low pressure fittings (Anachem Ltd).

Following on-line microwave digestion, cold vapour atomic fluorescence measurements were performed using a 10.023 Merlin atomic fluorescence detector (P. S. Analytical Ltd, Orpington, Kent). The principles of atomic fluorescence spectrometry and cold vapour generation are described in Sections 2.5 and 2.6.2 respectively. The layout of the AFS detector and the operating conditions are given in Figure 6.2 and Table 6.3 respectively. Following cold vapour generation and gas liquid separation in a 'type B' gas liquid separator (P.S. Analytical) the mercury vapour was swept by a stream of argon gas into the central core of the chimney interface. A semi-permeable Nafion membrane dryer tube (Perma Pure Products, Monmouth Airport, Farmingdale, NJ, USA) was placed between the gas liquid separator and the detector to prevent water vapour entering the detector and thus avoid base line drift and losses in sensitivity. A further argon flow sheaths the sample, which effectively constrains the mercury vapour to a laminar flow past the fluorescence detector. A series of lenses focus and collect light from the source, a specific high intensity mercury vapour discharge lamp, which is then collimated over the chimney interface. Fluorescence emission from the sample was filtered with a 254 nm interference filter to achieve wavelength isolation and detected using a side window

photomultiplier tube. Signal output was recorded on a chart recorder (Labdata Instrument Services Ltd., Carshalton, Surrey) and quantification achieved by measuring peak heights.

Figure 6.2: Schematic Diagram of the P.S. Analytical Merlin Atomic Fluorescence Detector

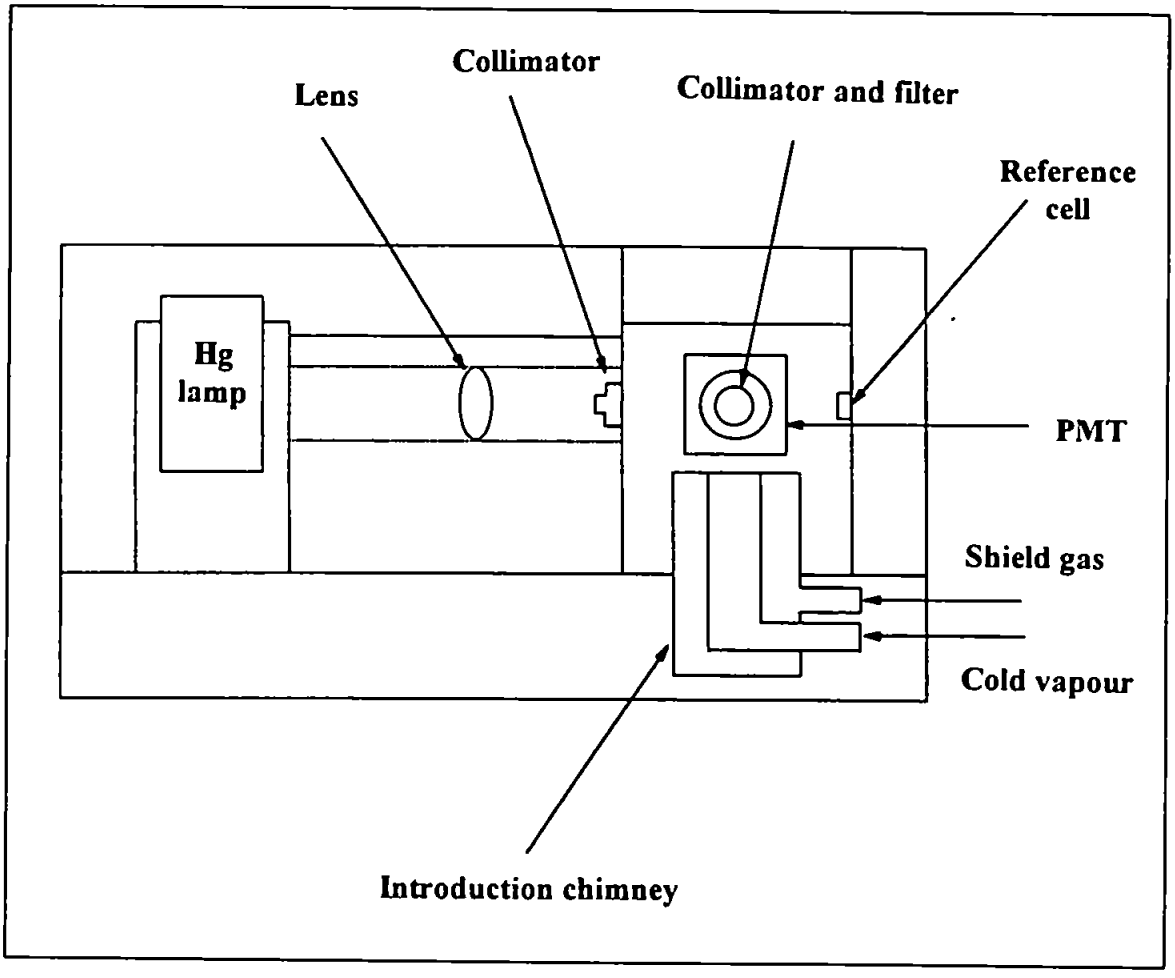


Table 6.3: Operating Conditions for the Merlin AFS Detector

Wavelength	254 nm
Read mode	Running
Mode	Emission
Integration time	0.25 s
Argon sheath gas flow rate	1 l min^{-1}
Argon carrier gas flow rate	250 ml min^{-1}

6.2.2 Reagents

Hg(II) standard solutions were prepared daily from a high purity 1000 mg l^{-1} stock solution of mercury nitrate (Spectrosol, Merck Ltd, Poole, Dorset). Methylmercury standard solutions were prepared by dissolving methylmercury chloride salt (98%, Merck Ltd) in deionised water. Solutions of tin (II) chloride were prepared daily by boiling tin chloride 2-hydrate salt (98%, Aldrich Chemical Company, Dorset) in hydrochloric acid, before diluting to volume with deionised water. Heating was undertaken to aid dissolution and remove traces of mercury from the solution by volatilisation. Hydrochloric acid (Aristar), hydrogen peroxide (Analar), nitric acid (Aristar), sulphuric acid (Aristar), hydroxylammonium chloride (Analar), potassium bromide (Analar) and potassium bromate (Analar) were obtained from Merck Ltd. All solutions were prepared in deionised water (18 MΩcm^{-1}) purified through a Millipore purification system (Millipore-Waters, Bedford, MA, USA).

Glassware was acid washed in 10% (v/v) nitric acid (Analar, Merck Ltd) for at least 24 h and rinsed in deionised water prior to use. For validation of the batch and on-line techniques the certified reference materials DORM-2 (dogfish muscle) and PACS-1 (harbour marine sediment) were obtained from the National Research Council Canada (Ottawa, Canada).

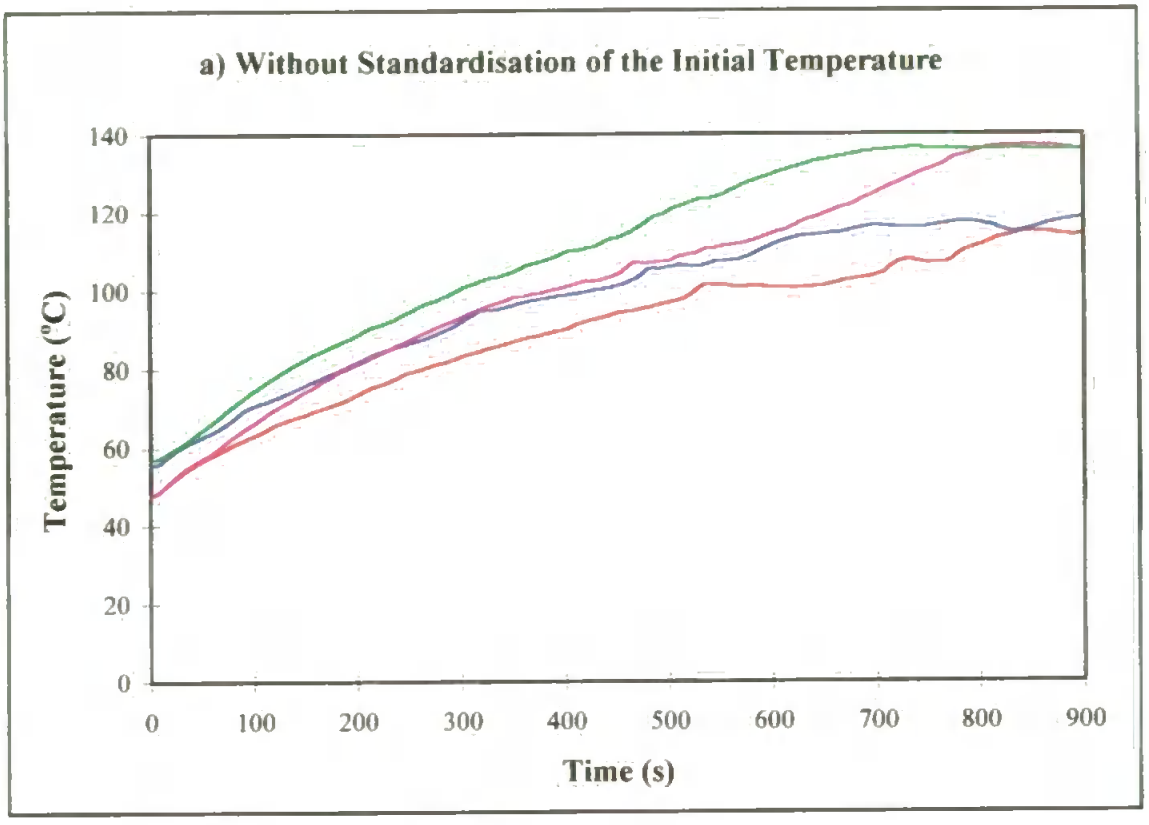
6.3 The Development of a Batch Microwave Digestion Method for the Determination of Mercury in Biological and Sediment Samples

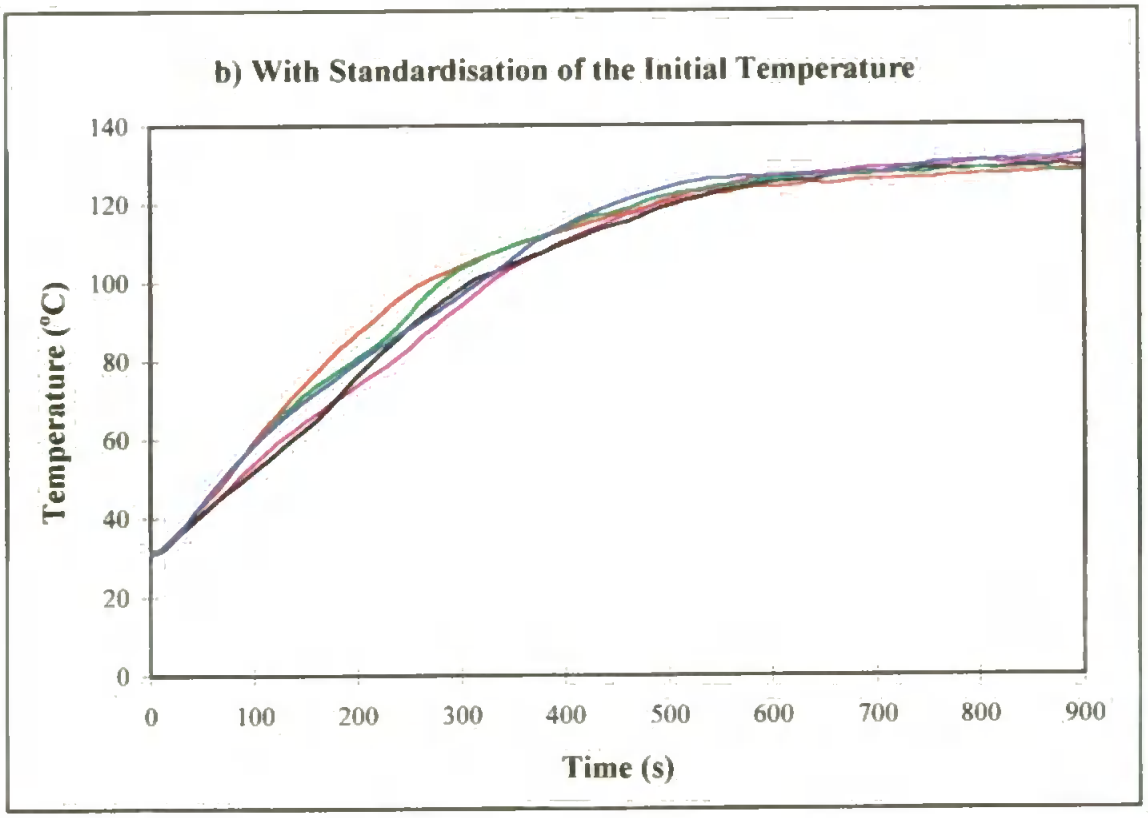
A batch microwave digestion method was developed for the determination of total mercury in sediment and biological samples. The effect of digestion with hydrogen peroxide, nitric and sulphuric acids was investigated and the digestion conditions (i.e. heating time and power setting) optimised to achieve complete oxidation of organomercury compounds in the samples whilst avoiding losses of mercury through volatilisation. This is of particular importance for open digestions, especially in the case of biological samples where strongly oxidising conditions are necessary for the decomposition of organomercury compounds. Throughout the heating and reagent additions care was taken to avoid venting off acid fumes which may have resulted in losses of mercury occurring. After cooling, samples were quantitatively transferred into 50 ml glass volumetric flasks and made up to volume with deionised water. Analysis was performed by FI-CV-AAS with calibration standards of Hg(II) prepared in 2% (v/v) nitric and 2% (v/v) sulphuric acid.

