ON-LINE SYSTEMS FOR AUTOMATED SAMPLE INTRODUCTION IN ATOMIC SPECTROMETRY

by

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ABSTRACT

Automation has become increasingly common in most areas of human endeavour. In the field of analytical chemistry, particular attention has been paid to on-line systems which offer high sample throughput. Among other attributes, such systems also offer advantages in terms of ease of automation and coupling to a variety of analytical detection systems. This thesis describes a number of automated approaches that have been developed to form either complete systems, or units which may be incorporated into hyphenated techniques.

Two automated speciation systems incorporating a microwave reduction step followed by hydride generation have been developed and evaluated. The first is a conventional system employing atomic absorption detection, whilst the second system employs high performance liquid chromatography to separate the species prior to detection by atomic fluorescence. Selenium has been used to evaluate both systems, the latter offering detection limits of 0.2 and 0.3 ng ml^{-1} for selenium(IV) and selenium(VI) respectively.

Kinetic studies on the reduction process of selenium(VI) to selenium(IV) were carried out. The reduction took 6 minutes at 70° C, which is faster than previously reported in the literature. Thus the use of on-line systems employing a reduction step are supported for this element.

Photolysis was also evaluated as a means of transforming organo-metallic species on-line. The results indicated that photolytic treatment of seleno-methionine may be effective in cleaving the carbon-selenium bond, and provide the basis for an on-line system to pretreat organo-selenium compounds.

On-line pyrolysis followed by pre-concentration on a gold trap was employed to determine mercury. This technique was incorporated into a system employing gas chromatography-pyrolysis-atomic fluorescence and used to determine a range of mercury species. In addition the approach was also used to determine mercury in sediment samples. Good agreement was obtained with two certified reference materials (NIST 1646 and NIST 8406.

An automated on-line pH adjustment system was also designed and developed. This device featured the use of ammonia gas to avoid contamination of samples prior to trace analysis.

Finally, a number of further applications of the above systems are discussed and a number of avenues for future work suggested.

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Chapter 1

Introduction

1.0 INTRODUCTION

Over recent years, many factors have contributed to the need for an ever increasing number of routine chemical tests to be carried out in laboratories of many types. In industry, quality control checks on incoming raw materials, outgoing products and waste effluent all require monitoring if government regulations are to be met and competitive viability is to be maintained. In medicine, as more is discovered about health and disease, and how each is affected by traces of a particular element or compound, more and more sensitive tests need to be carried out. The need for more analyses in government and other regulatory body's laboratories is obvious. Since the sensitivity of the analytical techniques is often pushed to the limits, better instrumentation is required to keep pace with demand. This increase in workload has led to a demand for the development of more automated systems.

Fortunately for the instrument manufacturers, this demand has coincided with enormous advances in the fields of electronics, computing, mechanical engineering and optics. It is in these areas that the instrument makers have invested massive resources which in turn have produced the advanced instruments which are currently appearing. Fifteen years ago, only the more advanced instruments contained, or were operated through, a computer. Now, even the most humble atomic absorption spectrometer has an on-board microcomputer which

monitors the operation of the instrument, and slightly more advanced instruments use microcomputer integrated circuits for data handling as well as controlling the operation of the instrument. Many larger instruments interface directly with the ubiquitous IBM Personal Computer or similar, and are completely operated and controlled by it - in many instances it is now the case of no computer - no instrument.

1.1 Why automate?

Throughout the world, more automated systems are being developed in all areas of human endeavour. In some cases, it could be supposed that these are introduced to avoid humans being subjected to dangerous environments, whilst in others, it could be argued that automation avoids the need for skilled personnel to do a boring repetitive job. In truth, almost all automation is introduced primarily for financial reasons. In the case of say the nuclear power industry, the fact that an automated radio-active fuel rod handling system does avoid the need for a man to enter the hostile environment in which the rods are situated is a beneficial side effect. If no such system were available, very much higher wages would be demanded by the operators who would have to handle the rods, and there would be exceptionally high additional costs in such things as insurance policies, compensation payments and so on.

It is true that automated systems do avoid the requirement of people doing boring and repetitive jobs, but again the prime motive for introducing the automation is financial. Bored people are likely to make mistakes and mistakes cost money. It is also true that for the company concerned, the installation of an automated system may have a high initial capital cost, but once purchased the system should be able to carry out it's function continuously except for periods set aside for maintenance. Unlike people, who like to eat, sleep and take holidays occasionally, machines will work twenty four hours a day, three hundred and sixty five days a year. They do not get ill, do not argue with the management and in many cases offer improved precision over analyses carried out by personnel.

The social impact of mechanisation and automation was predicted to be beneficial to all, providing a release from drudgery and more free time to be enjoyed by the general population. In the field of chemistry, it was suggested that automation would free the chemist from routine evaluations and enable him to carry out more interesting work[1,2]. This may be true in an ideal world, but employers are not primarily concerned with whether or not their employees are doing work which interests them, but whether they are earning profits for the company. An alternative view is that machines replace the more skilled and highly qualified workers, and thus downgrade them to the status of machine minders, whilst the less skilled and less qualified personnel are no longer required at all[3].

Examples of this theory appear to be more abundant than examples of the former, not only in the area of chemistry, but in most other areas of industry. Fortunately, the social impact of mechanical advances are not within the scope of this study - advances in the field of automation are absolutely inevitable and it is the job of governments and not scientists to cope with the effects of these changes.

1.2 What is automation?

It is important to define exactly what is meant by automation and mechanisation when applying these terms in the field of chemistry. One of the main regulatory bodies in chemistry is the International Union of Pure and Applied Chemistry (IUPAC) and it's definitions are that "mechanisation is the use of mechanical devices to replace, refine, extend or supplement human effort", and that "automation is the use of combinations of mechanical devices to replace, refine, extend or supplement human effort and facilities in the performance of a given process, in which at least one major operation is controlled without human intervention, by a feedback system".

The benefits of employing automated and mechanised systems have already been touched on. Apart from the obvious financial and managerial advantages of not having to employ people to carry out routine functions, there are other very considerable benefits in terms of accuracy and precision. Since one of the

most accurately measurable dimensions is that of time, timed processes may be controlled to whatever level of accuracy may reasonably be required. Most currently available computers have clock speeds in the range of 25 - 120 MHz, so direct timing from these clocks enables timing to be carried out to sub-microsecond accuracy. Vastly more accurate and faster clocks are available to scientists if these are required, although except for one or two notable exceptions such as in the areas of photochemistry and radiochemistry, present timing from computer clocks is generally accurate enough, and certainly many orders of magnitude better than a person with a stopwatch.

The advent of stepper motors and other electromechanical devices have also enabled mechanised and automated systems to deliver more accurately and with greater repeatability, measured quantities of chemicals and solutions to reaction vessels, hospital patients, etc.

Such has been the pace of advance in these areas, that chemists are becoming totally reliant upon the instrument, and there is a great danger that without a detailed understanding of the processes involved in a particular determination or reaction, errors of great magnitude may be made. So called "smart systems" are being incorporated into various manufacturers software in an attempt to minimise such errors, but it remains vital that personnel with sufficient understanding of what the instrument is actually doing and

what it's limitations are, should have overall control. The oft quoted example of the near nuclear war starting due to the American radar system detecting the rising moon and thinking it was a massive attack force, should be recalled by all who depend on the results of instruments.

1.3 From sampling site to results

In most cases there is a set path followed by a sample from it's point of collection, through to the final results required by the end user. The automation of these various stages differs in complexity and viability, and in general, it has been in the handling of samples within the laboratory which has received most attention from instrument manufacturers. A typical routine following , for example, a water sample from a river, for pollutant analysis may proceed as shown below.

Collection of sample from river - initial on-site treatment of sample to stabilize it - return of sample to laboratory - splitting of sample for organic and inorganic analysis - sample pre-treatment - analysis of sample by instrument(s).

Clearly, the first three stages are difficult to automate, and only when the sample reaches the laboratory, can any automation commence. At the point of entry, the sample may be bar-coded, so that it's progression through subsequent

processes may be monitored automatically. Splitting of the sample may be carried out automatically, as may transport throughout the laboratory itself. Sample pre-treatment will vary, depending on what information is required. It is in this area, and the final presentation to the measuring system in which most automation will be possible.

Remote on-site and on-line monitoring is now being exploited more often than previously possible, again thanks mainly to advances in electronics. The power requirements of on-site instrumentation have been reduced with the advent of complementary metal oxide silicon (CMOS) integrated circuits which exhibit very low power consumption, and this, combined with improvements in battery performance and capacity, have enabled developments in on-line instrumentation to proceed rapidly. This type of monitoring is presently restricted however, to a limited number of parameters of interest - the possibility of a remotely deployed inductively coupled plasma-mass spectrometer is still some way off. The results from the remote measuring system are either kept in the memory of an on-board computer for subsequent downloading to a portable computer, or are transmitted via a radio link to the laboratory. Thus whilst there are numerous applications for which remote sensing and monitoring may be the answer, it is still true that most determinations are, and will continue to be for some time, carried out in the laboratory.

1.4 Batch versus continuous flow

Once the sample has been received, the analyst has to decide how best to proceed with the analysis. When small numbers of samples are being processed for a limited number of elements, batch processing is the main method employed. The chief advantage of the technique is that it allows the sample to remain as a discrete entity throughout the analytical process, and so cross contamination is avoided. Small sample sizes are also more easily handled by the batch method. Automated batch analyzers are capable of high throughput rates, but automation is more difficult, requiring robotic auto-samplers and other mechanical systems to transport the sample from container to instrument.

The idea of continuous flow systems was first introduced by Technicon with their Autoanalyser - a device in which sample solutions and blanks are separated from one another by air bubbles, whilst they travelled through glass capillary tubing[4]. Reagents are added on-line, and detection is also carried out on-line, as the now coloured sample passes a detector. Such systems are now routinely employed in many laboratories which handle large numbers of samples which have a similar matrix, and which require the determination of the same elements or compounds. Continuous flow systems are relatively simple to automate. They do however suffer from the fact that cross contamination can occur, although by attention to design and operating procedures, this may usually

be kept to acceptable levels. The output from the detector system usually takes the form of sharp peaks, quantitative determinations being based on peak height measurements.

Hybridised instruments which employ both batch and on-line principles are now appearing, and offer advantages over single method systems.

An alternative to the air segmented system described above is flow injection analysis (FIA). In this technique, a very precise volume of analyte is injected into a moving stream which is unsegmented by air bubbles. This technique was first employed in electrochemical determinations[5], in which analyte samples were magnetically stirred following introduction into a moving stream. The method was later modified by two groups working independently of one another[6,7], who realised that mixing of sample and reagent could be accomplished by flow induced dispersion, and that this avoided the excessive dilution required when using the magnetically stirred system.

Whilst it was initially thought that air segmentation was necessary to prevent dispersion, promote mixing and clean the walls of the carrying tubing, this was found not to be the case[8]. Advantages obtained from not having segmentation include higher throughput, quicker response times, shorter start up and close down periods, and generally more simple and flexible equipment. There is now a considerable number of publications dealing with the technique including many

reviews[9,10].

1.5 Outline of thesis

The work described in this thesis falls into four distinct sections, related by the common threads of automation and on-line analysis.

Much work was carried out on hydride generation systems with particular emphasis on the study of selenium speciation. Whilst existing techniques could be operated in a partially on-line way, a fully on-line system has not been described. Since pre-reduction of selenium(VI) is required when employing hydride generation, the development of a microwave induced reduction system has enabled a number of hyphenated techniques to be produced and evaluated. These have proved to be both useful and reliable for the determination and speciation of selenium.

Since the determination of trace organo-selenium compounds by atomic spectroscopy relies upon the destruction of the carbon-selenium bonds and consequential analysis by hydride generation, work was carried out to examine the possibility of employing on-line photolysis to achieve bond cleavage. This work is fully described in Chapter 3.

Considerable attention is currently being paid to mercury in the environment, both in it's bioavailability and it's biogenic fate. Chapter 4 contains details of work employing pyrolysis and pre-concentration techniques for the determination of this element. Such methods are of interest since mercury is a difficult element to determine, due to the extreme problems of losses of analyte and also contamination. Thus any system which reduces sample preparation whilst still maintaining good sensitivity is to be considered.

The development of an on-line pH adjusting system is described in Chapter 5. This system was designed to provide automated pH adjustment of acidified aqueous samples, prior to trace analysis. As such, minimal contamination was a prime requirement, and was achieved by using gaseous ammonia as the adjusting reagent, thus avoiding the introduction of contaminants to the sample from solid or liquid reagents.

Chapter 2

Hydride generation, instrumentation and reagents

2.1 Hydride generation

In 1969, Holak[11] reported a technique for the determination of arsenic by the generation of arsine, and it's subsequent detection using an atomic absorption spectrometer. Holak employed metallic zinc and hydrochloric acid to generate the hydride, which was then cryogenically trapped before introduction into the spectrometer. Other reduction systems were also investigated including aluminium - hydrochloric acid[12], and magnesium, zinc and titanium(III) chloride - hydrochloric acid[13,14]. The reaction is shown below in Equation 1.

Equation 1

$$Zn + 2\,HCl \rightarrow 2H^{\circ} + ZnCl_2$$

$$(n+2)\,H^{\circ} + E^{m+} \rightarrow EH_n + H_2 \text{ (excess)}$$

(m and n may be equal)

All of the metal - acid reactions suffered from being slow, and this made it necessary to trap the hydrides produced, before subsequent analysis. A number of different trapping regimes have been evaluated including rubber balloons[15,16], plastic bags, pressurised containers[17,18], and bubbling the hydride into either silver nitrate solution[19], or silver diethyldithiocarbamate- ephedrine[20]. However, these trapping techniques all suffered from one disadvantage or another - for example the acid vapour degraded the rubber from which the balloons were made, etc. Whilst cryogenic trapping was one of

the more trouble free techniques available, it also had the advantage that it separated the hydride from the excess hydrogen generated, so presenting a greater concentration of the hydride to the detector.

After the introduction of sodium borohydride as the reducing medium, direct transfer became the method of choice, since the reduction using this reagent is very much faster than with zinc-hydrochloric acid. It also has the advantages that it generates hydrides from all the hydride forming elements, these being antimony, arsenic, bismuth, germanium, lead, selenium, tin and tellurium. It also has a superior reduction yield and suffers less contamination of blanks. The reaction is shown in Equation 2:

Equation 2

$$BH_4^- + H^+ + 3\,H_2O \rightarrow H_3BO_3 + 8H^\circ$$

$$(n + 2)\,H^\circ + E^{m+} \rightarrow EH_n + H_2 \text{ (excess)}$$

(m and n may be equal)

Since the use of sodium borohydride obviates the need for trapping, it has enabled automated hydride generators to be constructed, using peristaltic pumps[21] or pressurized reagent pumping systems[22]. Systems employing peristaltic pumps may be more easily automated, since less operator interference is required, and are operated in flow injection or continuous flow modes. The pressurized reagent pumping systems allow greater control over reaction conditions[23], and are mainly used

in the batch mode.

2.1.1 Interferences and limitations

Of the two forms of interference affecting determinations using hydride generation, chemical interferences are more problematic than spectral interferences. Since the analyte of interest is detached from it's matrix, spectral interferences are rarely encountered.

Chemical interferences do present many problems however, and great care must be employed to obviate their effects. An unexpected chemical interference in which traces of nitric acid present in the matrix produced an enhancement in the signal level during selenium determinations is reported in Chapter 3.2. A large volume of literature has reported on the effects of different interferences, but there are often conflicting views on the severity of these effects, due in part to the processes involved in the hydride generation conditions employed by individual workers. A number of classical studies[24-29] are however frequently referred to and these provide much information on interferences. A new work[30] contains an overview of most of the published work on the subject of hydride generation, and clearly illustrates many apparently conflicting results obtained by workers in this field.