6.3.1 Temperature Measurements

Throughout the course of the digestion, temperature measurements were taken to examine whether the recoveries obtained for each digestion procedure could be correlated, in part, to the temperature of the digestion. To obtain good reproducibility between replicate samples it was critical to ensure that the starting temperature was the same in each case. Figures 6.3a and 6.3b compare the reproducibility between the replicate heating of a mixture of sulphuric acid (6 ml) and nitric acid (5 ml) at 20 W power, with and without standardisation of the initial heating temperature. With a range in the initial temperatures of less than 10°C for 5 samples reproducibility was very poor (Figure 6.3a), leading to a final temperature difference of more than 25°C. This is because the absorption of microwave energy is dependent on the

Figure 6.3: Comparison of Temperature Profiles for the Replicate Microwave Heating of a Mixture of Nitric and Sulphuric Acid





temperature of the solution (see Section 1.3.1.3). Thus in the absence of a common starting temperature the rate of heating of the different samples is not uniform. However if the starting temperature is standardised, in this case at 30°C (Figure 6.3b), reproducibility is far more acceptable. Reproducibility was also dependent on the volume of reagents in the sample flask. Figures 6.4a and 6.4b compare the reproducibility of the replicate heating of 5 ml and 10 ml of water respectively. As can be seen reproducibility was far better with the larger volume of 10 ml. This is because the temperature probe is not completely immersed when the volume of sample is very small.

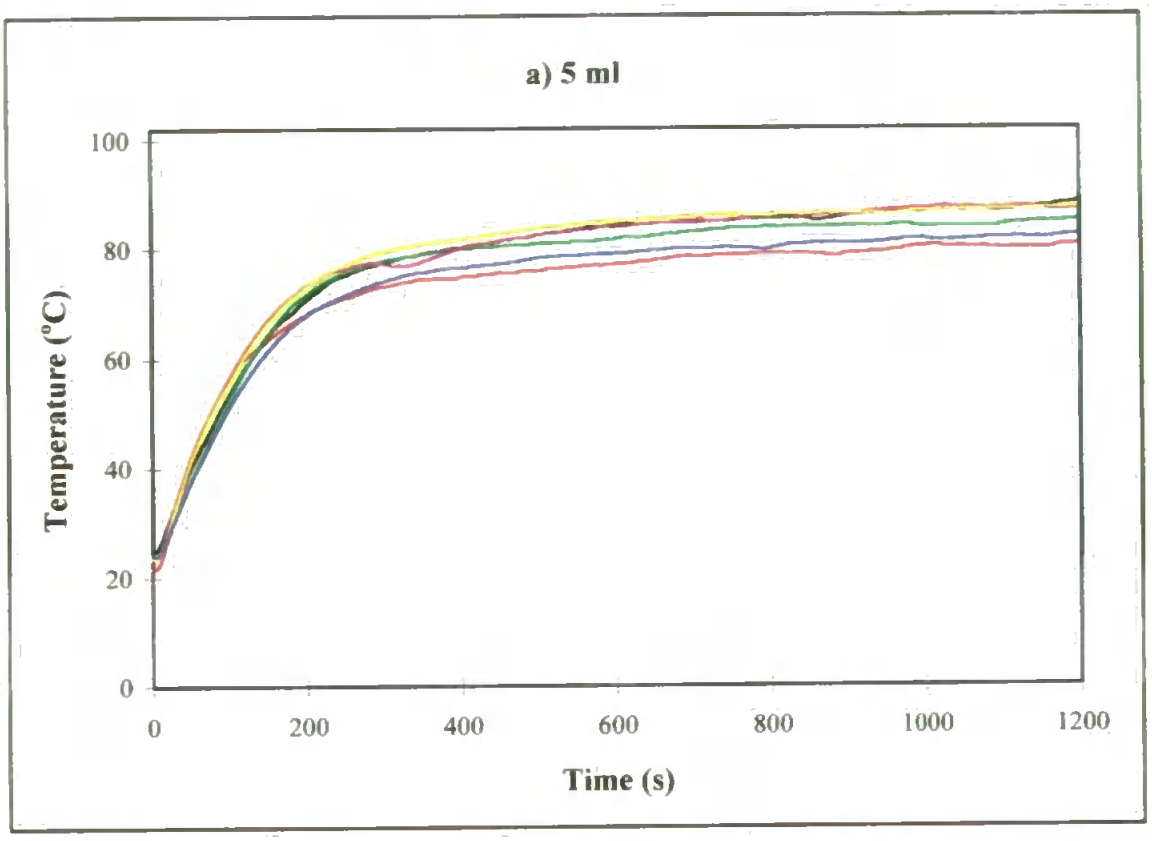
6.3.2 Optimisation of Batch Microwave Digestion Conditions

6.3.2.1 Biological Samples

Optimisation of the microwave digestion conditions for biological samples was undertaken by analysis of the certified reference material NRCC DORM-2. For this purpose nine different microwave digestion procedures were evaluated. Details of each procedure and a comparison of the results obtained (n=1) with the certified values are given in Tables 6.4 and 6.5 respectively.

The temperature profiles obtained for digestion Procedures 1, 2, 3, 5 and 7 and Procedures 7-9 are given in Figures 6.5 and 6.6 respectively. At a power setting of 20 W the temperature initially rises very quickly but decreases towards the end of the first heating step to almost reach a plateau. This effect is also seen when heating nitric acid, sulphuric acid and hydrogen peroxide individually (see Figure 6.7). In each case the temperature fails to reach the boiling point of the solution (121°C, 330°C and 150.2°C respectively) but tends to plateau off at a lower temperature. Presumably the power supplied is just sufficient to maintain the plateau temperature after accounting for heat losses to the surrounding environment. Following the

Figure 6.4: Temperature Profiles for the Replicate Microwave Heating of Different Volumes of Water at 20 W Power



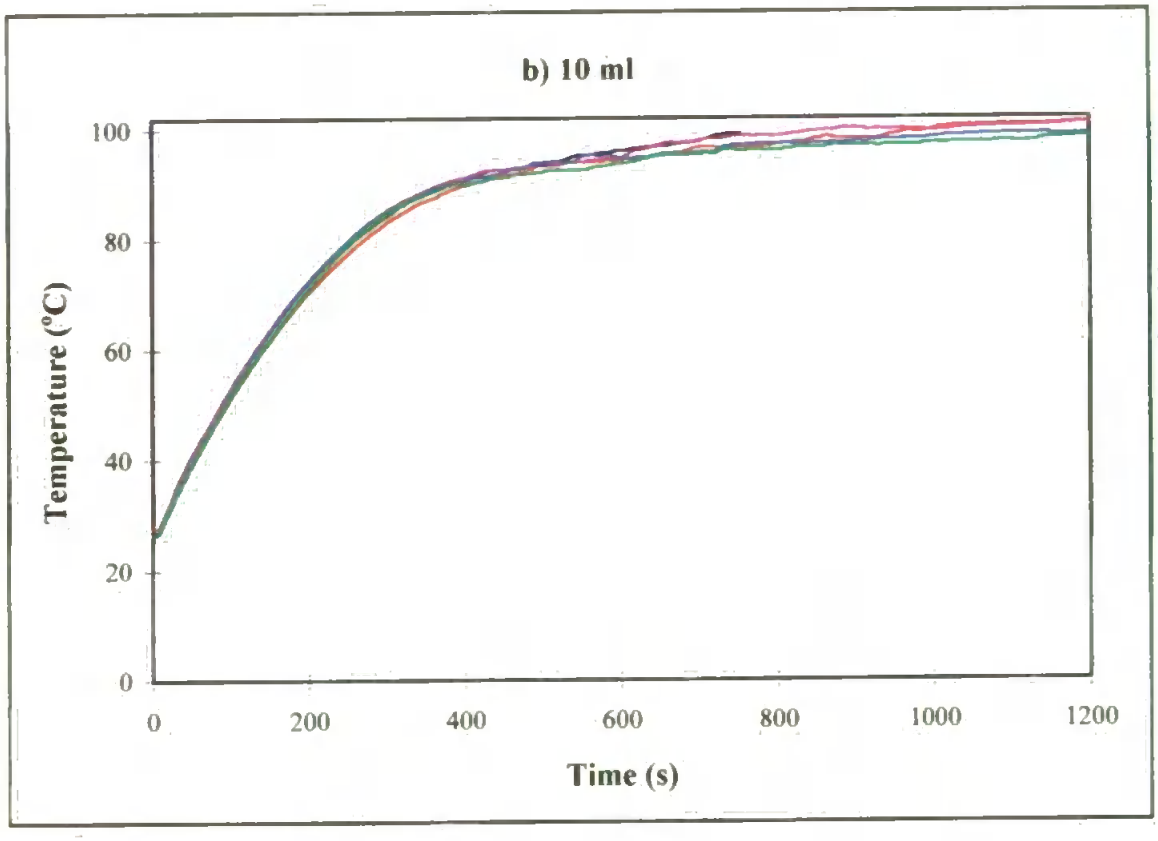


Table 6.4: Details of the Different Microwave Digestion Procedures Evaluated for the Determination of Mercury in Biological Samples

Procedure	Step 1	Step 2	Step 3
1	5 ml HNO_3, P=20 W[1], t[2]=5 min	2 ml H_2O_2, P=20 W, t=5 min	-
2	5 ml HNO_3 + 3 ml H_2SO_4, P=20 W, t=5 min	2 ml H_2O_2, P=20 W, t=5 min	-
3	5 ml HNO_3 + 3 ml H_2SO_4, P=20 W, t=3.5 min	2 ml H_2O_2, P=20 W, t=3 min	-
4	5 ml HNO_3 + 3 ml H_2SO_4, P=20 W, t=7.5 min	2 ml H_2O_2, P=20 W, t=7.5 min	-
5	5 ml HNO_3 + 3 ml H_2SO_4, P=20 W, t=10 min	2 ml H_2O_2, P=20 W, t=10 min	-
6	5 ml HNO_3 + 3 ml H_2SO_4, P=20 W, t=5 min	3 ml H_2SO_4, P=20 W, t=5 min	2 ml H_2O_2, P=20 W, t=5 min
7	5 ml HNO_3 + 6 ml H_2SO_4, P=20 W, t=15 min	2 ml H_2O_2, P=20 W, t=10 min	-
8	5 ml HNO_3 + 6 ml H_2SO_4, P=40 W, t=20 min	2 ml H_2O_2, P=40 W, t=10 min	-
9	5 ml HNO_3 + 6 ml H_2SO_4, P=20 W, t=15 min	Cool, t=10 min	2 ml H_2O_2, P=20 W, t=10 min

Notes:
1: P = power setting (W).
2: t = time (min).