The causes of chemical interferences are thought to be twofold. The first is the preferential reduction of the interferent ion either to a different oxidation state or to the free metal. This can give rise to slow reaction kinetics for the species of interest, co-precipitation, adsorption of the hydride or it's catalytic decomposition. Interferences due to the transition group metals fall into this category. It has been suggested[27-29] that these interferences could be reduced by the use of a low concentration of sodium borohydride combined with a high concentration of hydrochloric acid. It is postulated that the high acid concentration would keep the interfering element in solution, and it would be reduced less, thus allowing the hydride forming reaction to proceed more favourably.

The second cause of chemical interference is due to the formation of compounds in the relatively cool argon-hydrogen flame, in the cases of atomic absorption and atomic fluorescence detection. This mode of interference explains the mutual interference most hydrides have on one another[24].

A number of precautions to avoid the problems caused by interferences have been suggested by different workers, and these may be summarised as the use of standard addition, varying the concentration ratios of the sodium borohydride and hydrochloric acid, the use of masking agents, the use of releasing elements such as iron, mercury etc. and pre-

treatment to separate the analyte from the interfering compounds.

2.1.2 Speciation studies employing differential reduction

As more has been discovered about the various chemical processes which occur in nature, it has become increasingly recognised that the form in which the element is present plays a vital role. This has important implications in many areas of interest. The toxicity, bio-availability, bio-accumulation and transportation of the element through the ecological system critically depends upon the physico-chemical form in which the element is presented[31].

Some of the hydride forming elements exist in more than one oxidation state, these being As(III) and As(V), Pb(II) and Pb(IV), Sb(III) and Sb(V), Se(IV) and Se(VI) and Te(IV) and Te(VI). Even though sodium borohydride provides a much faster reduction than zinc-acid, there are still noticeable differences in the rate of hydride formation between the different species. Indeed, the oxidation state of the analyte plays a crucial role in the formation of the hydride[32]. In the cases of selenium and tellurium for example, it is only possible to derive the hydride from the lower oxidation state, and pre-reduction must be employed if the total element content is to be determined. This apparent disadvantage may be used to advantage in the determination of individual

species, by first analysing for the more reduced moiety, and then carrying out a reduction step and re-analysing for total concentration. The concentration of the more highly oxidised form is obtained by difference.

In the case of arsenic, there is a two stage process in the conversion of arsenic(V) to arsine[33], and this has a redox potential which is dependant upon pH. At a pH of 4.5, only arsenic(III) produces arsine, subsequent reduction in 5 mol l^{-1} hydrochloric acid yields total arsenic. Again, arsenic(V) may be determined by difference. The selective determination of antimony may be carried out in the same way[34].

Thus it may be seen that the determination of individual species present in a sample may be achieved using hydride generation, and there are a number of examples of the use of this technique in the literature[35-37].

2.1.3 Instrumentation

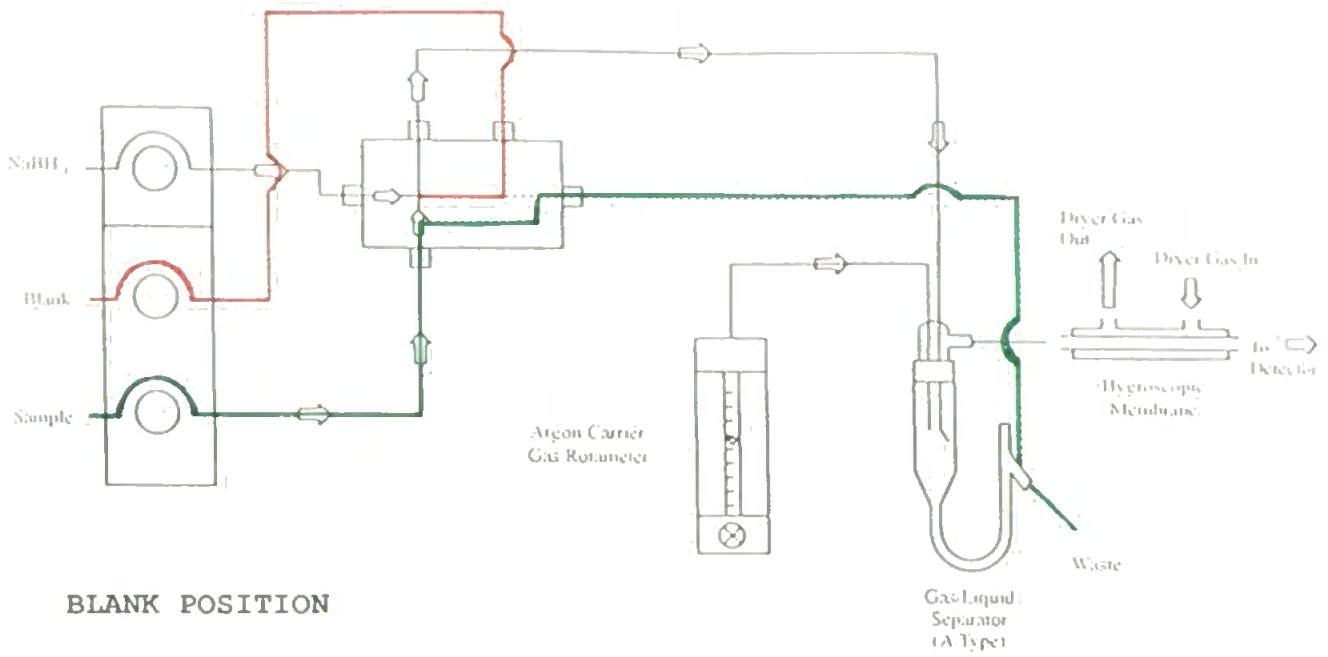
During the course of this study, a number of instruments have been employed.

2.1.3.1 Hydride generator

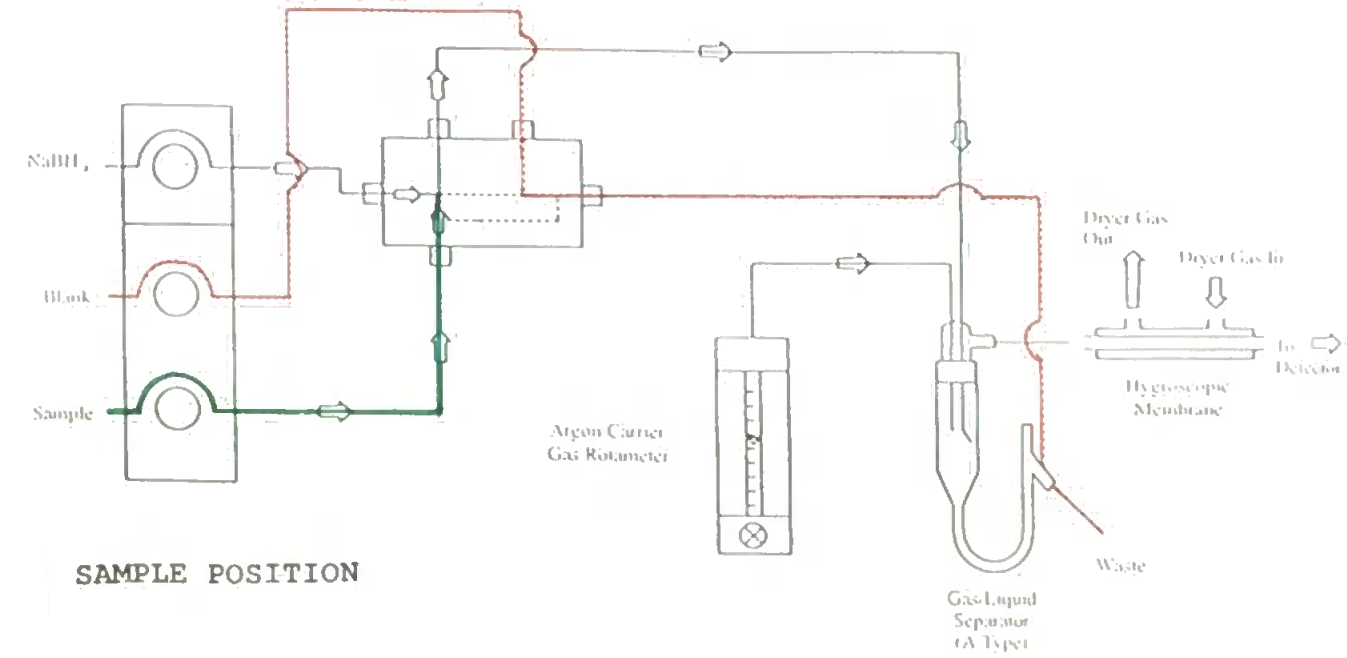
Early work was carried out using a continuous flow hydride generator (Model 10.003, PS Analytical Ltd., Orpington, Kent) and this was later upgraded to a fully computer controlled system (Model 10.004, PS Analytical Ltd.). Both units are similar mechanically, employing a peristaltic pump to supply reductant, acid blank and sample via a switching valve to a gas-liquid separator. The basic layout of the system is shown in Figure 2.1. The difference between the two models is that in the earlier model, the timing is set from the front panel, using rotary switches which allow only a limited number of pre-set timing periods. The later model derives it's timing from the computer software, and has a much greater flexibility over the range of timing periods which may be set by the operator. This facility became essential when using the atomic fluorescence detector in "flow injection" mode, as described in Chapter 3.7.

With both systems, the operation is the same and follows a four stage process. The first stage is the delay, during which

Figure 2.1 Diagram of a flow injection hydride generator



BLANK POSITION



SAMPLE POSITION

time the sample reaches the switching valve. This is followed by the rise period, during which the signal reaches a steady state. There then follows the analysis time, during which the detector makes measurements, and finally the decay period, during which the signal returns to the baseline. Each of these periods may be varied, depending upon the kinetics of the reaction being investigated. The later model (Model 10.004, PS Analytical Ltd.) provides total versatility in the control of these timing periods.

The same generator may be employed as a cold vapour generator for use in the determination of mercury, although a slightly different gas-liquid separator is then recommended.

2.1.3.2 Detectors

The atomic absorption spectrometer used throughout this study was a model SP 9 (ATI, Cambridge). The basic theory regarding atomic absorption spectrometry is given in Section 2.2.1, and the functioning of the instrument is more fully described there.

The atomic fluorescence instrument used is known as the Excalibur (PS Analytical, Orpington, Kent). It's operation and theory is given in more detail in Section 2.2.2.

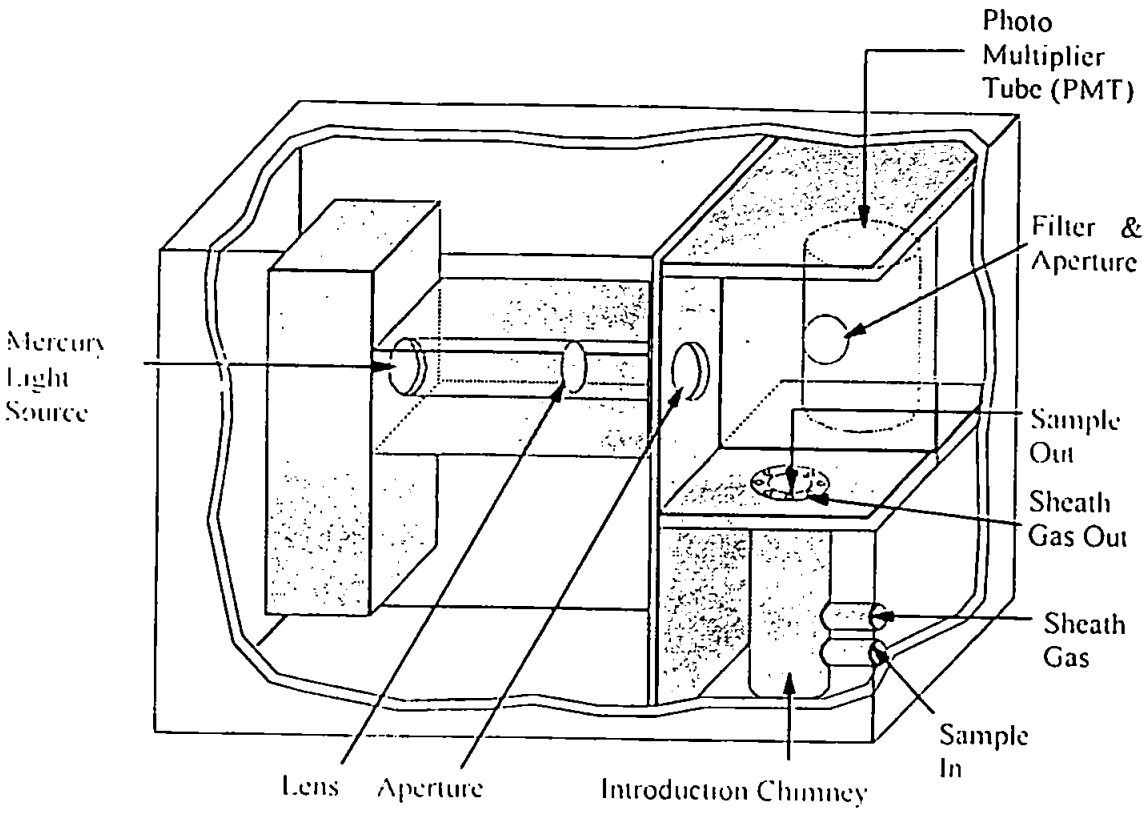
The mercury fluorescence detector used throughout the study was a Merlin (Model 10,023, PS Analytical Ltd., Orpington, Kent). This unit is shown in Figure 2.2, and features a fluorescence measuring system which may be employed either as a stand alone instrument, or as part of a fully integrated computer controlled analytical system. When using the fluorescence detector for the determination of mercury, high sensitivity with routine determinations below 1 pg ml^{-1} and a wide linear range over seven orders of magnitude are available.

Finally, an inductively coupled plasma-atomic emission spectrometer was also used. The instrument employed was a Liberty 200 (Varian Instruments) and further details of ICP-AES instruments are given in Section 2.2.3.

2.1.3.3 Microwave heating system

The microwave heating system employed was a Microdigest 301 (Prolabo, Paris, France). This unit employs a focused microwave beam, has full power control in 5% steps and control of the heating period from seconds to hours. The system was primarily designed for the digestion of individual samples, contained in discrete tubes in the microwave cavity area. In the applications presented in this thesis, it was operated as a continuous flow heating system, with the heating coil being

Figure 2.2 Mercury fluorescence detector



contained in a blank tube within the cavity area. It is operated via a simple multifunction keypad, on the control unit which houses the microprocessor system and other control electronics. The basic system is shown in schematic form in Figure 2.3.

2.1.3.4 Photolysis source

The high power UV source used was constructed in house, and a diagram of it is shown in Figure 2.4. The diagram shows the horizontal cross-section through the instrument. The UV source is an axially mounted 400 watt mercury discharge lamp, with the unit being cooled by a high flow rate forced air system.

2.1.3.5 Pyrolysis oven

The pyrolysis oven employed was a model 10.550 (PS Analytical Ltd., Orpington, Kent). The unit is shown in diagrammatic form in Figure 2.5. It features a very rapid heating cycle, precise control of temperature, and is fully programmable. It has been engineered in such a way that the ends of the silica sample tube remaining cool to the touch, even with sample temperatures of 800 °C.