Figure 6.5: Comparison of Temperature Profiles for Microwave Digestion Procedures 1, 2, 3, 5 and 7

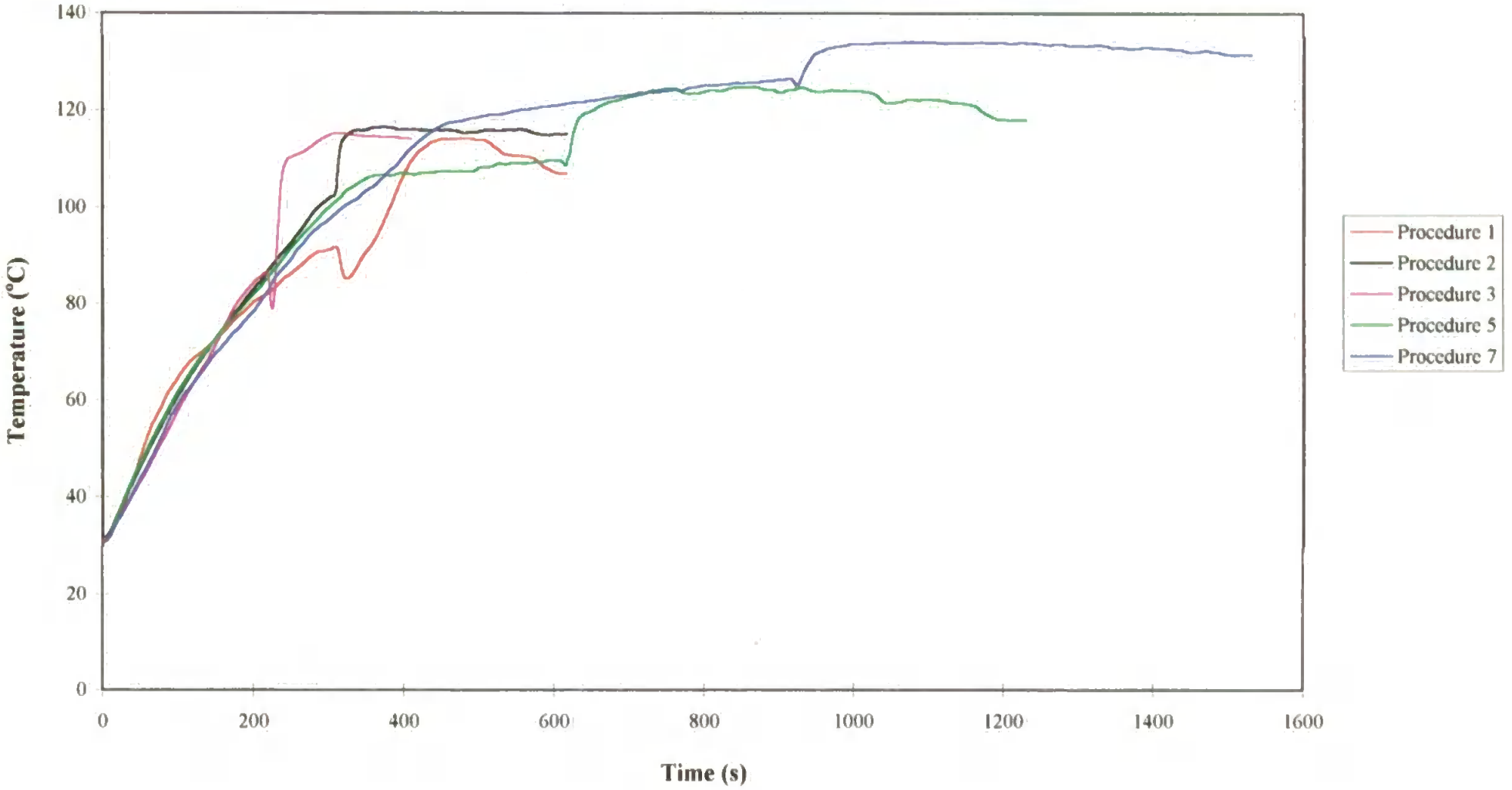


Figure 6.6: Comparison of Temperature Profiles for Microwave Digestion Procedures 7-9

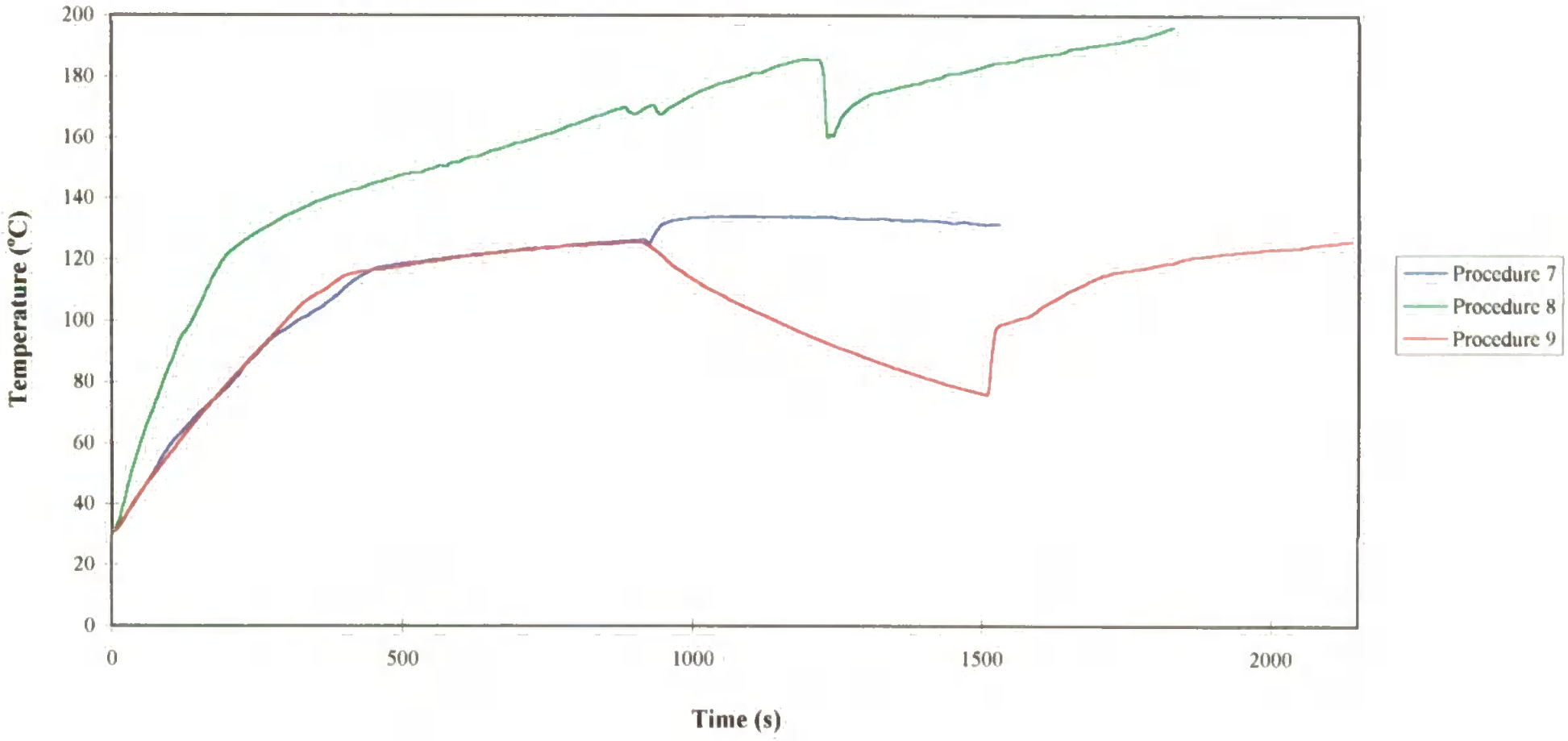
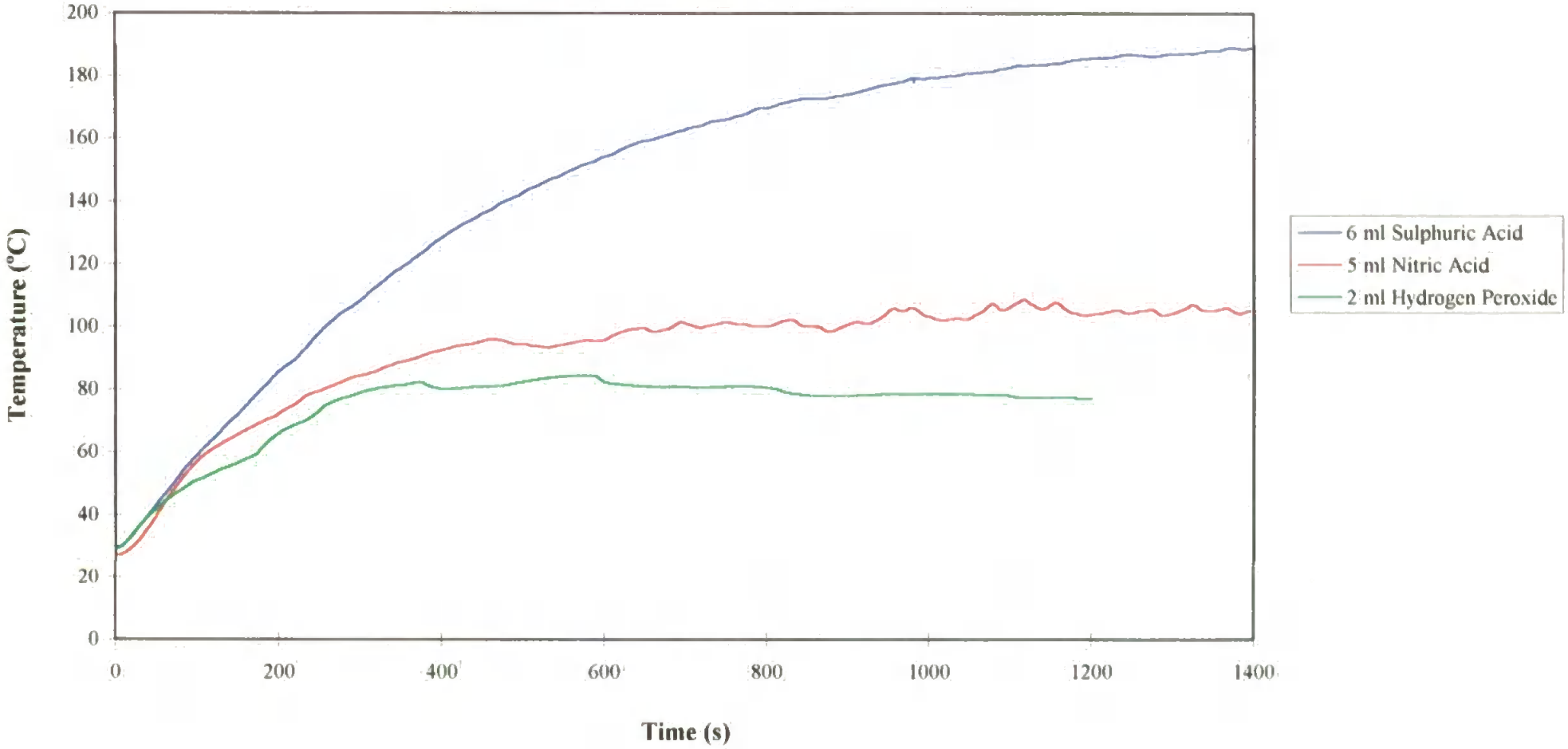


Figure 6.7: Temperature Profiles Obtained for the Microwave Heating of Hydrogen Peroxide, Nitric and Sulphuric Acid at 20 W Power



addition of hydrogen peroxide to the digestion the temperature rises very quickly by approximately 10°C (see Figure 6.5 and 6.6) due to the exothermic nature of the reaction and then almost reaches a plateau again for the duration of the second heating step.

Table 6.5: Results for the Batch Microwave Digestion of NRCC DORM-2

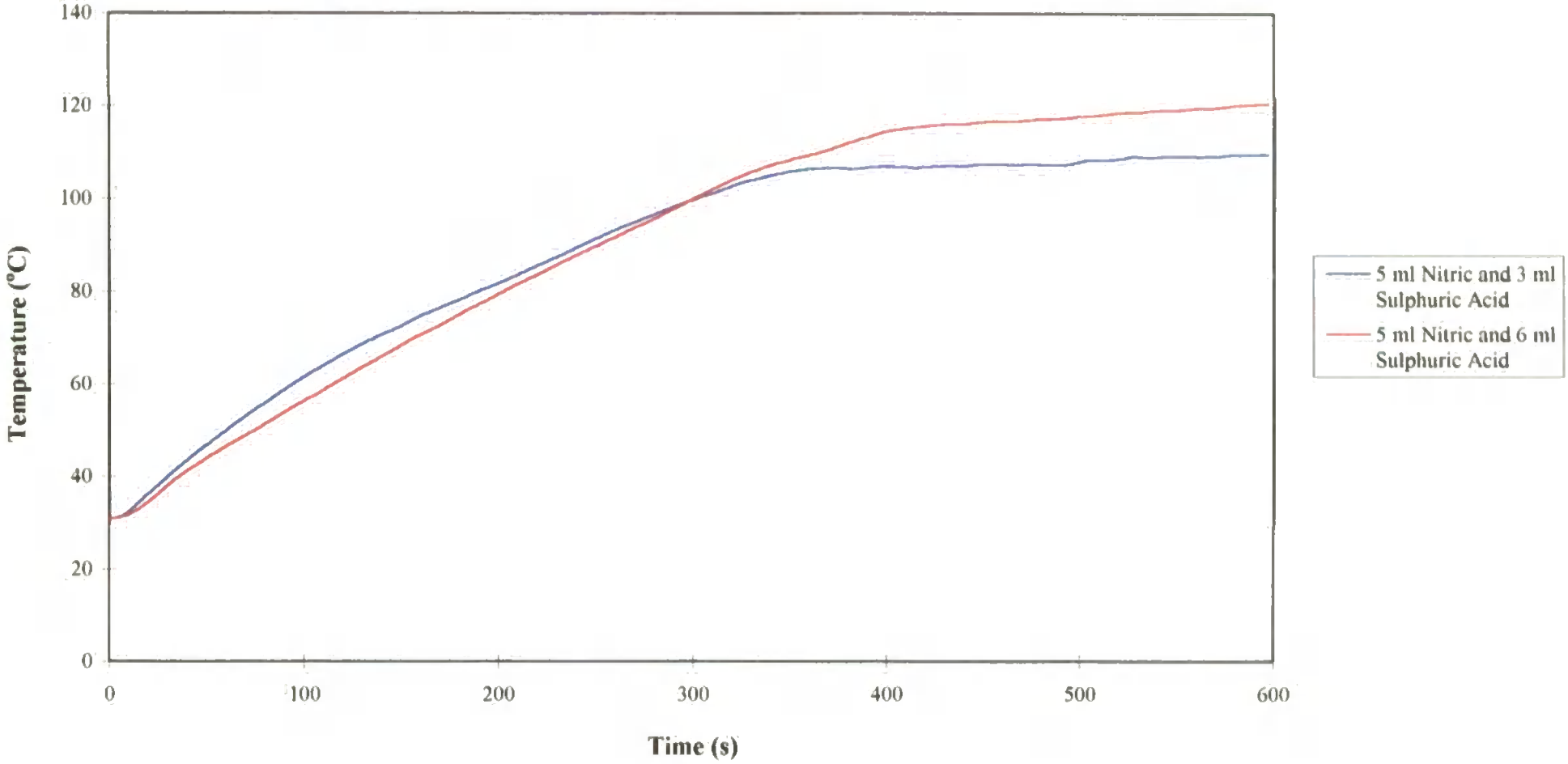
Procedure	Hg ($\mu g\ g^{-1}$)	Recovery (%)
1	1.29	27.8
2	3.20	69.0
3	2.61	56.3
4	3.45	74.4
5	3.41	73.5
6	3.88	83.6
7	4.12	88.8
8	3.87	83.4
9	4.55	98.1
Certified Value	**4.64 ± 0.26**	-

Digestion procedure 1 involved the initial heating of 0.25 g of sample with 5 ml of nitric acid for 5 minutes, followed by the addition of 2 ml of hydrogen peroxide and further heating for 5 minutes. This resulted in a clear solution, indicating complete liberation of the mercury species from the sample matrix, however recoveries for mercury were very low (28%). The cause of the low results can probably therefore be attributed to incomplete oxidation of organomercury compounds to Hg(II). Tinggi *et al.*[319] also obtained low results for mercury in biological tissue following digestion with nitric acid and hydrogen peroxide in a closed vessel.

The strong oxidising properties of sulphuric acid were demonstrated by the results of Procedure 2, in which 3 ml of sulphuric acid was included in the initial heating step with nitric acid. Although the heating times were the same as in Procedure 1 the presence of sulphuric acid significantly improved recoveries to 69%. The temperature of the digestion was higher than in Procedure 1 (see Figure 6.5) and thus the possibility that the low recoveries were due to losses of mercury through volatilisation was investigated by reducing the heating time of the digestion to a total of 6.5 minutes (Procedure 3). This resulted in a reduction in the recovery of mercury to 56% although the maximum temperature of the digestion was less than for Procedure 2. This finding indicated that the low results obtained for Procedure 2 were probably due to incomplete oxidation of organomercury species rather than to losses of mercury through volatilisation. Only a small increase in recoveries (to 74%) was achieved by increasing the total digestion time to 20 minutes (Procedure 5) and thus the effect of increasing the volume of sulphuric acid to 6 ml was investigated.

Figure 6.8 compares the heating profiles obtained for the heating of biological tissue with 5 ml of nitric acid and 3 ml/ 6 ml of sulphuric acid at a power setting of 20 W. As can be seen, the initial heating rate is similar in both cases and the temperature almost reaches a plateau after 6-7 minutes. However with 6 ml of sulphuric acid the temperature after 10 minutes is 11°C higher which is obviously due to a greater proportion of the higher boiling point acid in the mixture. The effect of an increase in the volume of sulphuric acid (Procedure 6) improved recoveries to 84%. Further improvements were also made by increasing the digestion time to a total of 25 minutes (Procedure 7). This resulted in a recovery of 89%. However it should be noted that a substantial amount of acid fumes were evolved during the vigorous reaction on addition of hydrogen peroxide to the digestion vessel.

Figure 6.8: Comparison of Temperature Profiles for the Microwave Heating of Biological Tissue with 5 ml of Nitric Acid and 3 ml/6 ml of Sulphuric Acid at 20 W Power



For Procedure 8 the effect of increasing the power setting to 40 W was investigated, however this reduced recoveries to 83%. From the temperature profile (see Figure 6.6) it is evident that this is probably due to losses of mercury. At 40 W power the initial temperature gradient is far steeper than at 20 W power and the temperature exceeds the maximum obtained in earlier procedures (130°C) after only 4 minutes heating. Also after the addition of hydrogen peroxide the temperature does not reach a plateau as is experienced at 20 W but continues to rise until the cessation of the microwave energy. It is of little doubt that at the final temperature of almost 200°C that mercury is being lost from the sample flask.

Further work continued following the conditions of Procedure 7 (i.e. 20 W power) but with the addition of a 10 minute cooling step before the hydrogen peroxide addition (see Procedure 9). During cooling the temperature fell to approximately 75°C, increased rapidly on the addition of hydrogen peroxide to 90°C and then steadily climbed to reach a final temperature similar to the final 'plateau' temperature of Procedure 7 (125°C). The effect of the cooling step was to significantly control the vigorous reaction on addition of hydrogen peroxide and thus reduced the amount of acid fumes vented off i.e. when losses of mercury are likely to occur. This approach proved successful, resulting in a recovery of 98% for mercury in NRCC DORM-2.

The requirement for digestion with a mixture of hydrogen peroxide, nitric and sulphuric acid to achieve quantitative results for mercury in biological samples is in agreement with the results of Tinggi *et al.*[319] and Schnitzer *et al.*[315] who both reported low results for nitric acid, alone and in combination with sulphuric acid. The latter reported however that a total digestion time of only 10 minutes was required for complete recovery of mercury in IAEA Lyophilised Fish Muscle Tissue following digestion in an open focused microwave system

similar to that used in this work. DORM-2 is certified as containing 92% methylmercury and thus will obviously require strongly oxidising conditions to achieve complete oxidation to Hg(II). The methylmercury content of IAEA Lyophilised Fish Tissue was not however stated and thus valid comparisons between the two procedures are difficult to make.

Figure 6.9a compares the temperature profile obtained for the optimised procedure (Procedure 9) in the presence and absence of biological tissue (n=5 in both cases). As can be seen the temperature was approximately 6°C lower in the presence of the sample. For comparison a sediment sample was also analysed by the same procedure (see Figure 6.9b) and again the same trend was observed. This is opposite to the findings of Kingston and Jassie[325] following the closed microwave digestion of a bovine liver sample with nitric acid, for which the temperature elevation was explained in terms of absorption of microwave energy by polar compounds in the sample. However in this work the reason for the lower results obtained in the presence of the sample is uncertain.

6.3.2.2 Sediment Samples

For the certified reference sediment sample NRCC PACS-1, a considerably shorter digestion time was required to obtain complete recoveries for mercury. Results in agreement with the certified value (see Table 6.6) were achieved following microwave digestion for a total of only 10 minutes at a power setting of 20 W (see Procedure 2). The effect of increasing the heating time to a total of 20 minutes (Procedure 5) neither increased recoveries by improving digestion efficiency or reduced recoveries due to losses of mercury from excessive heating. Presumably long digestion times are unnecessary as sediment samples contain low levels of organomercury compounds (generally less than 1.5% of the total mercury content[326]) and thus the requirement of the digestion is simply to liberate the mercury species from the sample matrix without the need for oxidation to Hg(II).

Figure 6.9a: Comparison of Temperature Profiles for the Optimised Microwave Digestion Procedure in the Presence and Absence of Biological Tissue

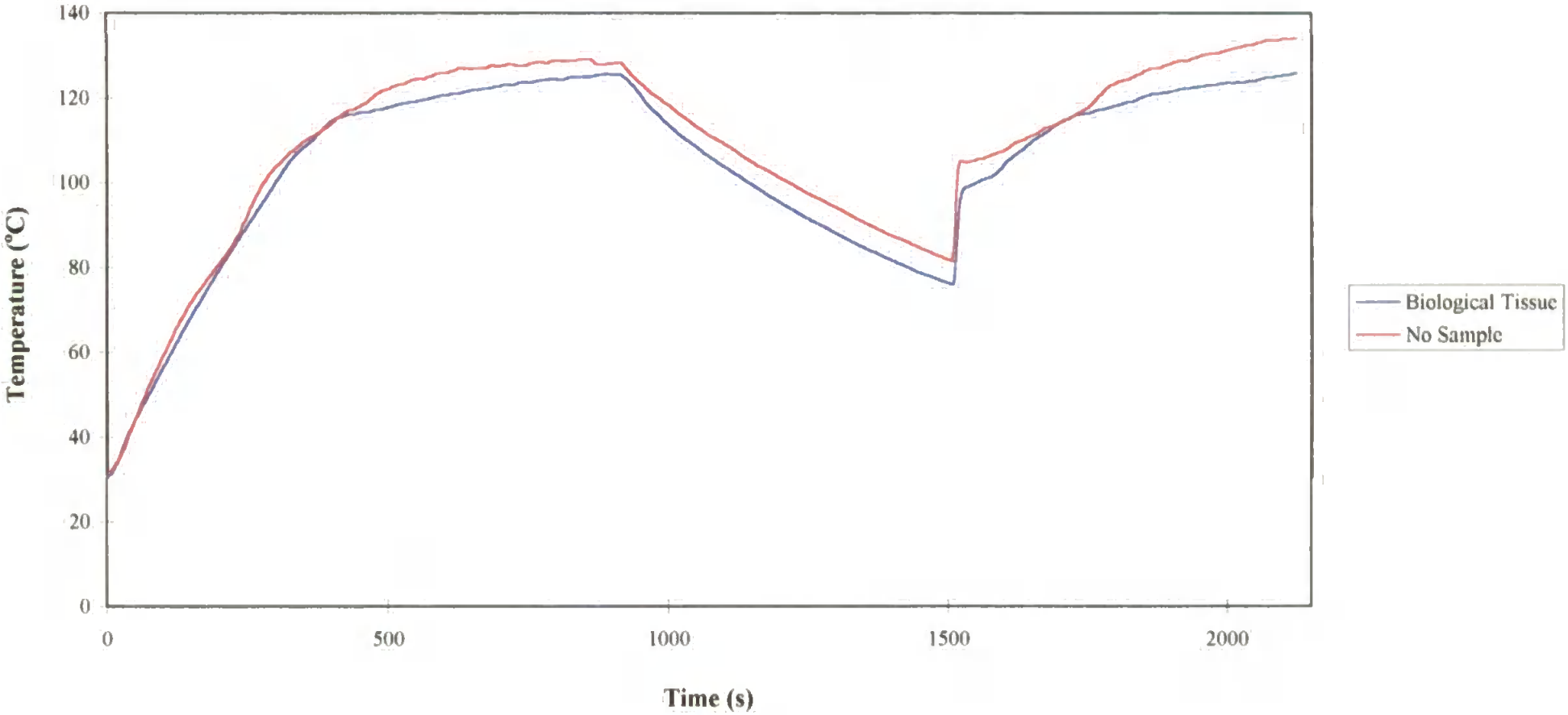


Figure 6.9b: Comparison of Temperature Profiles for the Optimised Microwave Digestion Procedure in the Presence and Absence of Sediment Sample