Figure 2.3 Microwave heating system

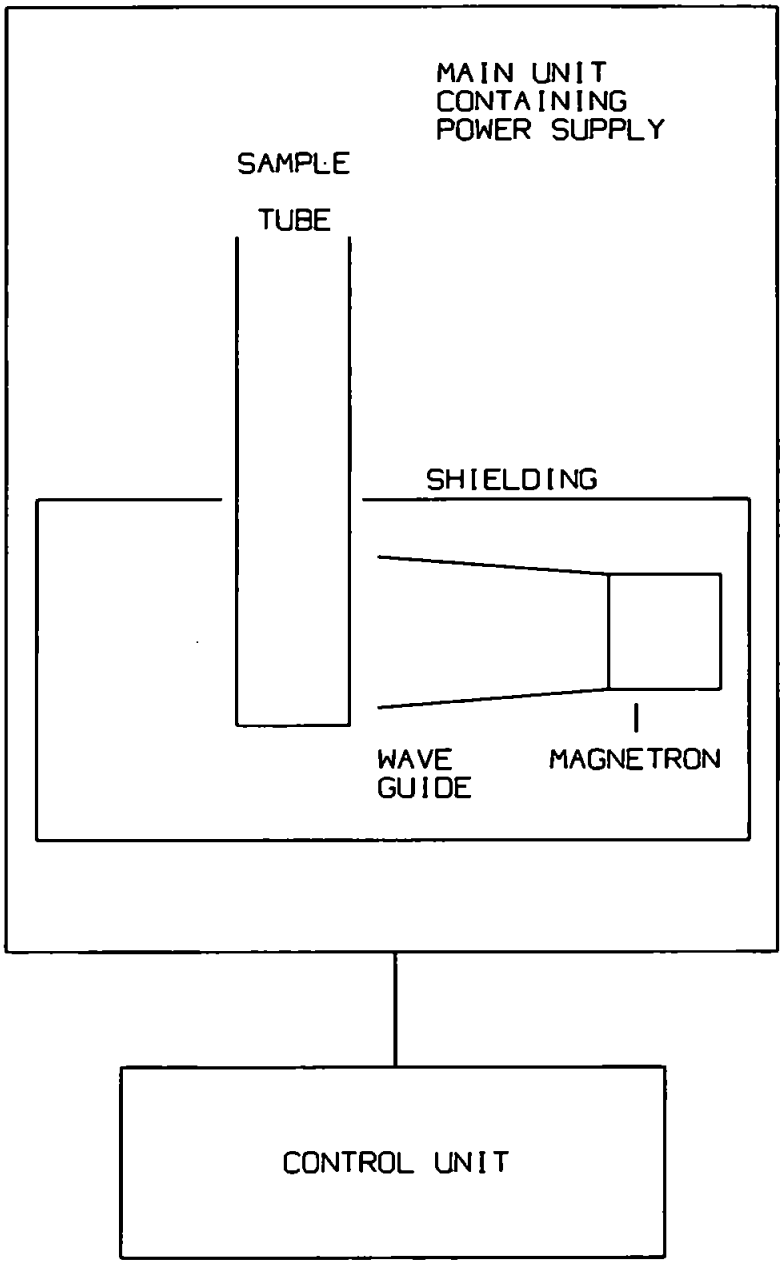


Figure 2.4 Photolysis source

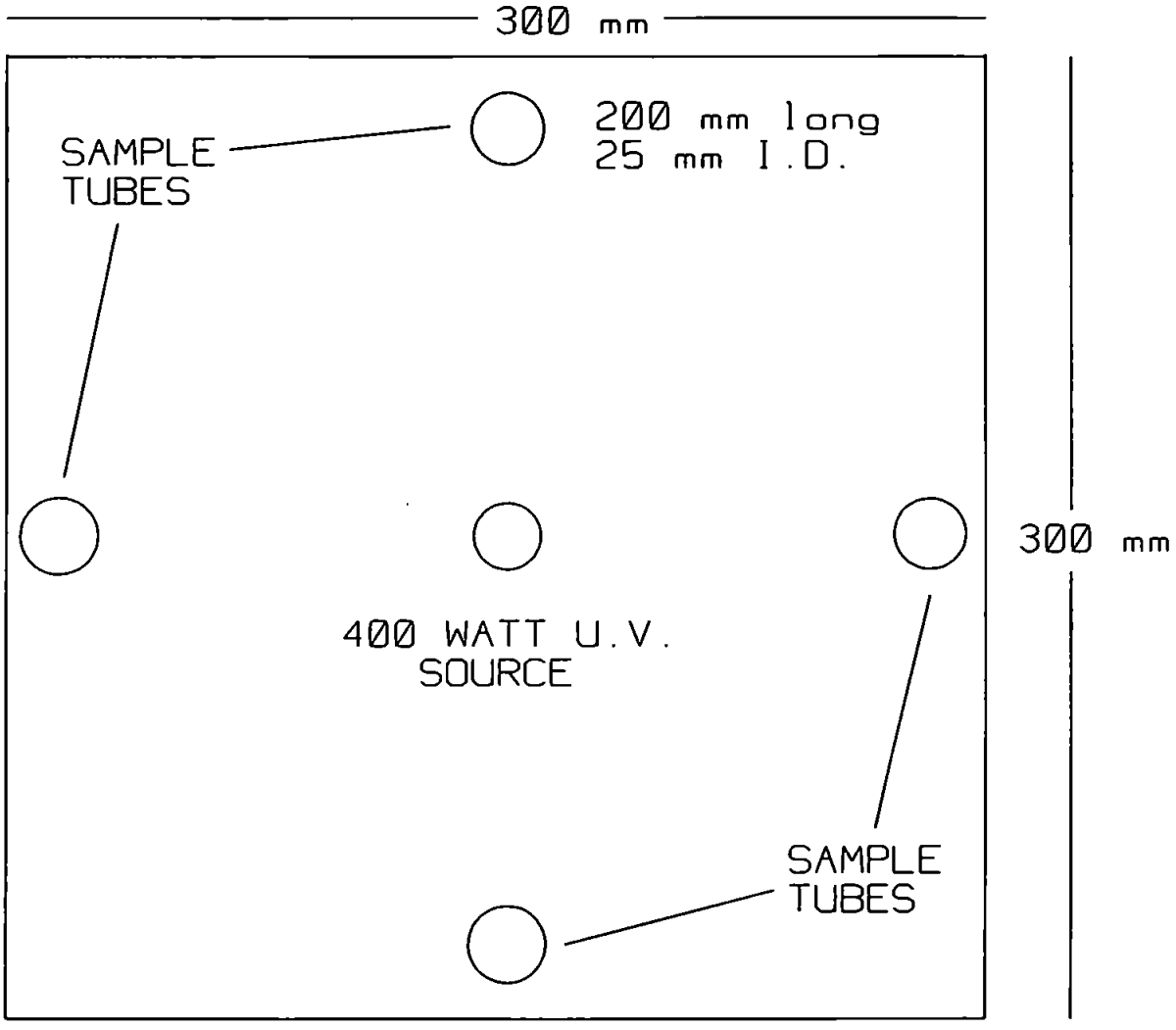
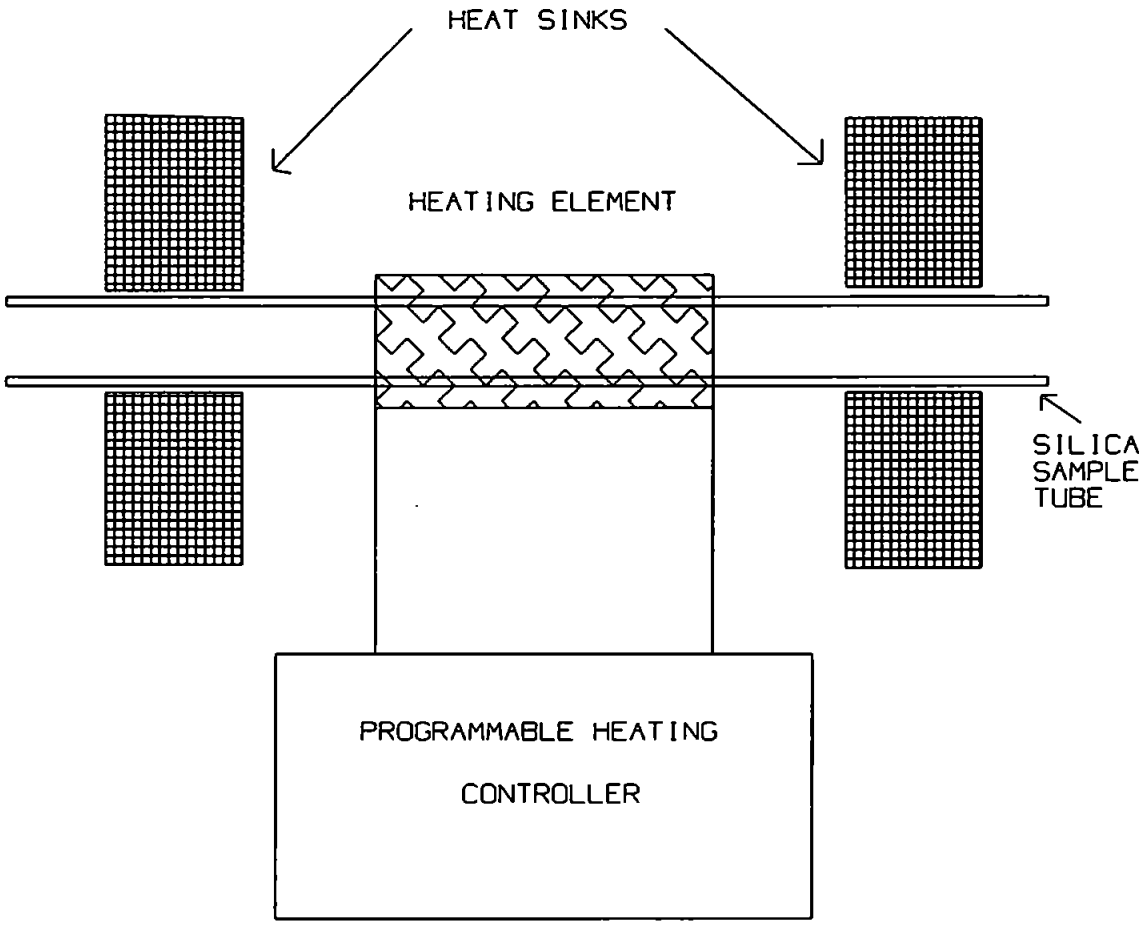


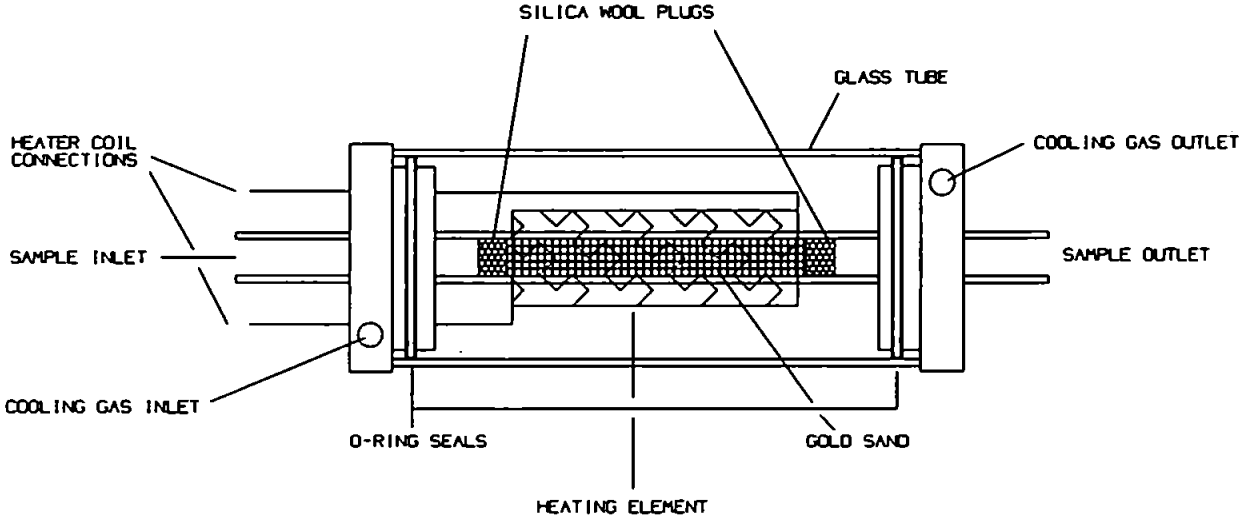
Figure 2.5 Pyrolysis oven



2.1.3.6 Mercury pre-concentration unit

The instrument used was a Galahad (Model 10.500, PS Analytical Ltd., Orpington, Kent). This unit features the trapping of mercury vapour on a gold - sand trap. Trapping may take place for a prolonged period if necessary, after which the mercury is released from the amalgam formed by heating. The mercury vapour is then swept from the trap into the detector in a stream of carrier gas, usually argon. The layout is shown in Figure 2.6. Other models of mercury pre-concentrating units are available from the same manufacturer, in which automatic computer controlled valving systems are employed for dedicated analytical requirements.

Figure 2.6 Mercury pre-concentration unit



2.2 Background and theory behind the choice of detection systems

There are four detection systems which are commonly employed in conjunction with hydride generation. These are atomic absorption spectrometry (AAS), atomic fluorescence spectrometry (AFS), inductively coupled plasma-atomic emission spectrometry (ICP-AES) and inductively coupled plasma-mass spectrometry (ICP-MS). Other techniques have been used, but are now rarely employed.

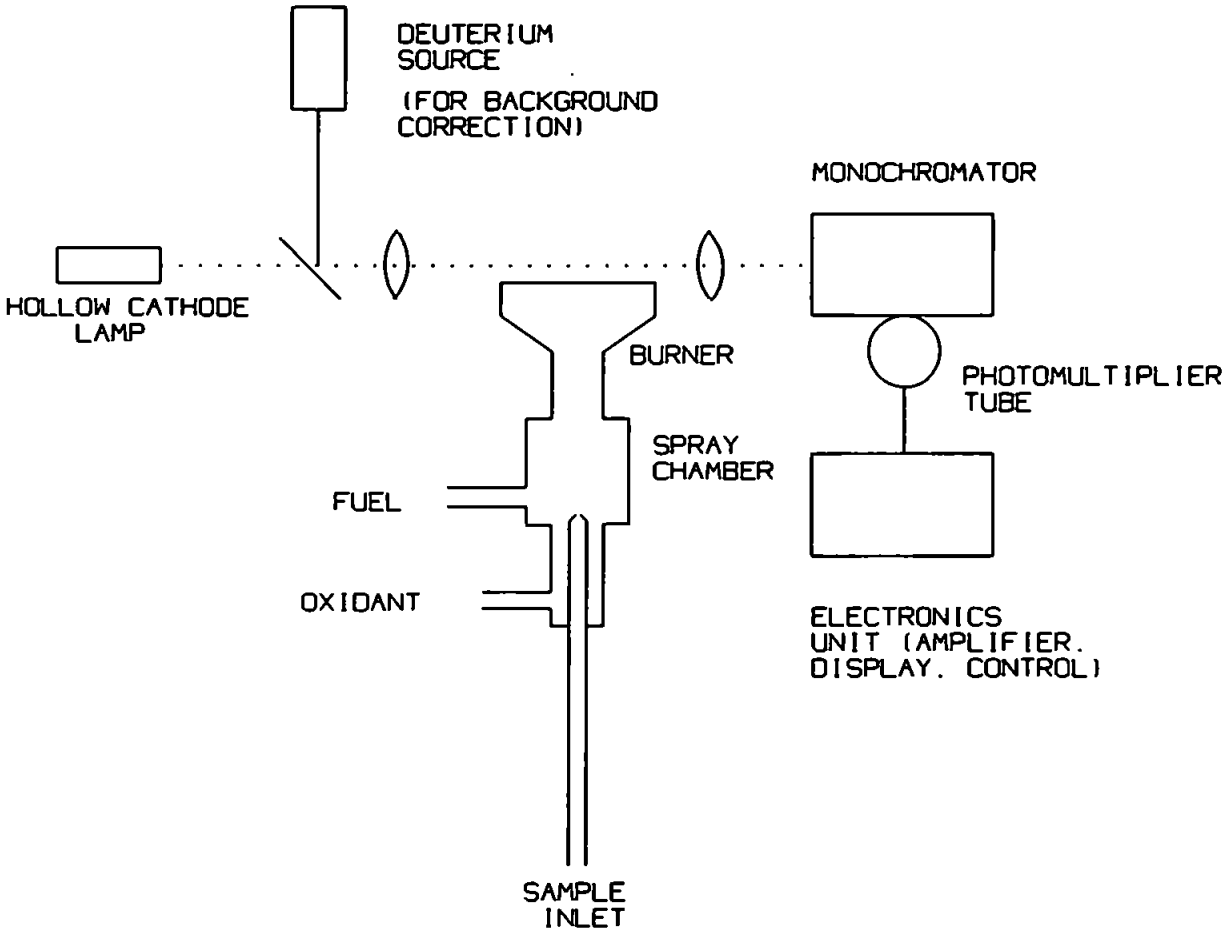
2.2.1 Atomic absorption spectrometry

This technique relies upon the absorption of electromagnetic energy, at a wavelength specific to the element concerned, by free atoms of that element. Fraunhofer[98] noticed dark lines in the spectrum obtained from sunlight, and in 1823, measured their wavelengths. He, and a number of later astronomers were observing atomic absorption lines. Although the method was considered theoretically possible for use in the laboratory, it was of little importance in analytical chemistry due to the very high resolution required to make quantitative measurements. Monochromators capable of a resolution of 0.001nm, would be required to isolate typical atomic absorption lines. These would be prohibitively expensive to produce, even if their production were possible. Thus the application of atomic absorption spectrometry from a continuum source was not feasible.