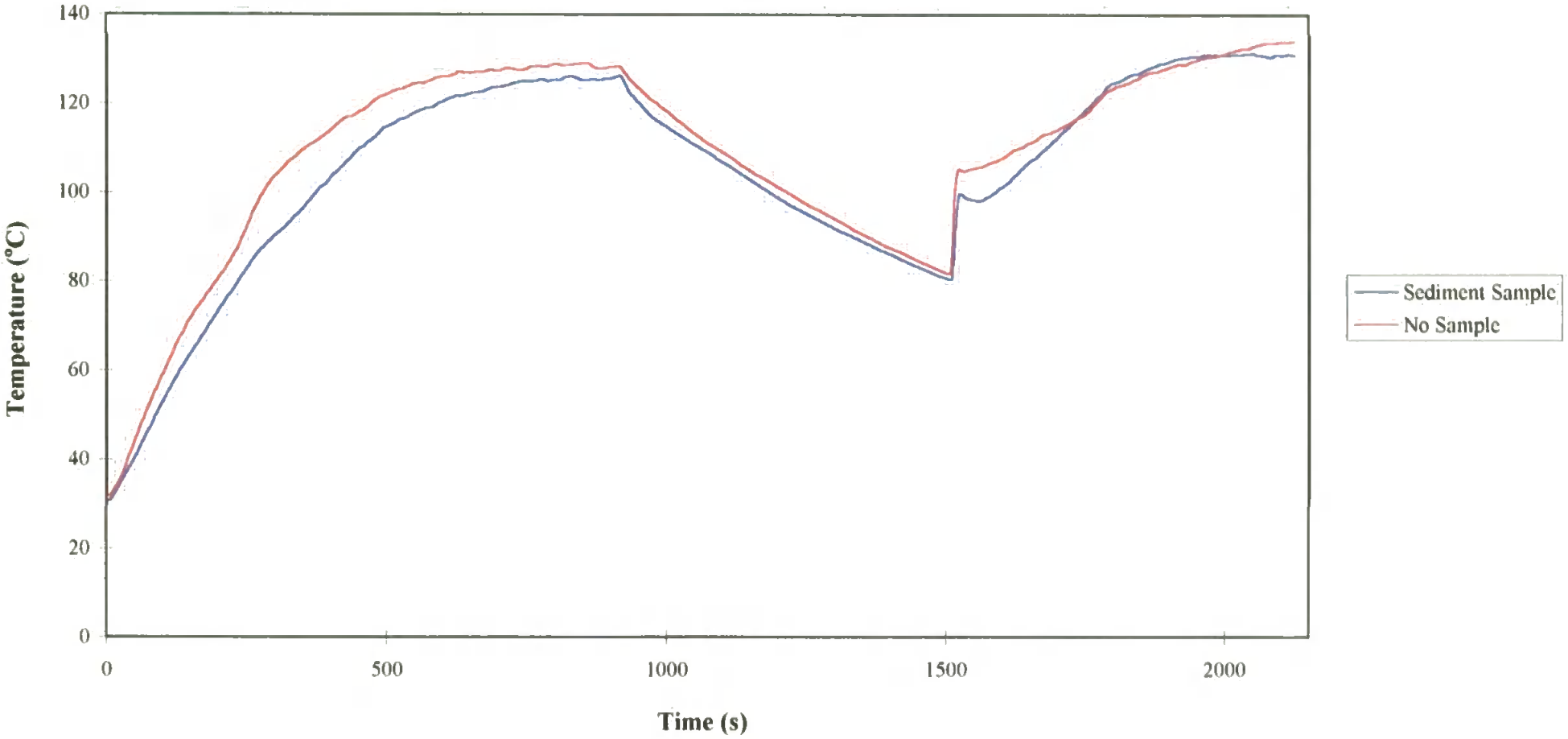


Table 6.6: Results for the Batch Microwave Digestion of NRCC PACS-1 (n=1)

Procedure	Hg ($\mu g\ g^{-1}$)	Recovery (%)
2	4.56	99.8
5	4.56	99.8
Certified Value	4.57± 0.16	-

6.3.3 Details of the Optimised Batch Microwave Digestion Procedures

Full experimental details of the optimised batch microwave digestion procedures for biological and sediment samples are given in Tables 6.7 and 6.8 respectively. For biological samples 0.25 g of sample was digested with 5 ml of nitric acid and 6 ml of sulphuric acid at a power setting of 20 W for 15 minutes. The sample was left to cool for 10 minutes before the drop wise addition of 2 ml of hydrogen peroxide and the continuation of heating for a further 10 minutes, again at a power setting of 20 W. For sediment samples 0.25 g of sample was heated with 5 ml of nitric and 3 ml of sulphuric acid at a power setting of 20 W for 5 minutes. Hydrogen peroxide (2 ml) was carefully added and heating continued at a power setting of 20 W for a further 5 minutes. In both cases, after cooling the sample was quantitatively transferred into a 50 ml volumetric flask and made up to volume with deionised water before analysis by the FI-CV-AAS technique. Sample blanks were routinely prepared and used to correct for potential contamination of the samples.

6.3.4 Results

The certified reference materials PACS-1 and DORM-2 were digested in replicate (n=4) by the optimised batch digestion procedures. The results of the FI-CV-AAS analysis are shown in Table 6.9 and are in good agreement with the certified values for both samples.

Table 6.7: Details of the Optimised Batch Microwave Digestion Procedure for the Determination of Mercury in Biological Samples

Step	Procedure
1	Heat 0.25 g of sample with 5 ml of HNO_3 and 6 ml of H_2SO_4. Power = 20 W Time = 15 min.
2	Allow to cool for 10 min.
3	Add 2 ml of H_2O_2 drop wise and continue heating, Power = 20 W, Time = 10 min.
4	Allow samples to cool. Quantitatively transfer into 50 ml volumetric flasks and make up to volume with deionised water.

Table 6.8: Details of the Optimised Batch Microwave Digestion Procedure for the Determination of Mercury in Sediment Samples

Step	Procedure
1	Heat 0.25 g of sample with 5 ml of HNO_3 and 3 ml of H_2SO_4 Power = 20 W, Time = 5 min.
2	Add 2 ml of H_2O_2 and continue heating, Power = 20 W, Time = 5 min.
3	Allow samples to cool. Quantitatively transfer into 50 ml volumetric flasks and make up to volume with deionised water.

Table 6.9: Results for the Batch Microwave Digestion of NRCC PACS-1 and DORM-2 by the Optimised Procedures

Sample	Certified Value ($\mu g\ g^{-1}$)	Batch Microwave Digestion ($\mu g\ g^{-1}$)
DORM-2	4.64 ± 0.26	4.37 ± 0.24[1]
PACS-1	4.57 ± 0.16	4.62 ± 0.32

Notes:

1: Uncertainty values for the experimental results are calculated as twice the standard deviation.

6.4 The Development of an On-Line Microwave Digestion Technique for the Determination of Mercury in Biological and Sediment Samples

A schematic design of the on-line microwave digestion system is shown in Figure 6.10. Details of the development and optimisation of the various components of the system are discussed in the following sections.

6.4.1 Optimisation of Microwave Digestion Parameters

Initial studies were undertaken to assess the efficiency of the oxidation of organomercury compounds in a standard solution. This was investigated by comparing the response obtained for a 1.25 ng g^{-1} sample of methylmercury (prepared in 1.0 M HCl) with that of the corresponding inorganic mercury standard. Samples were injected into a carrier stream of 1.0 M HCl (1.6 ml min^{-1}) by means of a rotary injection valve equipped with a 200 µl sample loop. After mixing with a solution of 0.5% (m/v) potassium bromide and 0.14% (m/v) potassium bromate[316,317] (1.6 ml min^{-1}) the sample flowed into the CV-AFS detector. A recovery of only 18% was obtained due to incomplete oxidation as a consequence of insufficient time for the reaction to reach completion. In order to improve the kinetics of the reaction and thereby improve recoveries the application of microwave energy to the sample was investigated. This was achieved by passing the sample through a PTFE coil situated in the microwave cavity of the microwave digestion unit.

Figure 6.11 shows the results of the optimisation of the microwave reaction coil length and power settings for the oxidation of a sample of methylmercury. As can be seen optimum conditions were obtained at a power setting of 20 W or greater with a 1 m heated coil followed by a 1 m reaction coil placed outside the microwave cavity (referred to as a post-microwave reaction coil). A recovery of 97% for the methylmercury standard was obtained, indicating that the oxidation of methylmercury to Hg(II) was complete. In the absence of a

Figure 6.10: Schematic Diagram of the On-Line Microwave Digestion System for the Determination of the Total Mercury Content of Biological and Sediment Samples

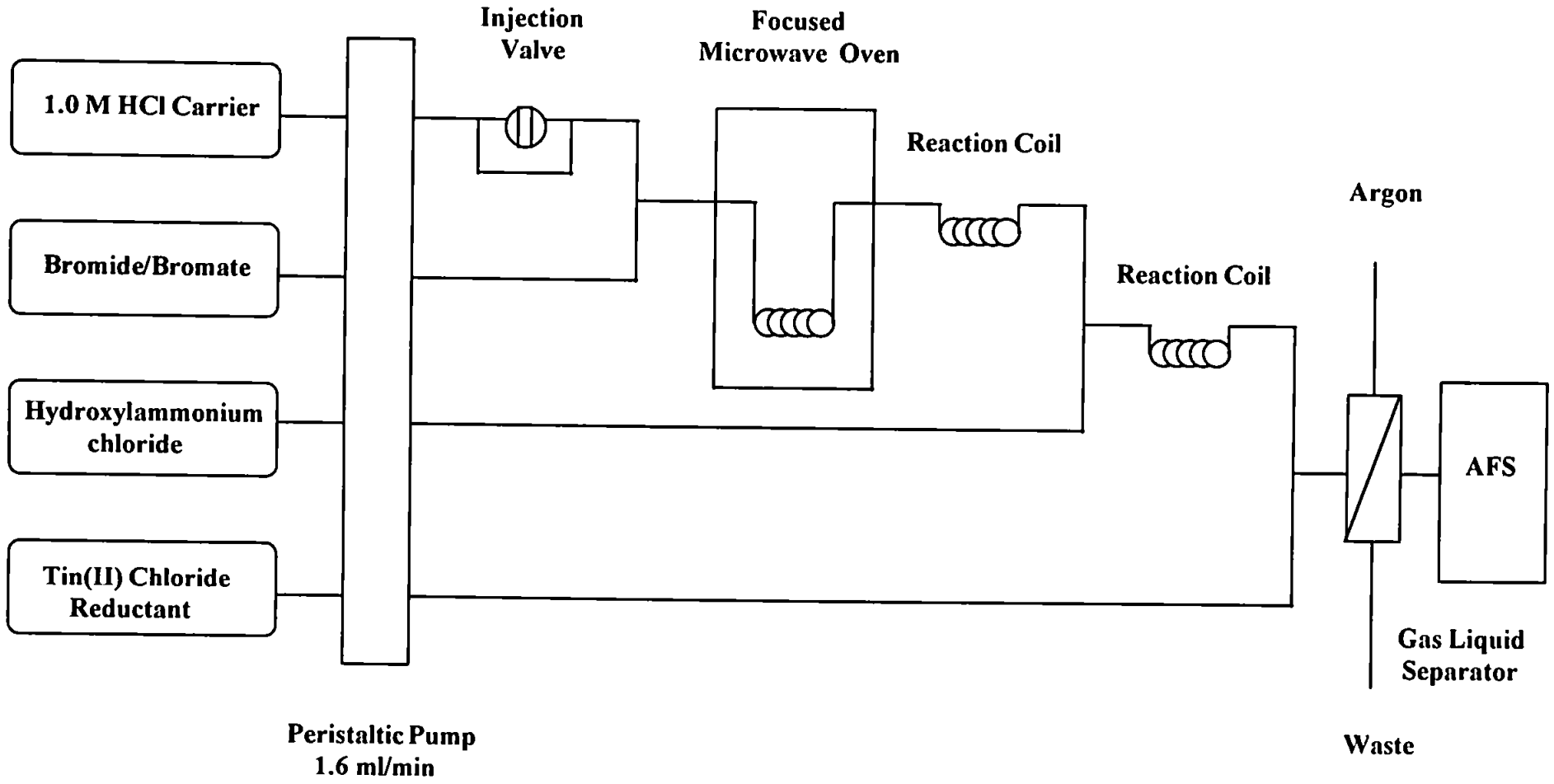
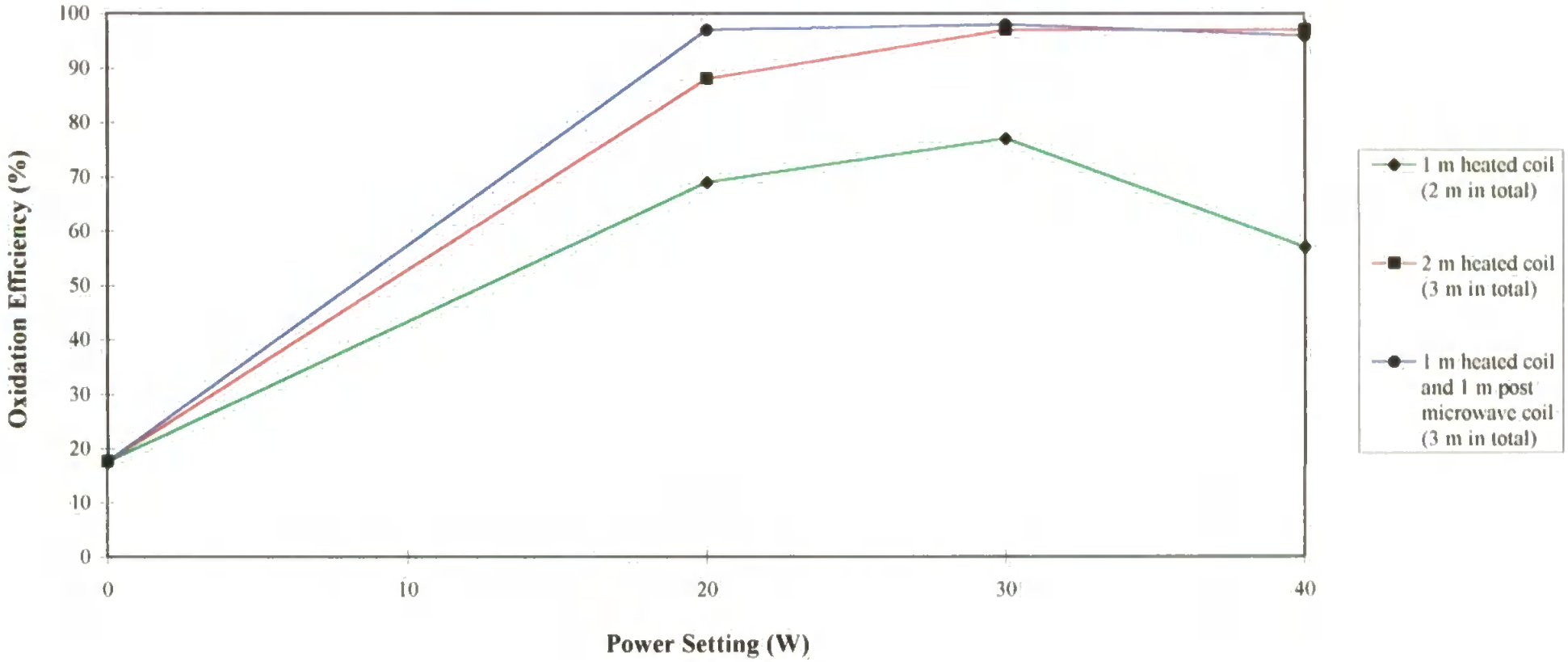


Figure 6.11: Optimisation of the Power Setting and Reaction Coil Length for the Bromide/Bromate Oxidation Reaction

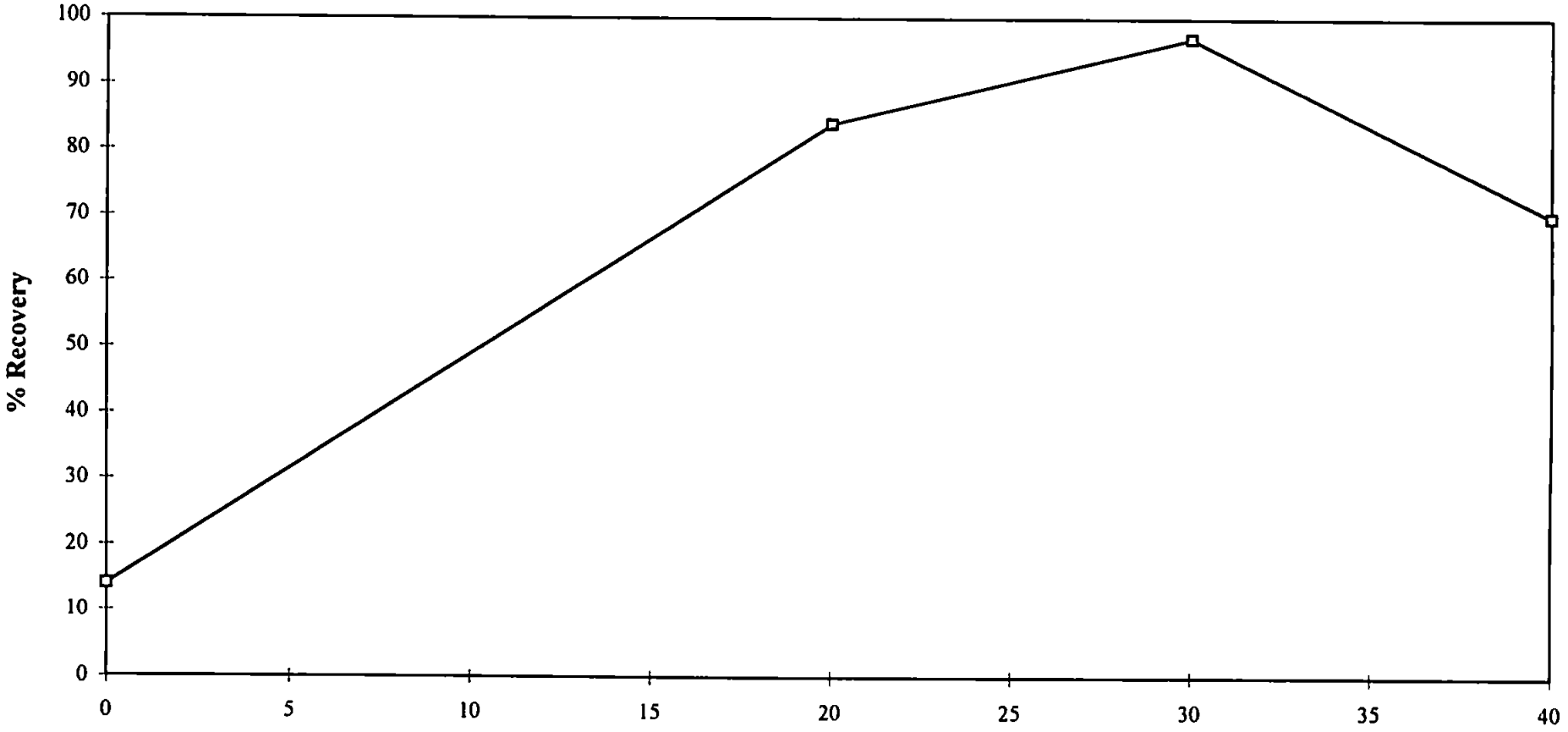


post-microwave reaction coil (1 m heated coil, 2 m total) recoveries were somewhat lower since the reaction did not have time to reach completion even at higher power settings. In fact, above 30 W power the oxidation efficiency was actually impaired due to boiling of the sample, causing instability in the system.

Complete recoveries for methylmercury were also obtained with a 2 m heated coil (no post-microwave reaction coil) but a higher power setting of 30 W was required. With the longer digestion coil the sample tended to overheat within the cavity resulting in a pulsation effect. The sample was displaced from the microwave cavity more quickly than with a shorter coil, thus effectively reducing the digestion efficiency. The optimum conditions i.e. 1 m heated coil followed by a 1 m post microwave reaction coil (3 m in total) maintains the stability of the system and provides sufficient time for the reaction to reach completion.

For the analysis of solid samples, optimisation of the oxidation coil length and power settings was again undertaken. Figure 6.12 shows the results of the optimisation of the power setting with a 1 m oxidation coil and a 1 m post-microwave reaction coil (3 m in total) for the NRCC DORM-2 certified reference material. Complete recoveries (97%) were obtained at a power setting of 30 W which is higher than the 20 W previously needed for the oxidation of the methylmercury standard. It is assumed that a higher power setting is required because the microwave digestion procedure must not only oxidise the organomercury compounds to Hg(II), but before doing so must effectively liberate them from the sample matrix. At power settings above 30 W, recoveries were reduced due to boiling of the sample which causes the sample to be displaced from the microwave cavity before the reaction was completed, as discussed previously. The effect of a microwave heated extraction coil prior to mixing with the bromide/ bromate reagent was also investigated, but with no beneficial result.

Figure 6.12: Optimisation of the Power Setting for the On-Line Microwave Digestion of NRCC DORM-2



6.4.2 Sample Grinding and Slurry Formation for Solid Samples

In order to introduce a homogenous sample into the on-line microwave digestion system a slurry was produced. For this purpose grinding of the samples was necessary. However grinding in a pestle and mortar to produce particles to pass through a <63μm mesh, did not produce particles with a suitable size distribution to maintain a stable slurry. As a result low recoveries (<70%) were experienced on analysis of the samples. Finer particles (<10 μm) however were produced following grinding by the 'bottle and bead' method[327]. A number of different dispersants including Triton-X 100 (Aldrich Chemical Co.), tetrasodiumpyro-phosphate (Analar, Merck Ltd), cetrimide (Aldrich) and Aerosol-OT (Merck Ltd) were evaluated to assess their effect on the grinding procedure, stability of the slurry and behaviour during microwave digestion and analysis. However no benefits were experienced from the presence of a dispersant and so samples were ground in deionised water and slurries prepared in the absence of a dispersant.

Details of the 'bottle and bead' method adopted for the grinding of solid samples are shown in Table 6.10. The procedure involved weighing 0.075 g of sample and 10 g of vitrified zirconia beads (Glen Creston, Stanmore, UK) into a 30 ml nalgene bottle (Merck Ltd). Deionised water (4 ml) was subsequently added before tightly capping the bottles and leaving on a shaker overnight. Sample blanks were also prepared to correct for potential contamination of the samples. Prior to analysis samples were quantitatively transferred to a 100 ml glass beaker, filtering off the beads, and made up to a mass of 50 g. Stable slurries for injection into the on-line microwave digestion system were maintained by continuous stirring with a magnetic stirrer.

Table 6.10: Sample Grinding Procedure by the Bottle and Bead Method

Step	Procedure
1	Weigh 0.075 g of sample and 10 g of zirconia beads into a 30 ml nalgene bottle.
2	Add 4 ml of deionised water and tightly cap the bottle.
3	Leave to shake overnight.
4	Quantitatively transfer into a 100 ml beaker, filtering off the beads and make up to a mass of 50 g with 50% HCl.

6.4.3 Optimisation of the Hydrochloric Acid Concentration for Slurry Preparation

The hydrochloric acid concentration of the sample necessary for complete recovery of mercury in the certified reference material NRCC DORM-2 was also optimised. Investigations were undertaken at the previously optimised conditions (30 W power, 1 m oxidation coil and 1 m post reaction coil). The results in Table 6.11 indicate that a concentration of 50% (v/v) hydrochloric acid was necessary for complete recoveries to be obtained. Standards of Hg(II) were also prepared by mass in 50% (v/v) HCl to ensure complete matrix matching between samples and standards.

The efficiency of the microwave digestion in the absence of the bromide/bromate reagent was also investigated. For a slurried sample of DORM-2 prepared in 50% (v/v) HCl a recovery of only 15% was obtained demonstrating the necessity for the bromide/bromate oxidation reaction in samples containing organomercury compounds.