In 1953, Walsh[99] achieved the breakthrough in the form of a line source, which enabled atomic absorption spectrometry to become the most widely used technique for the detection and determination of metallic and metalloid elements. Walsh realised that by replacing the continuum source with an atomic spectral source, the monochromator would only be required to filter out other lines produced by the source (e.g. from the lamp filler gas), which would usually be well separated from the lines of interest. This provided the basis for a practical atomic absorption spectrometer.

The layout of a typical instrument is shown in Figure 2.7. It consists of a source, a hollow cathode lamp (HCL) or electrodeless discharge lamp (EDL), an atom cell, a monochromator and a detection system. The source emits radiation characteristic of the element in question, and this radiation is directed into the atom cell. This may simply be a flame, but other cells have been developed to meet particular requirements. The purpose of the atom cell is to produce a cloud of atoms in the ground energy state. These ground state atoms then absorb photons from the source, and produce a reduction in the amount of energy reaching the detector when an analyte is present. The light passes through a monochromator, which normally has a resolution in the order of 0.2 - 0.02 nm, and on to the detector. The detector is usually a photomultiplier tube, but the suitability of solid state detectors is starting to be investigated.

Figure 2.7 The optical layout of a typical atomic absorption spectrometer



Figures 2.8 and 2.9 show the construction of the hollow cathode lamp and the electrodeless discharge lamp respectively. The latter type of lamp offers a higher intensity of light output, but are more complex to drive and construct, and therefore more costly to utilise in the instrument. Since high light output is not a requirement in atomic absorption spectrometry, the use of these lamps tends to be restricted to those elements for which hollow cathode lamps are unavailable due to poor performance.

With the huge improvements in the production of optical components which has occurred since Walsh's original work, the use of a continuum source has been exploited[38,39], but so far the work has been restricted to research laboratories possessing very high resolution monochromators. If such monochromators should become available in the future at very low cost, an instrument offering multi-element capability with background correction built in could become financially viable.

Figure 2.8 Diagram of a hollow cathode lamp

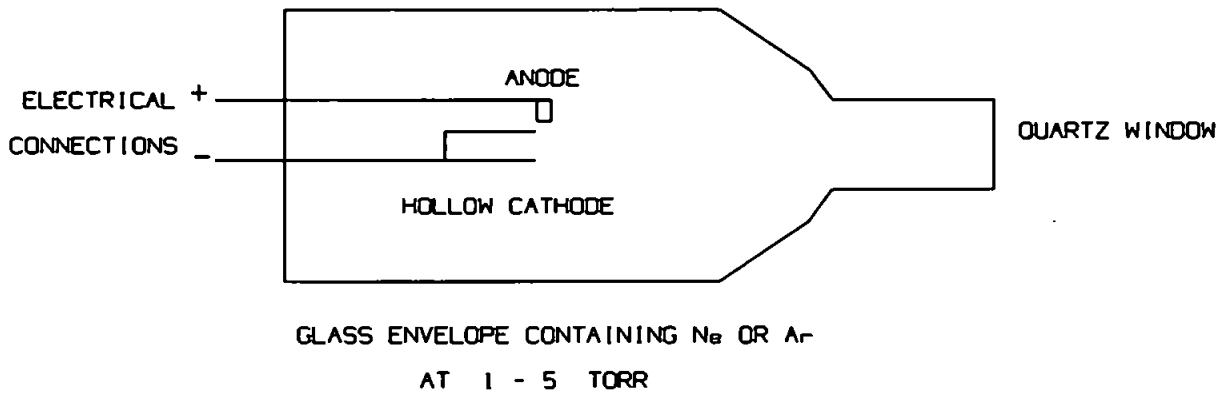
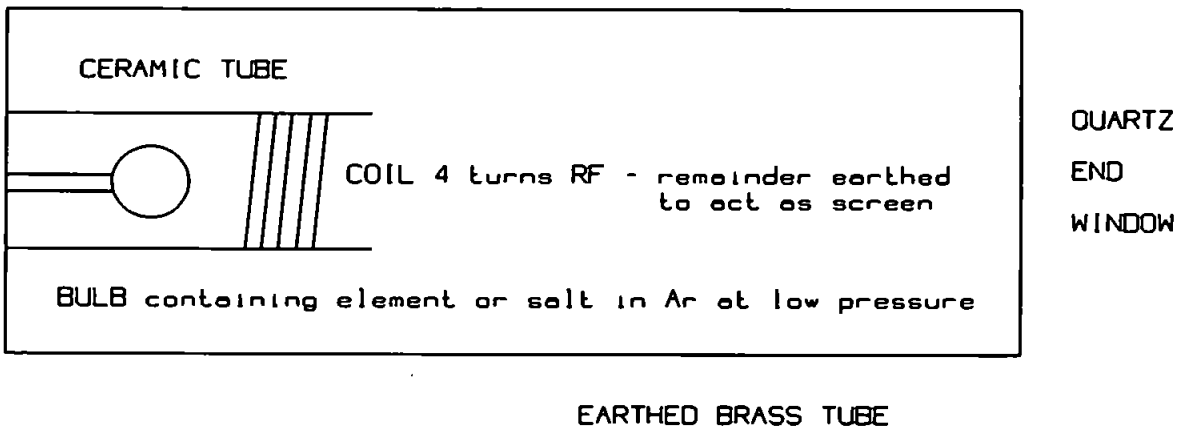


Figure 2.9 Diagram of an electrodeless discharge lamp



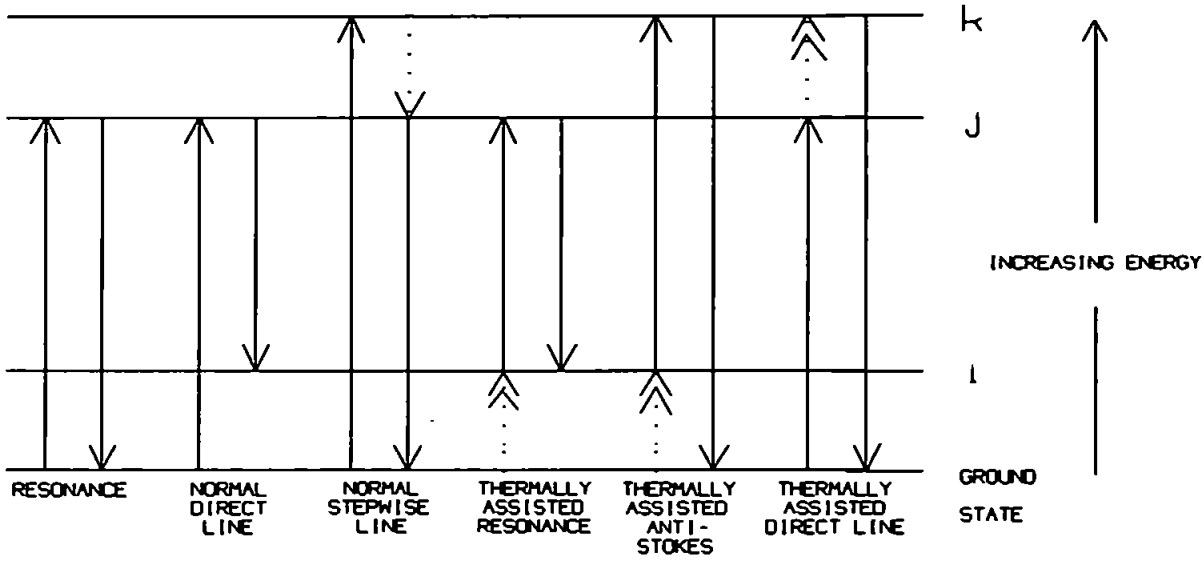
2.2.2 Atomic fluorescence spectrometry

Atomic fluorescence is a relative newcomer to the list of techniques available to the analyst. As the name implies, the technique relies upon light being absorbed and then re-emitted by the atoms of interest in the analyte. At low concentrations, the intensity of the emitted radiation is directly proportional to the concentration of the analyte, and to the radiant power of the source at the analytical wavelength. At high concentrations of analyte, self absorption can occur.

The two modes of fluorescence of interest are resonance and non-resonance. In the former, the light is emitted at the same wavelength as that used to provide excitation, and this is generally the more intense of the fluorescence processes. Figure 2.10 shows the transitions involved in both fluorescence processes[40], with solid lines representing radiational processes, and dotted lines representing non-radiational ones. In the non-radiational transitions, a single headed arrow indicates non-radiational deactivation, whilst a double headed arrow signifies a thermal activation process. When the emitted radiation is of a higher energy and therefore a shorter wavelength, the term Anti-Stokes is applied.

Since the fluorescence emission intensity is proportional to the excitation intensity, the need for a very stable, high intensity line source is apparent. It was this factor which

Figure 2.10 Transitions involved in the fluorescence processes



The solid lines indicate radiational processes. dotted lines indicate non-radiative processes. On dotted lines, a single headed arrow indicates a non-radiational deactivation and a double headed arrow, a thermal activation process. The term anti-Stokes is employed when the emitted radiation is of a shorter wavelength.

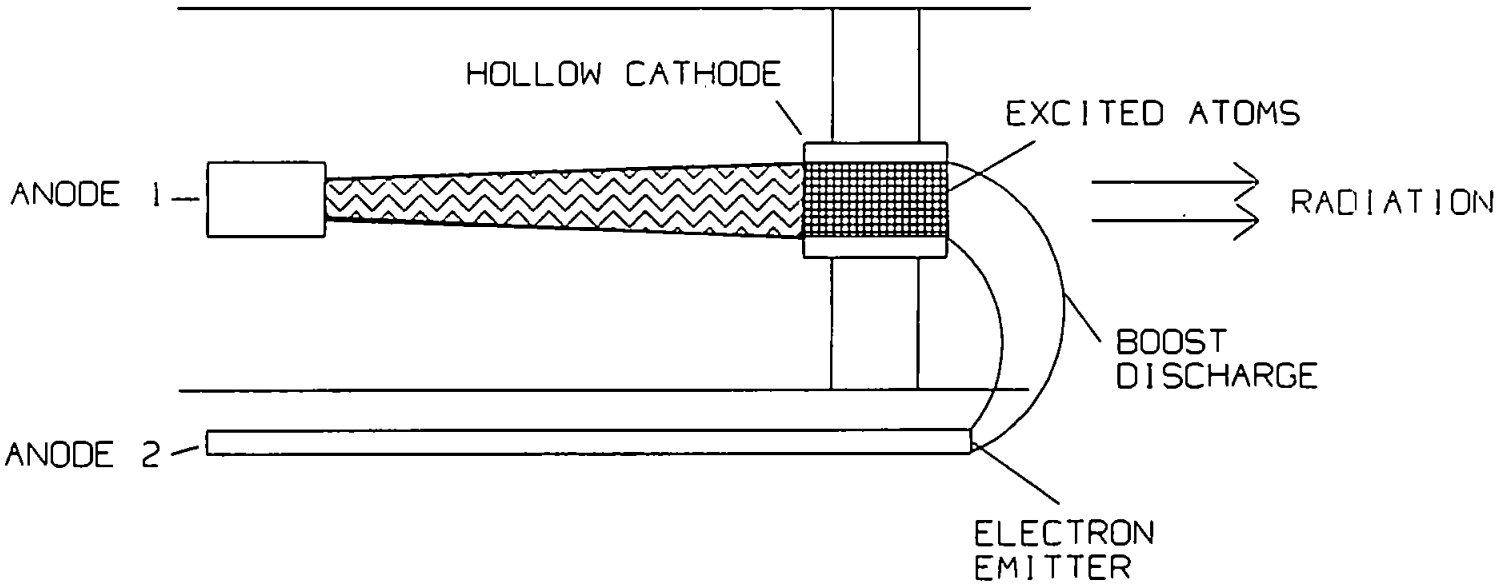
delayed the development of the technique. Continuum sources do not provide sufficient energy, particularly in the UV region of the spectrum. Xenon arc lamps have been employed, but problems of scatter do occur. Moreover, the intensity over the absorption half-width is relatively low when compared with line sources, in which all the radiated energy is concentrated in the few emission lines of interest.

In cases where suitable vapour discharge lamps exist, they have been employed with success, but the range of such lamps is severely limited.

The conventional hollow cathode lamp as employed in atomic absorption spectrometers do not provide sufficiently high intensity emissions, unless they are pulsed. Boosted discharge hollow cathode lamps (BDHCL) have overcome the low emission problem, and an example of such a lamp is shown in Figure 2.11. In these lamps, the intensity of emission is raised by superimposing a positive column discharge across the hollow cathode discharge[41]. This provides additional electrons for the hollow cathode plasma, ensuring that most of the atoms are excited, thus increasing radiated output without increasing sputtering. The absence of ground state atoms in the light path combined with the low voltage - high current discharge obviates self-absorption.

BDHCLs have overcome the low source intensity problem in many cases, and have allowed the development of commercial atomic

Figure 2.11 Diagram of a boosted discharge hollow cathode lamp

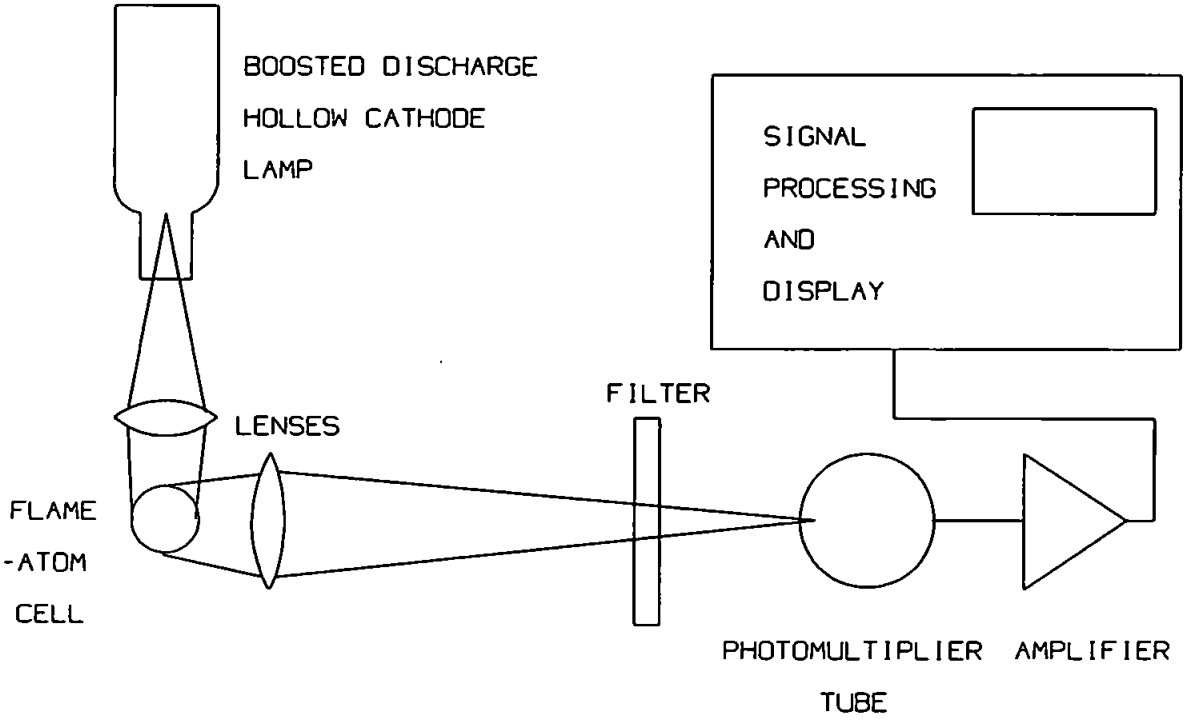


fluorescence instruments, such as the one used throughout this study. Other high intensity sources which have been investigated include microwave excited electrodeless discharge lamps, and lasers. The former are between 200 - 2000 times more intense than hollow cathode lamps, but have suffered from stability problems. These have been reduced by the operation of the lamps within thermostatted microwave cavities. Lasers have also been employed as sources, in particular the tunable dye laser. The output from lasers can be sufficient to cause saturation fluorescence, which nullifies the effects of quenching and self-absorption.

The design and operation of the atom cell is of vital importance in atomic fluorescence. Since energy is lost through quenching, the selection of the carrier gas is critical. Whilst nitrogen may be employed as the carrier for atomic absorption determinations, being diatomic it is usually avoided for fluorescence work. Fortunately, argon has a negligible quenching cross-sectional area, and is generally employed. Hydrogen also has a low value, and so the use of a hydrogen diffusion flame has been employed with great effect. The preference for circular flames for fluorescence studies is almost universal.

The basic layout of a typical non-dispersive atomic fluorescence instrument is shown in Figure 2.12. From the diagram, it can be seen that the instrument is basically simple in design, consisting of a source and a lens to focus

Figure 2.12 Diagram of a typical non-dispersive atomic fluorescence spectrometer



the beam into the atom cell. At right angles to this assembly is a collector lens, filter and a light detector (usually a photo-multiplier tube). The output from the detector is fed through an amplifier and into a readout device. The actual design of the optical assembly is critical, if optimum performance is to be obtained. Very small quantities of light are involved, and these must be collected as efficiently as possible, with efforts being made to avoid degrading the signal via scatter etc. In a dispersive instrument, the filter is simply replaced by a monochromator.

Atomic fluorescence has a number of advantages over atomic absorption and atomic emission. As may be observed from Figure 2.12, the instrumentation is less complicated than in either of the other techniques. Good sensitivity is obtained, particularly in the far UV region, where the other two techniques are relatively insensitive. The method is highly selective due to the employment of a line source, and has low spectral interference. The technique also enjoys excellent linearity over at least several orders of magnitude (seven in the case of the mercury fluorescence detector).

Apart from the disadvantages regarding quenching, scattering and self absorption at high concentrations already mentioned, the only other major limitation of the technique is reduced sensitivity for elements which absorb and emit in the visible region of the spectrum, when compared with atomic emission.

2.2.3 Atomic emission spectrometry

A number of atomic emission spectrometers have been employed as detectors following hydride generation. These include the direct current plasma (DCP), microwave induced plasma (MIP) and inductively coupled plasma (ICP). Whilst there is still some work being carried out using the DCP, most attention is now concentrated on ICP. MIP has a number of advantages over ICP, but as yet has still not been fully developed. The new generation of ICP instruments operate at higher frequencies (41MHz c.f. 27.12MHz), and are more tolerant to higher levels of gases such as hydrogen which is produced in the hydride generation reaction than were their predecessors.

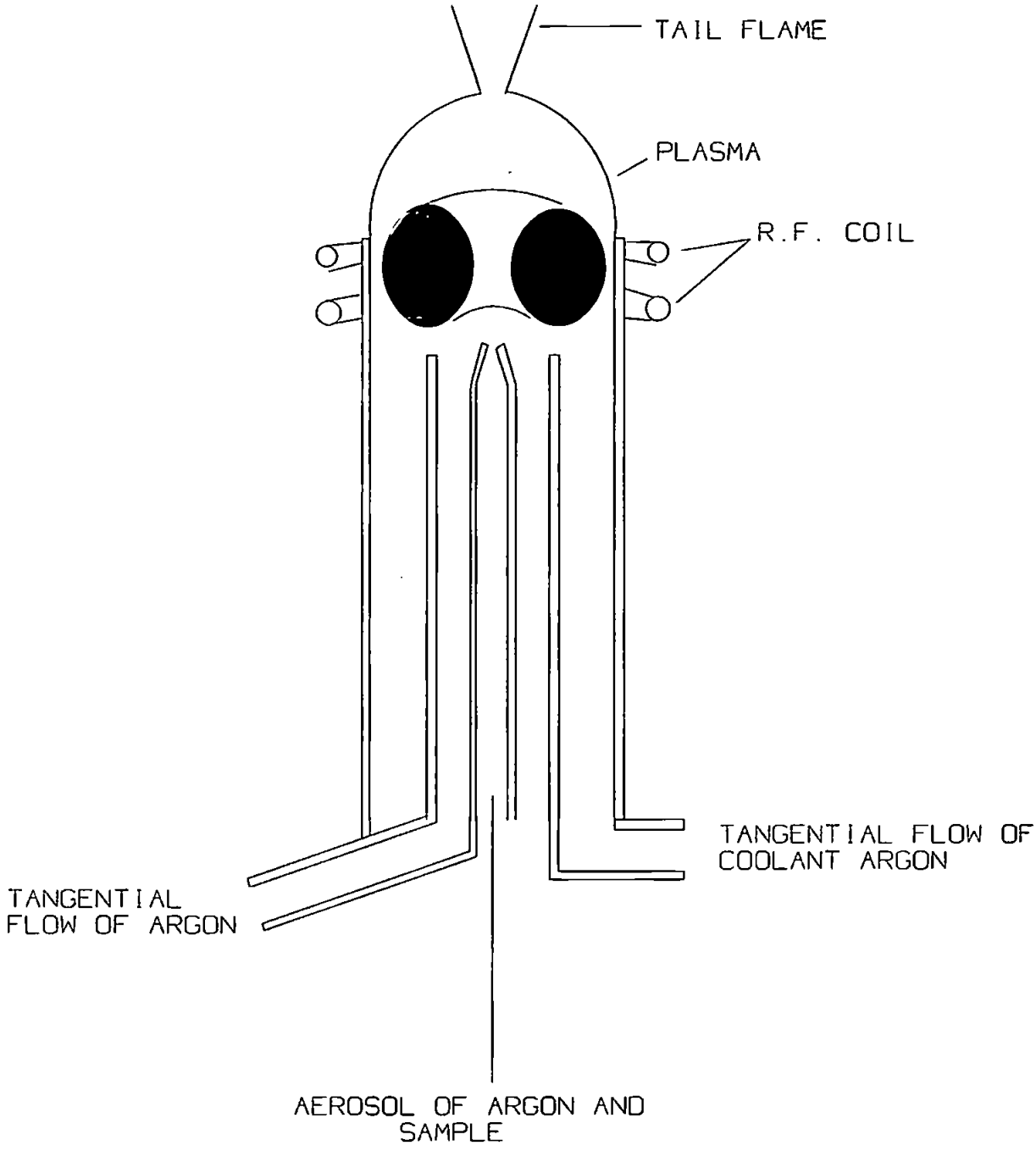
Atomic emission spectroscopy is the measurement of the light emitted by atoms, which have been excited by the input of energy, as they drop back to their ground states. Atomic emission may be observed at the simplest qualitative level in the "platinum wire flame tests" which will be familiar to any student of chemistry. The technique is also commonly employed in flame photometers for the quantitative determination of the alkaline earth elements. In these instruments, the sample solution is aspirated into the flame, and the increase in light emission due to the element of interest measured by a photocell, spectral selection being accomplished with a simple filter between the flame and the detector. Many atomic absorption spectrometers also have the facility for operation in the emission mode, again using the flame as the excitation

source.

A great advance was made in the late 1970's when two workers operating independently produced similar designs for an inductively coupled plasma torch. The main difference between the designs was that of the overall diameter - the Fassel[42] design has an outside diameter of 18mm, whilst the Greenfield torch has one of 27mm. A typical design of an inductively coupled plasma torch is shown in Figure 2.13. It consists of three concentric quartz tubes, with a water cooled two or three turn radio-frequency (RF) coil around one end. The inner tube carries an aerosol of the sample in a stream of argon, at a flow rate of about 1 l min^{-1}. The middle tube may have an auxiliary tangential flow of argon through it, this flow being optional in all-argon plasmas. The outer tube has another tangential flow of argon passing through it at a flow rate of 10 - 15 l min^{-1}, which provides argon for the plasma itself, whilst simultaneously providing cooling for the torch.

The plasma is struck by a high voltage discharge from a Tesla coil, which ionizes some of the argon, and provides a source of electrons. These ions and electrons then interact with the rapidly changing magnetic field induced by the RF coil, and this causes the ions and electrons to flow in a closed circular horizontal path. Collisional energy exchange then occurs between ions, electrons and the argon atoms, to produce a white hot fireball. The tangential flow of argon in the outer tube provides thermal isolation between the fireball and

Figure 2.13 Diagram of the inductively coupled plasma torch



the tube. The flow of argon and sample though the inner tube must be sufficient to punch a hole through the fireball, and thus an annulus is produced. The analyte atoms reside in the area of the fireball for about 2 milliseconds, during which they are subjected to temperatures of 6 - 8000°K. These times and temperatures are at least twice those experienced in nitrous oxide-acetylene flames, as used for refractory elements in atomic absorption spectrometers. The consequent atomization is more complete, fewer chemical interferences occur and ionization interferences are small or non-existent[43].

The plasma itself is a brilliant white, non-transparent doughnut shaped entity, topped by a flamelike tail. The core which extends a few millimetres above the top of the tube shows a continuum spectrum, produced by the re-combining of argon and other ions with electrons. This continuum diminishes and an optically transparent region occurs some 10 - 30 mm above the core, with spectral measurements made usually between 5 and 25mm above the coil.

The RF energy is supplied by a high power RF generator, capable of providing up to about 2 kW of power at 27 MHz. Higher frequencies are now being employed in some instruments, which result in a more stable plasma, which among other things, exhibits a higher tolerance to organic solvents and gases such as hydrogen which were problematic in earlier instruments. These later generation instruments operate at approximately 41 MHz.

As a source for atomic emission measurements, the inductively coupled plasma offers high stability, high sensitivity and freedom from interferences, but the capital and running costs are high. All such instruments are computer controlled, and are therefore well able to operate in an automated mode. Optimum viewing heights for all the elements are stored in the software, and may be automatically set by the instrument.

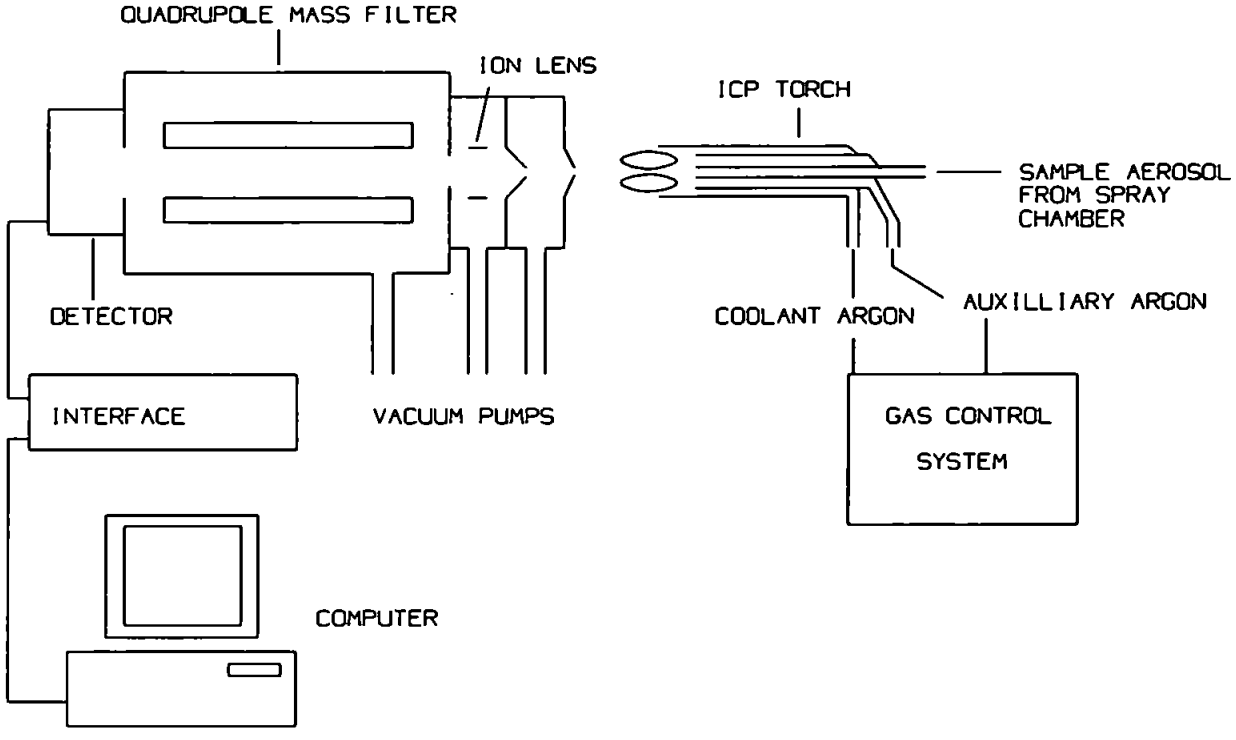
ICP-AES offers a wide choice of spectral lines, good linearity, and the possibility of simultaneous multi-element determinations. This latter feature has recently been more fully exploited by commercial manufacturers in their latest generation of instruments which employ solid state detectors.

2.2.4 Inductively coupled plasma - mass spectrometry

The inductively coupled plasma - mass spectrometer (ICP-MS) is undoubtedly one of the most sensitive and versatile instruments for trace element determinations currently available to the analytical chemist. It offers the facility to perform simultaneous determinations on most elements and also isotopic determinations.