Table 6.11: Optimisation of the HCl Concentration of the Slurry

HCl Concentration (%)	Recovery of Hg in DORM-2 (%)
10	76
20	77
30	75
40	86
50	97

6.4.4 Removal of Excess Bromine

Prior to analysis by CV-AFS it is necessary to remove the excess bromine evolved in the bromide/bromate oxidation reaction. This is undertaken to prevent quenching of the fluorescence signal which can lead to a loss of sensitivity and irreproducible results. Following microwave heating the excess of bromine was removed by reaction with 6% (m/v) hydroxylammonium chloride (see Reaction 6.3), delivered at a flow rate of 1.6 ml min^{-1}. The effect of a reaction coil was investigated due to the poor reproducibility obtained in it's absence. Optimum results were obtained with a coil of 1 m, giving reproducible results without excessive peak broadening. With longer reaction coils no improvement in signal reproducibility was observed, however the peak height decreased due to increased dispersion of the sample.

The sensitivity of the analysis is dependent on temperature and thus in the absence of a final cooling step the signal response initially increased as the temperature rose in the gas liquid separator. The effect of cooling the sample to a constant temperature in an ice bath however dramatically reduced sensitivity. It was observed though that after operation of the system for 10 minutes that the temperature in the gas liquid separator stabilised and thus the signal

response became constant. Therefore before commencing the analysis the system was allowed to operate for at least 10 minutes to allow thermal equilibrium to be established.

6.4.5 Details of the Optimised Technique

Table 6.12 summarises the optimised parameters for the on-line microwave digestion technique. Slurried samples and aqueous standards were prepared in 50% (v/v) HCl and a 200 µl aliquot injected into a carrier stream of 1.0 M HCl. After mixing with a solution of 0.5% (m/v) potassium bromide/0.14% (m/v) potassium bromate, the sample stream passed through a 1 m PTFE coil situated in the microwave cavity of the microwave system, followed by a 1 m post microwave reaction coil. The total length of tubing between the bromide/bromate and hydroxylammonium chloride additions was 3 m. The microwave was operated at a power setting of 30 W which was sufficient to achieve complete liberation of mercury from the sample matrix and oxidation of organomercury compounds to Hg(II). Following microwave digestion the removal of the excess of bromine was achieved by reaction with 6% (m/v) hydroxylammonium chloride and passage through a 1 m reaction coil. Finally the sample was mixed with a solution of 3% (m/v) tin (II) chloride in 15% (v/v) HCl before entering the gas liquid separator. Here the Hg(0) formed was effectively purged from the solution and swept into the atomic fluorescence spectrometer by a carrier stream of argon gas (250 ml min^{-1}). Signal output was recorded on a chart recorder and quantification was achieved by measuring peak heights.

Table 6.12: Optimised Operating Conditions for the On-Line Microwave Digestion Procedure

Sample matrix	50% (v/v) HCl
Carrier	1.0 M HCl
Oxidant	0.5% (m/v) potassium bromide/ 0.14% (m/v) potassium bromate
Digestion/oxidation coil	1 m heated, 1m post microwave reaction coil (3 m total).
Power setting	30 W
Removal of excess bromine	6% (m/v) hydroxylammonium chloride
Reaction coil length	1 m
Reductant	3% (m/v) tin (II) chloride in 15% (v/v) HCl
Reagent flow rates	1.6 ml min^{-1}

6.4.6 Results and Discussion

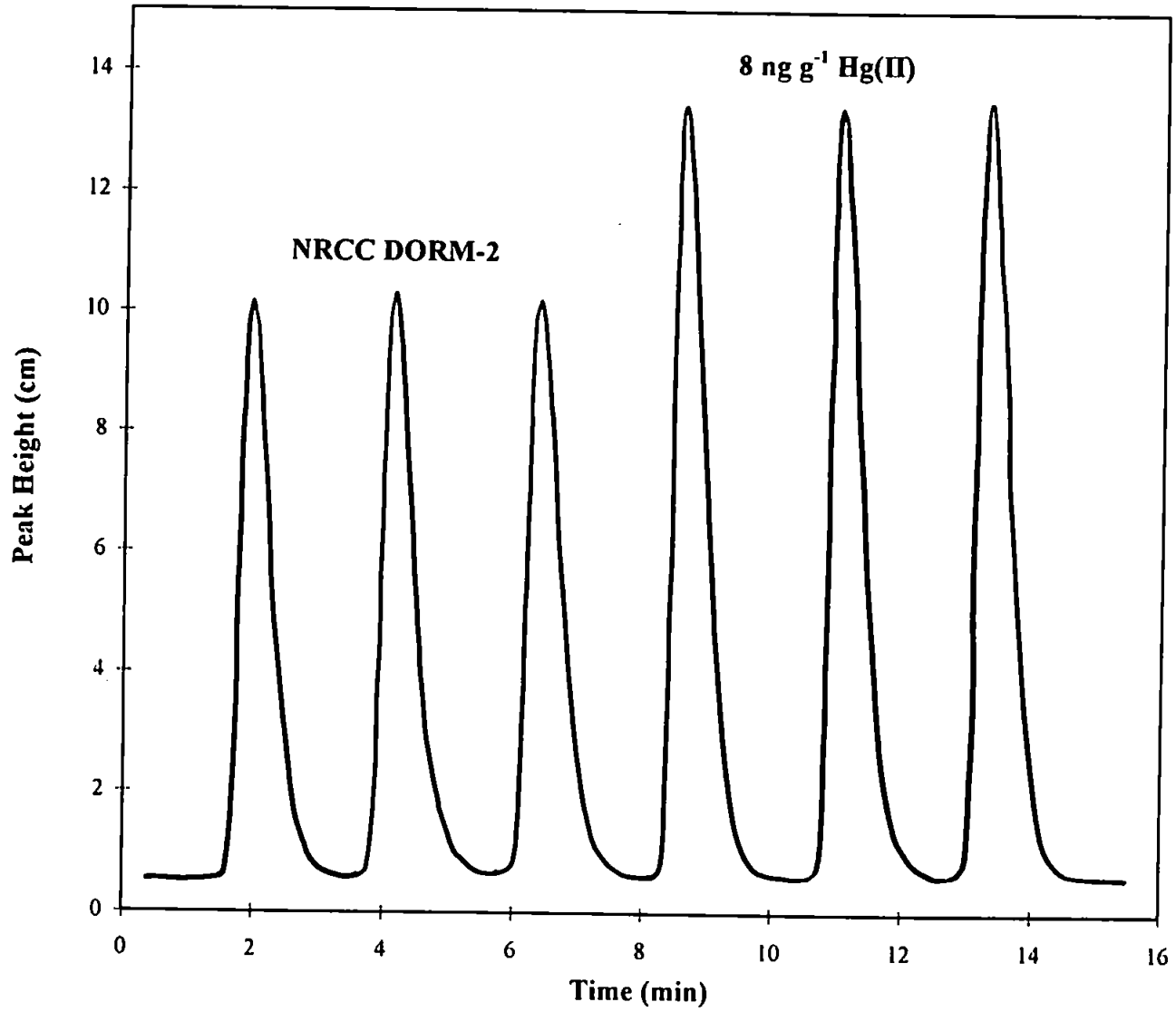
For the on-line microwave digestion technique the limit of detection ($3\sigma_{n-1}$) was determined as 13 ng kg^{-1} (8.7 ng g^{-1} dry wt) and linearity extended to 10 ng g^{-1} (r^2=0.9997). The typical response obtained from the replicate analysis (n=3) of a sample of NRCC DORM-2 and a standard solution of 8 ng g^{-1} Hg(II) is shown in Figure 6.13. As can be seen precision was good with typical relative standard deviations of 1.5% obtained at a concentration of 1 ng g^{-1} (n=3). Validation of the technique was undertaken by analysis of the certified reference materials NRCC DORM-2 and PACS-1. The results (n=4) are shown in Table 6.13 and are in good agreement with the certified values for both samples.

Table 6.13: Results for the On-Line Microwave Digestion of NRCC DORM-2 and PACS-1

Sample	Certified Value (μg g^{-1})	On-Line Microwave Digestion (μg g^{-1})
DORM-2	4.64 ± 0.26	4.46 ± 0.35[1]
PACS-1	4.57 ± 0.16	4.68 ± 0.11

Notes:
1: Uncertainty values for the experimental results are calculated as twice the standard deviation.

Figure 6.13: Replicate Analysis of 8 ng g^{-1} Hg(II) Standard Solution and NRCC DORM-2



6.5 Conclusions

A batch open focused microwave digestion technique was successfully developed for the determination of mercury in solid environmental samples. This demonstrated how by carefully controlling the digestion conditions the quantitative determination of mercury could be achieved following digestion in an open digestion vessel, even with a mixture of oxidant acids and reagents. From these investigations it is apparent that the heating time and power setting are critical parameters to consider. For biological samples the digestion time must be sufficiently long to achieve complete liberation and oxidation of organomercury compounds from the sample but must also avoid reaching temperatures where mercury is lost through volatilisation. Digestion at a low power setting (20 W) therefore proved most suitable for this purpose. At 20 W power the temperature of the digestion tended to plateau and thus heating could continue without further increase in the temperature which could otherwise lead to losses of mercury. For biological samples this enabled digestion times of sufficient length for the complete oxidation of the organomercury compounds present in the sample. Also care must be taken during the addition of hydrogen peroxide to avoid losses of mercury through volatilisation. For this reason the optimum procedure was developed with a cooling step before the addition of hydrogen peroxide. This had the effect of reducing the vigorousness of the reaction and thus minimised losses of mercury occurring.

For sediment samples a shorter digestion time was necessary due to the low levels of organomercury compounds commonly present (generally less than 1.5% of the total mercury[326]). Thus the requirement of the digestion procedure is to simply liberate the mercury species from the sample matrix without the need for their oxidation to Hg(II).

The developed on-line microwave digestion technique successfully illustrated how complete recoveries for mercury could be achieved by the combination of a microwave digestion and a bromide/bromate oxidation reaction. The linearity and good precision obtained for this technique demonstrate the suitability of the system for quantitative analysis. Although grinding of solid samples is a precursor to on-line microwave digestion, sample digestion is then achieved in less than one minute. The on-line analysis approach also reduces potential losses of mercury occurring during the delay period between sample digestion and analysis. This is of particular importance at the levels of mercury generally found in many environmental samples. Safety is also improved by avoiding the use of hazardous oxidising acids such as sulphuric acid and hydrogen peroxide. Finally the risk of losses of mercury through volatilisation are minimised in the fully enclosed system.

The batch and on-line microwave digestion techniques were validated by analysis of the certified reference materials NRCC DORM-2 and PACS-1 and in both cases produced results in good agreement with the certified values. The DORM-2 sample is certified to contain 92% methylmercury and so the results obtained demonstrate the suitability of the methods for the determination of total mercury in samples containing a high proportion of organomercury compounds.

7. Conclusions and Future Work

7 CONCLUSIONS AND FUTURE WORK

The past two decades have seen a remarkable increase in our understanding of the impact of trace elements in environmental and biological systems. This has led to an increase in the need for quantitative analytical techniques to accurately and rapidly determine a wide range of trace elements with sufficient sensitivity for application to a large number of different environmental samples. This requirement has led to major technological advances in the development of analytical instrumentation, however for sample digestion developments have been somewhat slower. Microwave digestion techniques have however, since their emergence in 1975, revolutionised conventional approaches to sample digestion and have rapidly gained widespread acceptance as an effective method of sample preparation. Using this approach not only have digestion times been dramatically decreased but other benefits such as a reduction in contamination, less reagent and sample usage, a reduction in the loss of volatile species and improved safety have been reported.

In Chapter 3 the suitability and simplicity of batch open focused microwave digestion procedures for the digestion of botanical samples was demonstrated. For the trace element analysis of tea leaves the optimised procedure involved digestion with a combination of nitric and perchloric acids. This approach enabled the complete digestion of samples within 35 minutes, a substantial time saving on conventional hotplate digestion techniques (in excess of 2 h). The digestion of a number of different samples from a range of origins highlighted the variability of their trace element content.

The digestion of seaweed samples is still largely undertaken by conventional dissolution procedures and is the subject of considerable debate over the suitability of the different acids employed. To date no microwave digestion procedures have been reported in the literature.

The developed batch microwave digestion technique offers an accurate and rapid method for the determination of a range of trace elements in seaweed samples. Digestion with nitric acid and hydrogen peroxide proved to be most effective and gave good results for As, Ca, Cu, Fe, K, Mg, Mn, Zn in NIES No. 9 Sargasso. However if the determination of iron is required the use of hydrofluoric acid may be necessary in order to decompose the resistant siliceous material in the sample. This possibility could be investigated in future work.