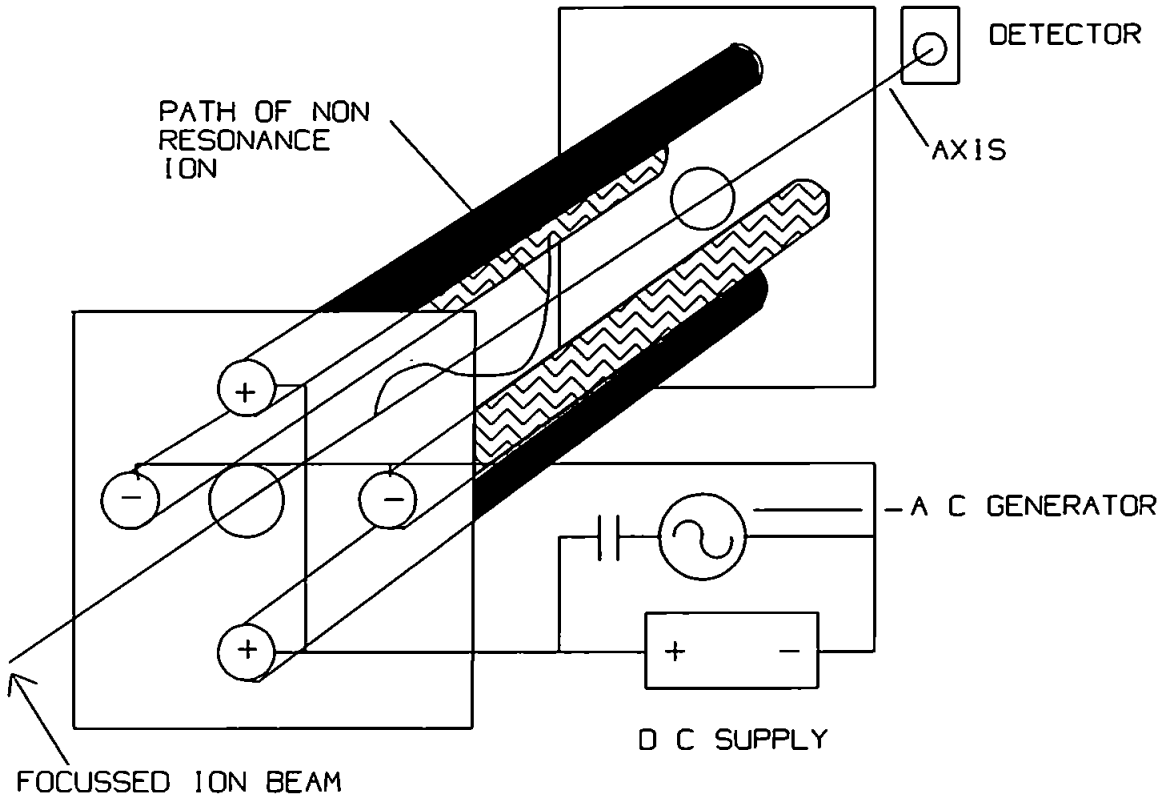
The layout of a typical instrument is shown in Figure 2.14. The ICP torch described in the previous section is employed

Figure 2.14 Block diagram of a typical inductively coupled plasma-mass spectrometer



in this case as an ion source, and is held in the horizontal plane. The stream of ions generated by the plasma is directed towards the first of two nickel (or platinum) cones, which are held axially with the torch. The first of these cones is the sampler, the second the skimmer, and these act in such a way as to produce a beam of ions into the quadrupole mass filter. There is a three stage pressure reduction between the torch which operates at atmospheric pressure, to the quadrupole mass filter which operates at a pressure of approximately 2×10^{-9} bar. After passing through the cones, the beam of ions then passes through accelerating electrodes before entering the mass filter. The layout of a quadrupole mass filter is shown in Figure 2.15. The filter consists of four precision ground metal rods, which are symmetrically arranged and parallel to the axis of the ion beam. The opposing rods are connected electrically to one another, and each pair is connected to a variable direct current (DC) supply. Each pair of rods is also connected to a variable radio frequency (RF) generator in such a way that each pair has a signal which is 180° out of phase with the other pair. Mass scanning is achieved by altering the frequency of the RF supply whilst holding the applied potential constant, or by altering the potentials of the two sources whilst keeping the ratio between these potentials and the frequency constant. The effect on the ions is to cause oscillations about the axis in the fields produced, and only the resonant ions i.e. those ions who's masses are of the critical value, pass through the filter to the detector - non-resonant ions collide with the rods.

Figure 2.15 Layout of a quadrupole mass filter



The technique does suffer from a number of interferences, one of which is due to overlap of elements having the same mass. This process is known as isobaric interference, and with reference to this particular study which has concentrated in particular upon selenium, this interference renders the use of this instrument problematic. This is due to the fact that naturally occurring selenium has six isotopes, and their masses correspond in some cases to the masses of argon compounds produced in the plasma, and from which they could not therefore be separated.

In general however, the technique is an extremely powerful one, and new instruments are being developed to further improve detection limits and specificity.

2.3 Reagents

A number of reagents have been employed during this study. These are listed below, with their respective source of supply :

Sodium selenite (99%)	-	Aldrich, Gillingham, Dorset.
Sodium selenate (99%)	-	Aldrich, Gillingham, Dorset.
Sodium Borohydride (98%)	-	Aldrich, Gillingham, Dorset.
Hydrochloric acid (37%)	-	Aldrich, Gillingham, Dorset.
	-	Merck, Poole, Dorset.
Hydrochloric acid (Aristar)	-	Merck, Poole, Dorset.
Nitric acid (Aristar)	-	Merck, Poole, Dorset.
Mercury AA standard (1000ppm)	-	Merck, Poole, Dorset.

Chapter 3

On-line hydride systems

3.1 Introduction

The work described in this chapter concentrates on the determination by hydride generation of the element selenium. Selenium is an essential trace element for mammals, avians, certain plants and bacteria, but is also highly toxic to these species. It is a constituent of the enzyme glutathione peroxidase, which affords protection to cells against oxidative damage[44]. In the environment, selenium may be found in the organic form as seleno-amino acids and seleno-enzymes[45] which are readily transformed into dimethylselenide and dimethyldiselenide[46]. Selenium has the narrowest "tolerance window" of any essential element[47-49]. The "tolerance window" is the difference between the minimum amount of an element required for good health, and the amount which will exhibit toxic effects. A selenium deficiency in man may result in cardiomyopathy[50]. Since this window is so narrow in the case of selenium, the need for accurate and sensitive analytical techniques for the determination of this element are readily apparent.

Selenium is a Group VIA metalloid, with an atomic weight of 78.96 and has oxidation states of +6, +4, +2, 0 and -2. It accounts for only 9×10^{-6} % of the earths crust, and occurs mainly as an impurity in sulphur, sulphide and sulphate deposits[51]. It has industrial use as a red colourant for glass, and owing to the fact that the metal-like form is very light sensitive, it is used in photocopying machines, and to a small

extent in the electronics industry. The concentration of selenium in sea water is low, i.e. 4 pg ml^{-1} in shallow water and 60 pg ml^{-1} in deep water as selenite, and 30 pg ml^{-1} and 120 pg ml^{-1} respectively as selenate[52]. Since it is estimated that 8000 tonnes of selenium are introduced into the sea each year[53], these figures may start to increase, particularly in localised areas.

Selenium has six naturally occurring isotopes, ^{74}Se (0.89%), ^{76}Se (9.02%), ^{77}Se (7.58%), ^{78}Se (23.51%), ^{80}Se (49.81%) and ^{82}Se (9.19%). The first choice technique for the determination of many trace elements is often ICP-MS, but this technique suffers from a number of interferences when selenium has to be determined. Isobaric interferences such as $^{40}Ar^{40}Ar$, $^{38}Ar^{38}Ar$, $^{38}Ar^{40}Ar$, $^{36}Ar^{40}Ar$ and $^{40}Ar^{37}Cl$ all limit the usefulness of ICP-MS in selenium studies. Fortunately, hydride generation provides a method which achieves virtually 100% conversion efficiency and combined with an atomic absorption spectrometer is able to determine selenium down to about 1-2 ng ml^{-1}. When atomic fluorescence is employed as the method of detection, an improvement in sensitivity of approximately three orders of magnitude is possible.

In inorganic compounds, selenium is most frequently encountered in either the +4 or +6 oxidation states. Of these, only the +4 state reacts to form the hydride, and therefore a reduction of any +6 form present in the sample is necessary. This property of selenium has been regarded as a problem in

many cases, adding a further step for the analyst to carry out when total selenium determinations are required. The perceived problem may however be turned to advantage when information regarding the individual species present is required. The sample may first be analyzed for selenium(IV), and then a reduction step carried out, before analysing again for total selenium. The concentration of selenium(VI) may then be calculated by subtracting the former figure from the latter.

3.2 An investigation into the apparent signal enhancement due to nitric acid when determining selenium by hydride generation

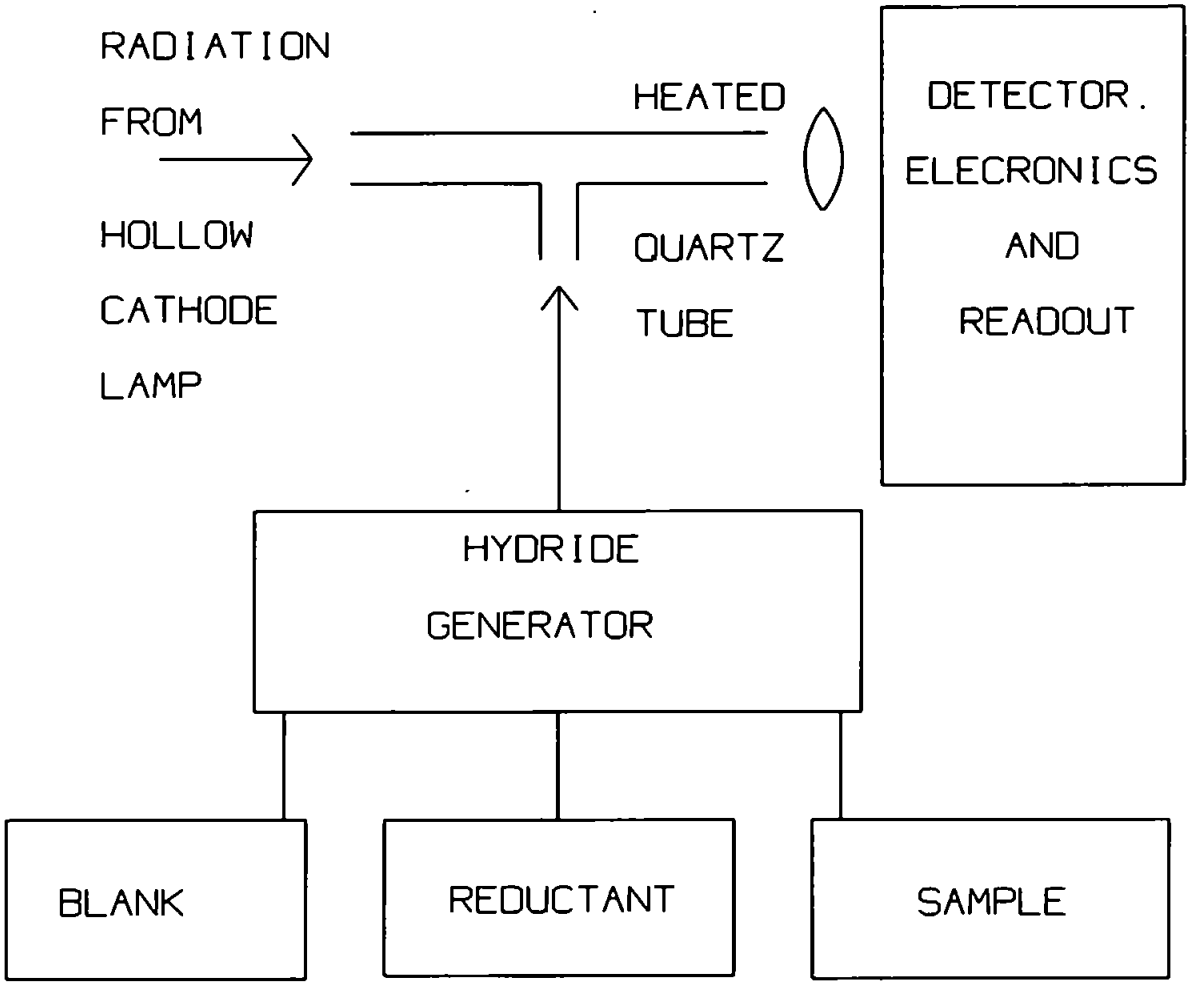
3.2.1 Background

A number of workers have reported an apparent signal enhancement when selenium has been determined by hydride generation [54]. The increase in signal only occured when nitric acid was present, as is the case when nitric acid is added to liquid samples at the time of collection to prevent loss of cations due to adsorption onto vessel surfaces (plate out). There are a number of reports in the literature suggesting that nitric acid is an interferent suppressing the signal in selenium hydride generation systems[24,25,36,55-57]. However no reference could be found reporting an enhancement effect, and so this was investigated further.

The apparatus used for this investigation is illustrated in Figure 3.2.1 It consists of a hydride generator, an atomic absorption spectrometer employing a quartz furnace, with data collection and processing by computer using Touchstone© software.

It was first necessary to evaluate whether the reported enhancement effect could be observed under laboratory conditions. This was accomplished by first preparing a standard containing 25 $\mu g\ l^{-1}$ of selenium as sodium selenite

Figure 3.2.1 Diagram of the basic hydride generation-quartz furnace atomic absorption detection system



in 3 mol l^{-1} hydrochloric acid. The signal obtained for this standard was measured (in replicate) and compared with a 3 mol l^{-1} hydrochloric acid blank. Two 25 ml aliquots of the standard were then taken. To one of these 25 μl of concentrated nitric acid was added, and to the other 25 μl of concentrated hydrochloric acid. These modified standards were then evaluated against the 3 mol l^{-1} hydrochloric acid blank. The solution containing the nitric acid was found to give an absorbance reading over 10% higher than the solution containing no nitric acid. This obviously established that an enhancement did occur, and so a systematic study was undertaken in an attempt to more fully understand the mechanisms involved.

3.2.2 Experimental

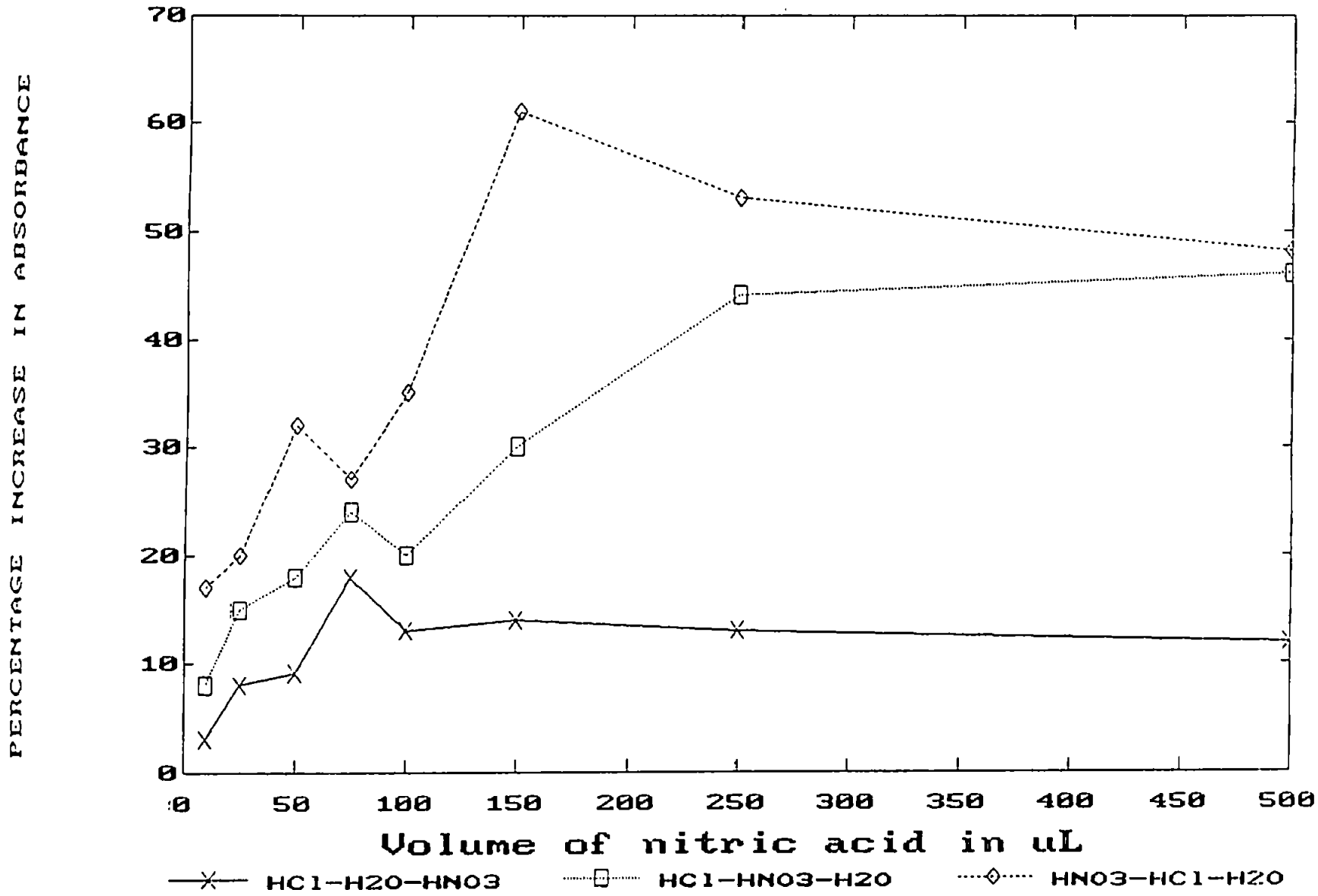
A series of solutions were prepared by taking 25 μl aliquots of a 130 mg l^{-1} solution of selenium as sodium selenite and adding them to 100 ml volumetric flasks. To each of these was added 26.7 ml of hydrochloric acid, to give a final acid solution strength of 3 mol l^{-1}. Analar nitric acid was then added in the range 10-500 μl to these solutions, prior to making up to volume with deionised water. They were then analyzed, again using the hydride generation - quartz furnace atomic absorption technique, against a standard prepared in a similar method, but containing no nitric acid. Determinations

were carried out by the bracketing method, that is standard, sample 1, standard, sample 2, standard etc. The reading obtained for the sample in each case was then compared with the mean value for the standards either side. This method compensates for instrumental drift. Again, enhancements were observed, although the increases in signal did not appear to be directly proportional to the quantity of nitric acid present.

A further series of experiments was performed in which the sample solutions were prepared in different orders. For the first series of solutions, the aliquot of concentrated stock was placed in the flask, followed by the nitric acid, the hydrochloric acid and finally the water. In the next series, the hydrochloric acid was added before the nitric acid, and in the final series, the nitric acid addition was made after the water. These solutions were then determined as previously, again using the bracketing technique. The results are shown in Figure 3.2.2. As may be observed, the order of sample preparation has a great effect upon the degree of signal enhancement observed.

Since there was a possibility of molecular species causing the enhancement, these experiments were repeated employing background correction. Similar results were obtained. Determinations were also made using two other detection systems, namely inductively coupled plasma - atomic emission spectrometry and atomic fluorescence spectrometry. Again

Figure 3.2.2 Diagram showing the effect of solution preparation on observed enhancement



similar results were obtained. Further experiments were performed in which other aspects of the system were investigated, including the gas-liquid separator, reagents and pump tubing. Using reagents from other suppliers made no difference to the observed enhancements. A U-tube type gas-liquid separator was fabricated from Quick-fit glassware in-house, and produced virtually identical results to those previously obtained. The effects of the pump tubing were then examined.

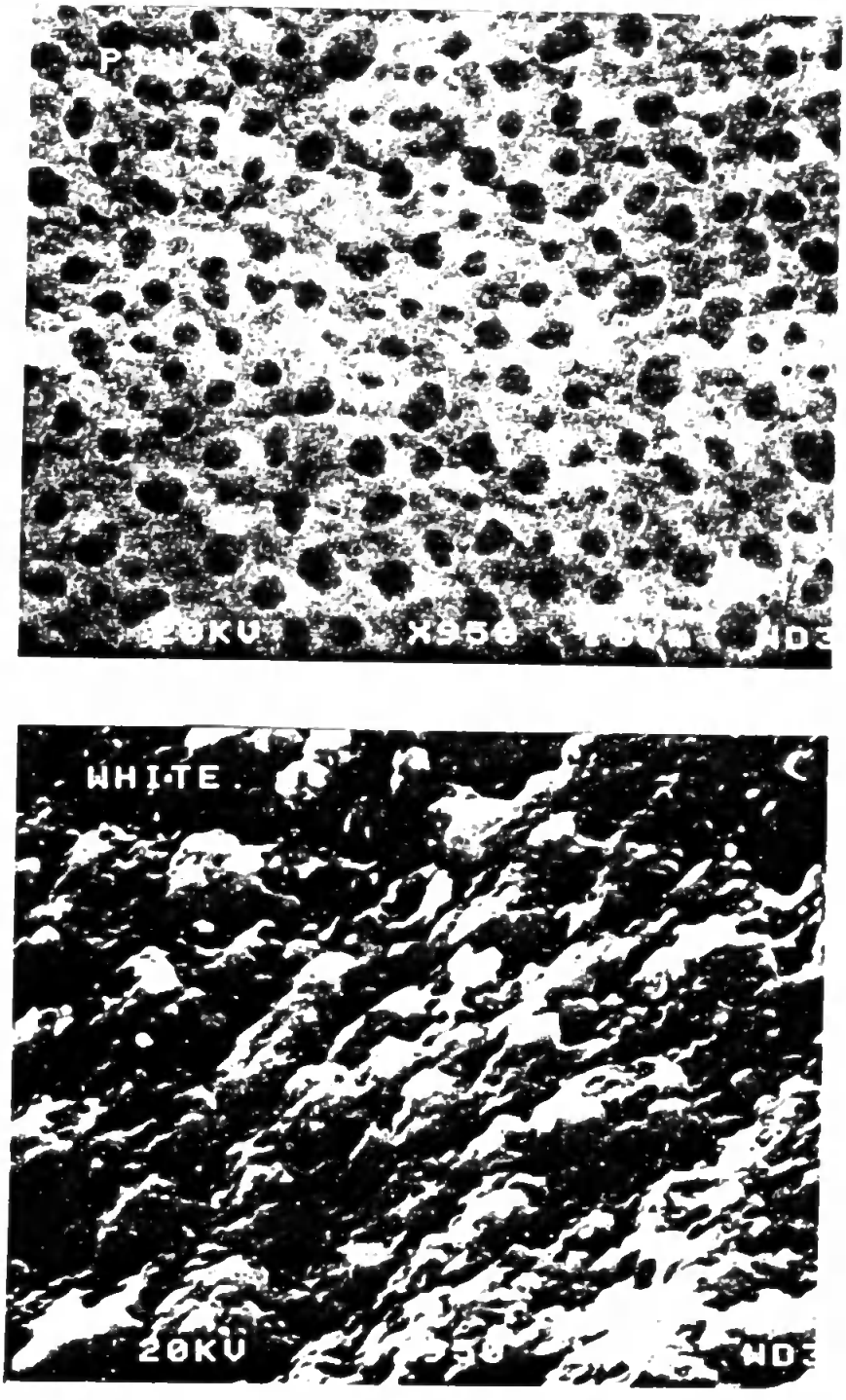
Up until this stage of the study, only one type of pump tubing had been supplied for use with the hydride generator system. This was the standard tubing supplied by the manufacturer of the hydride system, and was of a silicone rubber type of material, a pink transparent type being supplied for the sample and acid blank, and a blue transparent type for the reductant. This type of tubing had been successfully employed for the determination of selenium and other hydride forming elements for many years. However, because of it's rather fragile nature, it was subsequently replaced by 'Santoprene' tubing, which is physically more robust. This had the advantage that it provided a more constant delivery of reagents to the gas-liquid separator. The experiments were repeated using this tubing, and no enhancement effects were noted. With a section of the old tubing placed between the sample inlet and the pump, the enhancements were again observed.

3.2.3 Further examination of the pump tubing

An more detailed examination of the surface of both types of pump tubing was then conducted. An initial examination of the surface of the tubing was made by obtaining infra red spectra, to see if any trace of carbon-selenium bonds could be observed. This was carried out using a total internal reflectance attachment on a Perkin Elmer Fourier Transform spectrometer. No such bonds were detectable, but since the selenium concentration in the tubing would be extremely low if present at all, this technique would be unlikely to be capable of it's detection.

Samples of both types of tubing were examined using a scanning electron microscope. The photomicrographs are shown in Figure 3.2.3. The surface of the inside of the early tube type can clearly be seen to have what appears to be a honeycomb of holes in it's surface, although from these pictures, it is not possible to be certain whether these are holes in, or mounds on, the surface. Analysis of the tubing was also carried out using the X-ray back scattering attachment on this instrument. The results from this study were inconclusive - no selenium was found in either sample of tubing, although this was not surprising since the limit of detection on this instrument is in the order of 1%. The main difference observed between the spectra produced for the two tubings was the presence of high concentrations of aluminium in the Santoprene tubing. It is

Figure 3.2.3 Photomicrographs of the bores of the pump tubing



Upper - the older pink tubing

Lower - new Santoprene tubing

assumed that alumina is used as a filler in the formulation of this tubing, and this idea is reinforced by examination of the photomicrograph of this tubing. The surface is extremely rough with jagged peaks and troughs in the surface structure. Whilst this material may provide a surface at which reactions may occur, it clearly does not have holes in it into which selenium compounds may be trapped.

Since selenium diethyldithiocarbamate [$Se(S_2CNEt_2)_4$] may be used in silicone rubber as a vulcanizing agent[58] and other selenium compounds are used as colourants especially in the production of red coloured glass[51], the possibility of selenium being present in the matrix of the tubing itself could not be ruled out. Enquiries to the manufacturers were unsuccessful due to commercial confidentiality.

An attempt was made to digest the silicone rubber in a mixture of nitric and hydrofluoric acids, in a PTFE container. The resulting liquid was analysed for selenium, but again the results were inconclusive. Since selenium hexafluoride is a gaseous compound, it is possible that any selenium present was converted into this form, and was subsequently lost.

It would appear therefore that two possibilities could account for the enhancement. The first is that the former tubing contributes to the overall selenium level by releasing selenium into the analyte solution, this only occuring when nitric acid is present in trace quantities. This theory does

not explain why the enhancements are different, depending upon the order in which the solutions are prepared.

A second possibility concerns the apparent holes in the surface of the tubing. If selenium is attracted into these holes, then an equilibrium may be set up, in which there is an interchange between selenium in the solution passing through the tubing, and that held at the surface. When the standard solution is passed through the tube, a certain percentage will be retained on or in the wall of the tube. This will result in a given value being obtained by the detection system. If a nitro-selenium complex is formed, it is likely to be a large structure, and may be too great to enter the holes in the tubing. In such circumstances, more selenium will arrive at the detection system and will result in a higher reading, for exactly the same total selenium concentration. This could account for the observed effects regarding the order in which the samples were prepared. The largest enhancement effect occurred when the aliquot of concentrated nitric acid was added directly to the selenium stock solution. If the formation of a complex were to occur, it is likely to do so more efficiently at high concentrations of reactants. As dilutions occurred, e.g. by adding the nitric acid after the hydrochloric acid, the effects diminish. Clearly, further work would be necessary to prove this hypothesis, although this is outside the scope of this work. However, the study does demonstrate the need for caution when selecting appropriate tubing,

especially for use in on-line systems where a number of connections may have to be made.

3.3 Conditioning of selenium hydride generation systems

From the earliest experiments carried out during this study into selenium hydride generation, it was apparent that the response drifted with time in an upward direction i.e. a standard sampled at time zero would give a lower absorbance reading than the same standard run again say 30 minutes later. These early experiments all employed quartz furnace atomic absorption spectroscopy (QFAAS) as the detection system, with solution strengths in the 10 - 30 ng ml^{-1} range. It was noted that these apparent increases in sensitivity continued for an hour or so, after which time the system became more settled.

Instrument drift was at first suspected, and so it became the practice to set the instruments running at least an hour before any measurements were made, but this did not overcome the increase in sensitivity. It was noted that the monochromator in the atomic absorption spectrometer used did require frequent adjustment as it warmed progressively during use, but again this did not account for the change in sensitivity.

During conversations with other users of the hydride generation equipment, it became apparent that this effect was well known, although seemingly little documented. In most cases, users condition the system by running selenium solutions through the equipment a number of times before any

measurements are made. In addition, for highest accuracy, the procedure of bracketing should be employed (see section 3.2.2). The readings for the standards either side of the sample are averaged, and the reading for the sample calculated against this mean value. This is a very effective technique for overcoming drift, even fairly short term drift. It does however slow the rate of sample throughput, and since most workers use some kind of computer system for data capture and calculation of results, bracketing becomes something of a problem to fit into these systems. The Touchstone® software employed in these laboratories does allow for new standards to be run periodically, and will back calculate the concentrations obtained from the earlier results, but it does not do so relative to time. The user therefore obtains concentrations either based on the first set of standards, when the response was low or from the later set of standards, when the response has increased. The problem with this is that the increase in sensitivity occurs over a period, and since the exact rate of increase is unknown, it is impossible to calculate results obtained in the way described.

When using the QFAAS system, the detection limits obtained are generally in the low ng ml^{-1} region, and the variations due to the above effect are not too serious, except for the most accurate work. Once the atomic fluorescence system became available here this drift became more problematic, due to the much greater sensitivity of the instrumentation. During work in the BCR program to measure selenium in natural waters for

example, this increase in sensitivity became a real problem, even after conditioning of the equipment had been carried out in the way previously employed for use with QFAAS. Thus it was decided to investigate the process in more detail.

A 250 ml standard solution containing 1 ng ml^{-1} of selenium as sodium selenite in 3 mol l^{-1} hydrochloric acid was prepared. A 1.3 % solution of sodium borohydride in 0.1 mol l^{-1} sodium hydroxide was freshly prepared, as was a 3 mol l^{-1} solution of hydrochloric acid. A continuous flow hydride generation system was set up as described in Chapter 2, with the apparatus not having been employed for selenium determinations for at least four weeks prior to the experiment. This was coupled to an atomic fluorescence detection system. The detection system was switched on and allowed to warm up for two hours prior to use, and the hydride generator was allowed to operate pumping blank solutions for one hour prior to readings being taken. The selenium standard was then introduced in the normal way, and the results from making one determination every two minutes or so were recorded to produce the plot shown in Figure 3.3.1. The instrument conditions are shown in Table 3.3.1.

The commonly suggested explanation for the observed effect is that active sites on the surface of the glassware capture selenium hydride molecules and that this results in a diminution of the analyte reaching the detector. Gradually, these active sites are filled up, and thus an increase in the

Figure 3.3.1 Diagram of the signal drift against time

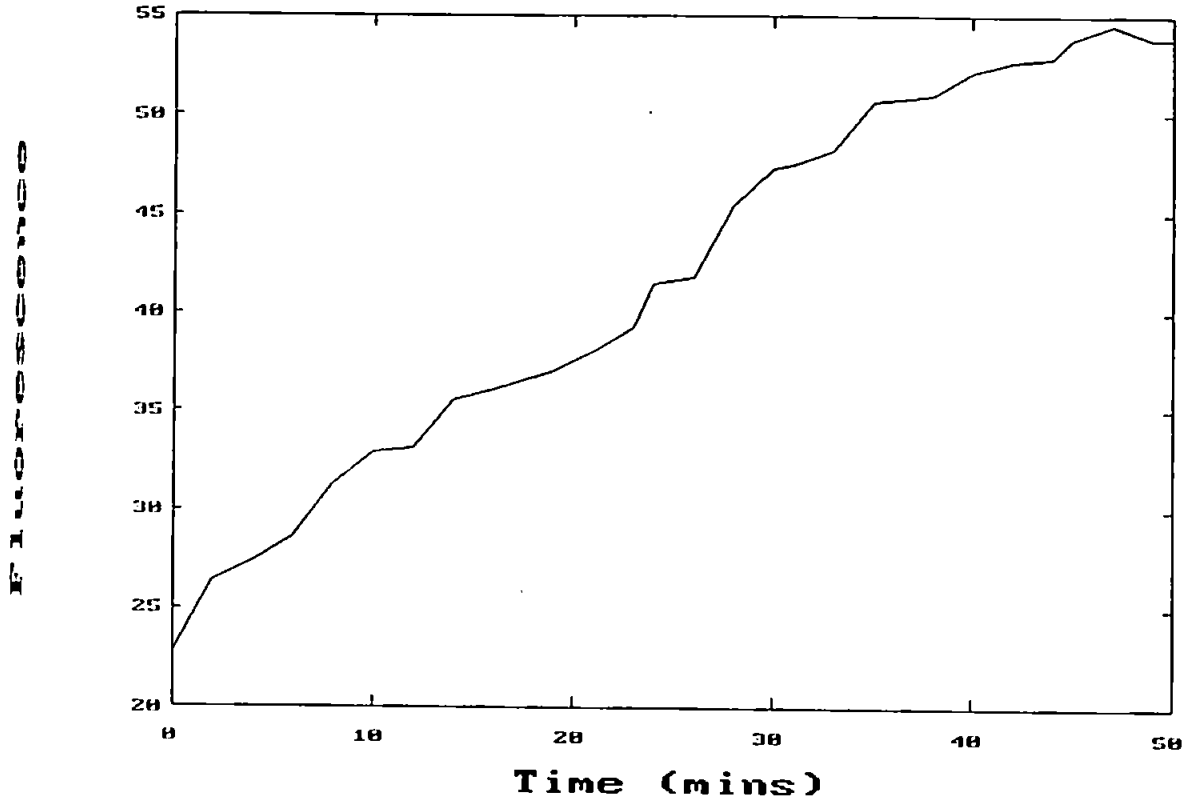


Table 3.3.1 Instrument operating conditions

Reductant flow rate	4 ml min^{-1}
Sample flow rate	8 ml min^{-1}
Blank flow rate	8 ml min^{-1}
Argon purge flow rate	200 ml min^{-1}
Instrument gain	100 x 10
Data collection mode	Peak height

numbers of selenium atoms reaching the detector occurs. Since the concentration of the selenium in these solutions is very low, the filling of the active sites is slow. This accounts for the observation that the effect is less of a problem when employing QFAAS - the higher detection limits of this system mean that higher concentrations of analyte are used, and therefore the sites are filled more quickly.