The complete digestion of sediment samples for trace element analysis still remains somewhat of a problem for routine analysis. This is because sediments consist of a complex combination of different materials including clay, organic matter, siliceous and other minerals and are thus one of the most difficult sample matrices to digest, proving very time consuming by conventional techniques. In addition there is a large variability between the composition of different samples making the development of a universal digestion method very difficult. In Section 4.1 a batch open focused microwave digestion technique was developed for the determination of Al, Ca, Cu, Fe, K, Mg, Mn, Ni, Pb, V and Zn in sediment samples. The low results obtained for Cr and Ti (both 77%) in NRCC MESS-1 reflect the problems commonly encountered for the determination of metals associated with the aluminosilicate matrix of sediments, even following microwave digestion with hydrofluoric acid under pressure[126,216,217,225]. In fact the results presented here for Cr and Ti were comparable to those commonly obtained using pressurised systems. In an attempt to decompose resistant Cr minerals it has been suggested that multiple addition-evaporation cycles of perchloric and nitric acid to the residues present following the initial digestion may be beneficial, although this may raise background levels. However in this work, heating for prolonged periods of time or further digestion of the residues did little to increase recoveries and thus it is difficult to see how improvements could be made.

The concentrations of pollutant gases, vapour-phase elements and the trace element content, size and morphology of airborne particles have been used to provide baseline information to identify pollution sources in urban areas[328]. Among them, the most commonly used method is the determination of the trace elemental composition of airborne particles. However their digestion is considered difficult because of the wide variety of their constituents e.g. organic compounds, oxides and silicates. It would thus be of considerable interest to apply the developed microwave digestion technique for sediments to the analysis of atmospheric particulate samples.

To address the well known problems associated with the extraction of organotin species from sediments for speciation studies, a microwave extraction procedure was evaluated (Section 4.2). Extensive investigations were undertaken to examine the cause of the low results obtained by identifying the losses occurring at the different stages of the extraction procedure. From these studies it was concluded that negligible losses occurred during rotary evaporation, redissolution of the sample in the HPLC mobile phase or during microwave heating of the sample. Losses were however identified during the back extraction step into toluene due to inefficient partitioning of the species in the organic phase and as expected was most pronounced for MBT due to its high polarity. The major cause of the low recoveries however can be attributed to inefficient extraction of the species from the sediment matrix during microwave heating. These studies therefore provide a good background for further work which is necessary to examine and fully optimise the microwave digestion parameters in more detail. This should include an assessment of the influence of the power applied, temperature and time of irradiation for each species.

Fundamental studies are also necessary to investigate the behaviour and stability of the different organotin species, during microwave heating. Some organotin species are particularly susceptible to microwave heating as observed in Section 4.2 for the degradation of TPhT. This is an interesting observation which requires further investigation since the decomposition products obtained did not follow the predicted degradation pathway for organotin species (progressive dealkylation to inorganic tin). However using the current chromatographic system the exact products could not be identified and thus further investigations, utilising an alternative chromatographic separation, are required to fully characterise these compounds.

The relative toxicity of the different forms of arsenic is of considerable importance in assessing the toxicity of food samples for human consumption, especially as the predominant arsenic species in fish, arsenobetaine, is considered to be non toxic. For this reason accurate and sensitive methods are needed for the speciation of arsenic in biological samples. In Chapter 5 an on-line HPLC-microwave digestion-HG-AAS technique was successfully developed for this purpose. This consisted of the on-line HPLC separation of AsBet, DMA, MMA and inorganic arsenic prior to the complete oxidation of organoarsenicals to inorganic arsenic and L-cysteine pre-reduction to As(III). In this way sample pre-treatment enabled detection to be performed by HG-AAS, thereby improving sensitivity over the use of FAAS alone. In future work it would be of considerable interest to apply the developed technique to the speciation of arsenic in urine samples. Urine is a particularly difficult matrix to analyse due to it's high organic content and would thus require strongly oxidising conditions to obtain complete decomposition of the organoarsenic compounds present in the sample.

Chapter 6 demonstrated how by carefully controlling the digestion conditions the quantitative determination of mercury in samples containing high levels of organomercury compounds could be achieved following digestion in an open digestion vessel with a mixture of oxidant acids and reagents. Temperature measurements were used to examine the effect of power, heating time and reagent additions during the digestion procedure. Further benefits are likely with the use of the Synthewave microwave digestion system (Prolabo Ltd) which is capable of taking direct temperature measurements throughout the course of the digestion and offers more control over the power applied to the sample (in increments of 2 W rather than 10 W for the Microdigest 301). The Synthewave system also allows digestion programmes to be executed in terms of temperature rather than power, thereby allowing complete control over the temperature throughout the digestion. These attributes are of particular importance, especially for the determination of volatile elements or for use in speciation analysis where the original form of the compound must not be changed during the extraction procedure. The possibilities arising from the use of the Synthewave microwave system are numerous, especially for use in fundamental studies to understand the processes occurring during the microwave digestion procedure.

Chapter 6 also showed that complete recoveries for mercury could be achieved in samples containing high levels of organomercury compounds by the combination of an on-line microwave digestion and a bromide/bromate oxidation reaction. As discussed, this approach offers a number of advantages over the more conventional approach of digestion with a mixture of oxidant acids and reagents. In light of the good results obtained for biological and sediment samples it would be of interest to investigate the application of the technique to the analysis of waste water, blood and urine samples. Blood samples are a particular challenge as a consequence of the low concentrations of mercury commonly present (sub ng g l^{-1} levels)

and to the difficulties experienced during analysis arising from the coagulation of blood in strongly acidic conditions, especially during heating. However with the use of a bromide/bromate oxidation reaction the acid concentration can potentially be kept at a level where this effect does not occur, whilst retaining the strongly oxidising conditions necessary for the oxidation of the organomercury compounds present in the sample.

The importance of speciation studies arise from the variation in toxicity of different species of the same element. Mercury is no exception with the organic forms exhibiting far greater toxicity than the inorganic. As discussed in Chapter 6, an on-line microwave digestion technique was developed for the determination of mercury in biological samples. Although successful, this method was only suitable for the determination of the total mercury content of the sample, and thus a logical progression would be to develop a technique capable of speciation analysis. It has been demonstrated in the literature that the total inorganic and organic forms of mercury can be separated on a column of sulphydryl cotton[329]. Future studies could therefore be undertaken to modify the existing on-line technique to a system capable of on-line microwave extraction followed by separation of the organic and inorganic forms of mercury on a column of sulphydryl cotton or some other suitable material. Following extraction of the species from the sample matrix, passage of the sample through the column would result in retention of the organic species, whereas the inorganic portion would pass straight through for analysis by CV-AFS. Following elution of the organic species with hydrochloric acid, a potassium bromide/bromate oxidation reaction could then be undertaken to oxidise the organomercury species to Hg(II) and thus enable their detection by the CV-AFS technique. The success of this approach would however depend on the stability of the organomercury compounds at the acid concentration necessary for their microwave extraction from the sample matrix. In addition the acid concentration of the extraction media

must be compatible with the optimum pH for the retention of the organomercury species on the sulphydryl cotton column (pH 3), unless adjustment of this parameter is undertaken before passage through the column. Although this approach would not result in true speciation i.e. complete separation and identification of all the mercury species present in the sample, it would allow the determination of the total organic and inorganic species, thereby giving a good indication of the toxicity of the sample.

The utilisation of microwave energy in preconcentration studies to aid the sorption of Cr(III) and Cr(VI) onto a polymeric Detata sorbent has been reported in the literature[330]. It was this idea that sparked a preliminary investigation (although not reported in this thesis) into the effects of microwave heating on the separation and elution characteristics of Cr(III) and Cr(VI) on an activated alumina mini column. The procedure was based on the method of Cox *et al.*[331] and exploited the fact that activated alumina (acidic form) has a high affinity for anionic Cr(VI) species, in contrast to that for Cr(III) (cationic or neutral). Following injection of a mixed standard of Cr(VI) and Cr(III) into the flow injection manifold, Cr(VI) was retained on the column, whereas Cr(III) passed straight through for detection by ICP-AES. Chromium(VI) was determined, after preconcentration, by eluting with 1 M ammonia. The effect of applying microwave energy (20 W power) to the alumina column during separation/elution was to improve peak shape (increase in peak height), however a similar effect was also observed following heating of the column in a water bath at 60°C. Therefore it can be concluded that the improvement to peak shape was purely a thermal effect due to improved reaction kinetics.

For future work it would be of interest to investigate the effect of microwave heating on the chromatographic separation of organic species. A possible separation which has been used in this laboratory by a number of workers is that of dichlorophenoxyacetic acid (2,4-D) and its metabolites (2,4-dichlorophenol, 4-monochlorophenol and phenol). This is undertaken using a reverse phase column such as silica bonded with octadecylsilane (ODS) groups, with a mobile phase of isopropanol in dilute phosphoric acid. By performing the separation on a column small enough to fit in the microwave cavity of the microwave oven (approx. 5 cm) the effect of the application of microwave energy to the separation could be investigated.

As outlined in this thesis there has been a growing trend in recent years toward the development of microwave assisted techniques, particularly for the digestion of environmental samples. However more recently other applications have been reported in the literature such as microwave extraction techniques for speciation studies[252-256] and for other uses not covered in this thesis such as organic synthesis[332], microwave assisted reactions[333] and the drying of samples[318,334]. The increasing need for automation in analytical chemistry has seen the advent of fully automated on-line microwave digestion and analysis techniques. The development of these methods offer much potential for time savings over batch microwave techniques and thus the benefits over conventional techniques are even more impressive. The on-line approach is well suited to the analysis of liquid samples and although the digestion of solid samples is complicated by the method of introducing the sample into the system (commonly in the form of a slurry), good results have been obtained for a number of biological and geological materials. Further developments are likely through both the adaptation of standard batch digestion methods to on-line applications and in the development of new chemistries suited to the on-line approach. It can thus be concluded that microwave assisted techniques

are an extremely promising area with much potential for further development and are thus likely to feature with increasing importance in analytical chemistry in the future.

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PUBLICATIONS

1 Lamble, K and Hill, S.J. The Determination of Trace Metals in Tea Using Microwave Digestion at Atmospheric Pressure and Inductively Coupled Plasma-Atomic Emission Spectrometry. *Analyst*, 1995, **120**, 413.

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PRESENTATIONS

1 *The Determination of Trace Metals in Tea Leaves Using Open Vessel Microwave Digestion and Inductively Coupled Plasma-Atomic Emission Spectrometry.* Poster presented at Research and Development Topics in Analytical Chemistry, University of Hertfordshire, 18-19 July 1994.

2 *The Determination of Trace Metals in Sediments Using Open Focused Microwaves and Inductively Coupled Plasma-Atomic Emission Spectrometry - A Comparison of Procedures.* Poster presented at 7th Biennial National Atomic Spectrometry Symposium, University of Hull, 20-22 July 1994.

3 *The Application of an Open Focused Microwave System for the Digestion of Tea Leaves Prior to Analysis by Inductively Coupled Plasma-Atomic Emission Spectrometry.* Poster presented at 7th Biennial National Atomic Spectrometry Symposium, University of Hull, 20-22 July 1994.

4 *The Utilisation of On-Line Microwave Digestion for the Determination of 'Non-Reducible' Species by Hydride Generation.* Poster presented at 1995 European Winter Conference on Plasma Spectrochemistry, Cambridge, 8-13 January 1995.

5 *Speciation of Arsenic by On-Line-HPLC-Microwave Digestion-Hydride Generation-Inductively Coupled Plasma-Atomic Emission Spectrometry.* Poster presented at 1995 Research and Development Topics in Analytical Chemistry, University of Hull, 10-11 July, 1995.

6 *Development of an On-Line Microwave Digestion-Cold Vapour Atomic Fluorescence Spectrometry Method for the Determination of Mercury in Solid Environmental Samples.* Lecture presented at 8th Biennial National Atomic Spectrometry Symposium, University of East Anglia, 17-19 July 1996.

7 *Determination of Mercury in Environmental Samples by On-Line Microwave Digestion and Cold Vapour Atomic Fluorescence Spectrometry.* Poster presented at Research and Development Topics in Analytical Chemistry, Nottingham, 22-23 July 1996.