Thus it is recommended from this work that several injections are made using a high concentration of sodium selenite (1 μg ml^{-1}) at the commencement of work. This should saturate the active sites, and if the system is then left for a period of fifteen minutes before running the standards, equilibrium will be re-established.

3.4 Development of a microwave reduction system

As discussed above (Section 2.1), certain elements must be in the appropriate oxidation state in order to facilitate the formation of the hydride. In the case of selenium, this is the +4 state. When determining total selenium by hydride generation it is therefore necessary to perform a pre-reduction step in order to reduce any selenium(VI) which may be present to selenium(IV). A number of systems may be used to achieve this reduction. D'Ulivo[59,60] and others[61] are advocates of hydrogen bromide reduction, but by far the most commonly employed technique uses hydrochloric acid at an elevated temperature in an open topped container. The reduction reaction proceeds as follows :

$$HSeO_4^- + 3H^+ + 2Cl^- = H_2SeO_3 + Cl_{2(aq)} + H_2O$$

The reaction is generally carried out at about 70 °C, although other temperatures have been reported, with greater or lesser times quoted for the reaction[62-64]. Analyte losses have been reported when the solution has been boiled, and these losses have been attributed to volatile selenium chlorides and oxo-chlorides such as $SeO.Cl_2$ and $SeO_2.2HCl$[65], partial reduction to metallic selenium[65] or adsorption onto the vessel surface[66]. Using the radiotracer ^{75}Se, Piwonka et. al.[67] found that when using 5M hydrochloric acid and boiling the mixture for 25 minutes or more, although all of the radio-tracer remained in the solution, the proportion converted into the hydride

decreased, indicating that some change in oxidation state had occurred, probably to Se(0).

Again employing radiotracers, Krivan et. al.[68] established that back oxidation occurred when the reduced solution were left before analysis, and this could explain some of the apparent losses which had been reported. After one week, 94% of the Se(IV) produced had been oxidised back to Se(VI), and was thus rendered undetectable by hydride generation (without a further reduction step). The back oxidation may be explained by the reaction :

$$H_2SeO_3 + Cl_2 + H_2O \rightleftharpoons H_2SeO_4 + 2HCl$$

Over a further period of four weeks, the 6% remaining as selenite did not change, and this may therefore be considered to be the equilibrium concentration. This back oxidation may be obviated to an extent by the use of an open topped vessel in which to heat the acidified sample, thus allowing the chlorine generated in the reduction reaction to escape.

When the determination of total selenium is required, the pre-reduction step may be considered to be a nuisance, since it involves considerable effort on the part of the chemist, and also provides opportunities for contamination to occur.

When speciation determinations of selenium are required, the

inability of selenium(VI) to form the hydride may be used to advantage. Since in the inorganic form, selenium exists only in the oxidation states of +4, +6 and 0 (elemental selenium), by first analysing the sample before reduction, the concentration of selenium(IV) is determined. If the sample is then subjected to a reduction step, and then re-analyzed, total inorganic selenium will be determined. The concentration of selenium(VI) may therefore be obtained by subtracting the selenium(IV) figure from the total selenium concentration. With the current interest in selenium speciation, it was decided to investigate the possibility of an automated on-line speciation system, capable in the first instance of determining selenium(IV) and selenium(VI) (via the total) but with the potential to be adapted for use with other elements with reducible species such as arsenic and tin.

3.4.1 Design criteria

The basic flow injection-hydride generation system is described in section 2.1.2, and this formed the basis for the work. It was recognised that the determination of selenium(IV) could be carried out on-line by simply segmenting the incoming sample stream. Whilst some peak spreading could occur, this is not a huge problem when determining selenium using hydride generation, since measurements are made when the system is in equilibrium. In addition, by measuring peak areas, all selenium hydride generated could be ascribed to the

appropriate sample. If part of the sample could be reduced on-line and subsequently analyzed, then a figure for the total selenium concentration could also be obtained. The main problem became one of heating the sample in order to achieve the desired reduction step.

It was the general practice in these laboratories to reduce a sample by adding an equal volume of hydrochloric acid, and then heating the mixture in a beaker in a water bath at 70 °C for 30 minutes. The sample was then cooled, any losses caused by evaporation made up with water, before the analysis was performed. Since some acid would also undoubtedly be lost during heating, this process was not ideal, since it is important to matrix match samples and standards as closely as possible if greatest accuracy is to be obtained. In designing the on-line system, the assumption was made that the reduction took in the order of 30 minutes. Assuming that the reaction time halved for each 10 °C rise in temperature, then it was supposed that it would be necessary to carry out the reduction in the gas phase in order to achieve acceptable reduction times to suit an on-line system.

A number of heating systems were considered including oil baths, "graphite baths" [69], and so on, but the obvious choice for such work was microwave heating.

3.4.2 Principles of microwave heating

There are three ways in which heat may be transferred from a source to a body, these being conduction, convection and radiation. Whilst almost all commonly employed heating systems are specified as relying on one of these heating methods, heat is in fact transferred from the heat source to the body being heated by at least two, and more generally all three systems. Using the example of heating a beaker of water on a hot plate for instance, most heat is transferred directly via conduction. However, since all bodies with temperatures above absolute zero emit infra red radiation, some heat transfer will occur through radiation from the hot plate being absorbed by the beaker and contents. There will also be convection currents in the air surrounding the top of the hot plate and the beaker, and these will contribute to the overall heat gain of the water and beaker. Within the liquid in the beaker, there will also be convection currents at work, transferring the heat throughout the body of the liquid. From this seemingly simple example, it can be seen that all three heat transfer systems make a contribution to the overall heating process.

Microwaves are a form of electromagnetic radiation, and have a frequency range from 300 MHz to 300 GHz. This radiation is non-ionizing, and causes molecular motion by migration of ions and rotation of dipoles. It does not however, cause any change in the molecular structure of the material being heated.

Materials range in their ability to absorb microwave radiation from those which are reflective, and do not therefore absorb any energy, through those which absorb some of the energy, to those which are totally transparent, and again do not absorb any of the energy. Only absorptive materials are heated by microwave energy. Reflective materials such as metals find use in the construction of the inside of the microwave oven, whilst transparent materials are used as containers for the materials which require heating.

Absorptive samples which are subjected to microwave energy will heat up partly in accordance with their dissipation factor, tan δ, which is the dielectric loss of the material divided by the dielectric constant of that material. The dielectric loss is a measure of a sample's ability to dissipate the input microwave energy in the form of heat, whilst the dielectric constant is a measure of the sample's ability to impede the microwave energy as it passes through the sample[11]. Thus when microwave energy enters a material, the rate at which the energy is absorbed depends upon the dissipation factor of that material. Transparent materials have a tan δ of infinity, and reflective materials have a value of tan δ of zero. Materials which have a high dissipation factor provide a resultant lower penetration of microwave energy at a given frequency. One way of characterizing penetration is by considering the half-power depth. This measure is the depth below the surface of the material at which the power density is half that at the

surface. The half-power depth varies with frequency, by approximately the inverse of the square root of that frequency, and is therefore specified at a given frequency.

As stated above, the two mechanisms by which microwave energy is transferred to a material are ionic conduction and dipole rotation, and in most practical applications, these processes occur simultaneously. Ionic conduction occurs as a result of the applied electromagnetic field, and is the conductive migration of dissolved ions, resulting in heating due to the electrical resistance of the sample to induced current flow. The absorption of energy due to ionic migration depends upon the size, charge and conductivity of dissolved ions, and is subject to ion interaction with solvent molecules. Thus the factors which affect ionic conduction are ion concentration, ion mobility and temperature. In an aqueous solution of sodium chloride, there is an approximately fourfold increase in tan δ between 0 molar (i.e. pure water) and a 0.5 molar solution.

The other cause of microwave heating is due to dipole rotation. When an electric field is applied, there is a partial alignment of molecules which have a permanent or induced dipole moment. In fact, the average time spent by the molecule in the aligned state is only slightly greater than the time it spends in a non-aligned position, and when the electric field starts to collapse, thermal disorder is restored. During the period of alignment, a small amount of energy is taken up by the molecule, and when relaxation of the

field occurs, this energy is absorbed by the system as heat. The efficiency of heating due to dipole rotation is dependant upon the sample's temperature and viscosity, which affect the dielectric relaxation time of the sample in question. At 2.45 GHz, one of the four frequencies allocated for microwave heating, alignment and randomising of the molecules occurs 4.9 x 10^9 times per second.

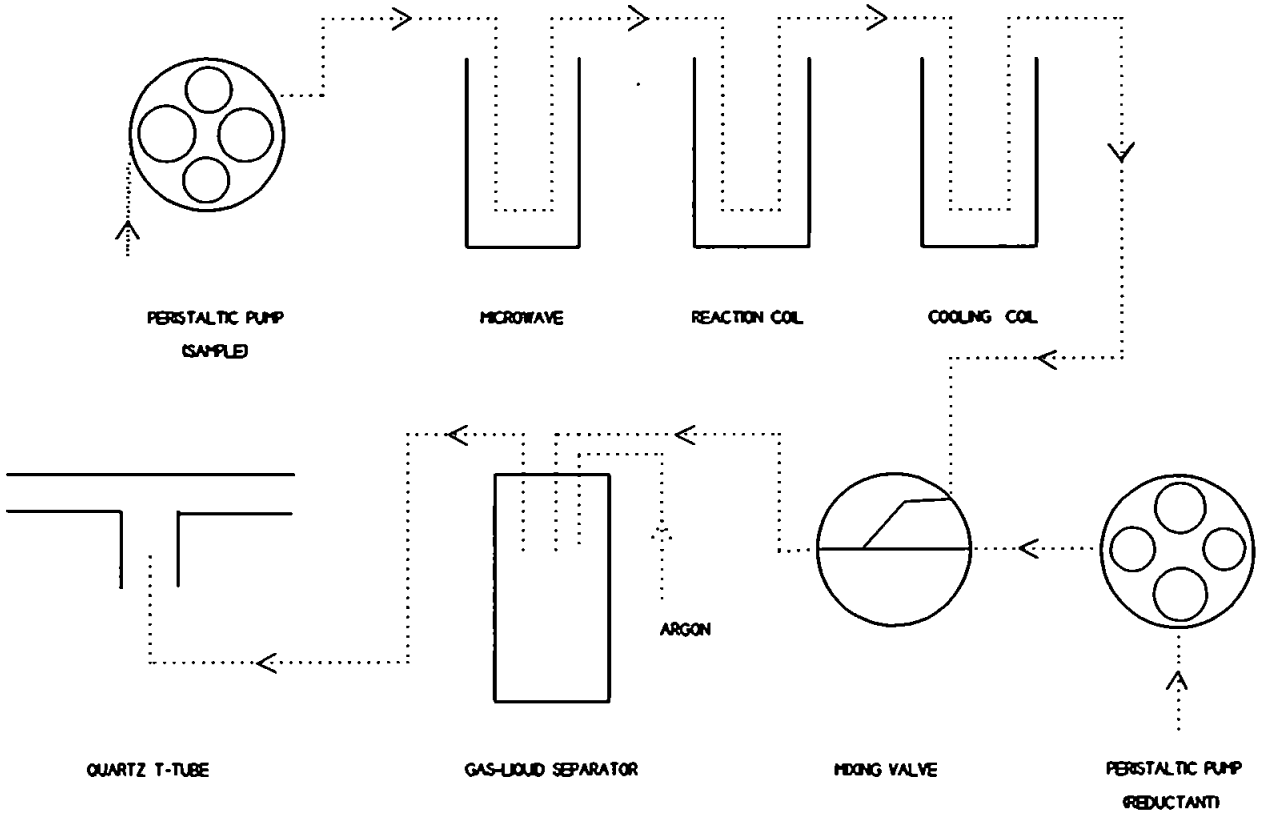
The relative contributions made by dipole rotation and ionic conduction in the heating of a sample vary with temperature, and with the composition of the sample. In ice, the molecules are rigidly held in a crystal lattice, and therefore molecular mobility is inhibited. The dielectric dissipation factor is therefore correspondingly low - 2.7 x 10^{-4} at 2.45 GHz, as opposed to the value of 12.2 for water at 27 °C.

3.4.3 The basic on-line microwave system

Once it had been established that heating by microwave did produce the required reduction of selenium(VI) on a batch analysis, a basic on-line system was devised. This is shown in Figure 3.4.1.

The acidified sample is introduced via a peristaltic pump to the heating coil contained in a microwave cavity. From here,

Figure 3.4.1 Diagram of the basic on-line microwave reduction system



the sample travels on through a reaction coil which is unheated, and thence to a cooling coil contained in an ice bath. After cooling, the sample enters the mixing valve, where it meets a stream of sodium borohydride solution, which is pumped using a second peristaltic pump. The reacting mixture then passes to a gas-liquid separator, from whence the hydrogen selenide produced is flushed into a flame heated quartz tube in an atomic absorption spectrometer.

The early problems encountered involved the aforementioned assumption regarding the temperature and residence time in order for the reduction reaction to take place. Attempts were made to produce a flow system which did not suffer from bumping, caused by uneven boiling of the reactants in the heating coil. The first heating coil employed is shown in Figure 3.4.2.a, but did suffer from violent bumping. A number of alternatives were evaluated, culminating with the design shown as 3.4.2.b. It was found that bumping was considerably diminished using this coil. In this design, the reactant solution passes through a mass of minute glass beads, which cause dispersion of the liquid and thus provide a very large surface area. As the liquid boils, there is also a damping effect by the glass beads, resulting in a much smoother boiling process. Unfortunately, even with the less violent boiling produced by this heating cell, the huge changes in volume produced by first boiling the liquid, and then cooling it back to about room temperature, resulted in a very unstable baseline.

Figure 3.4.2 Initial heating coil designs

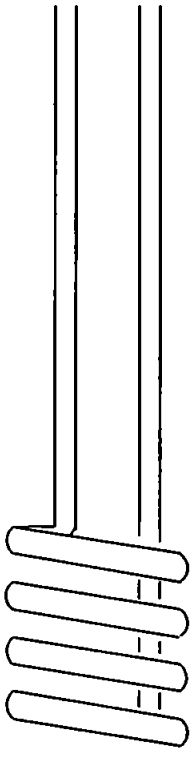


FIGURE 3.4.2a

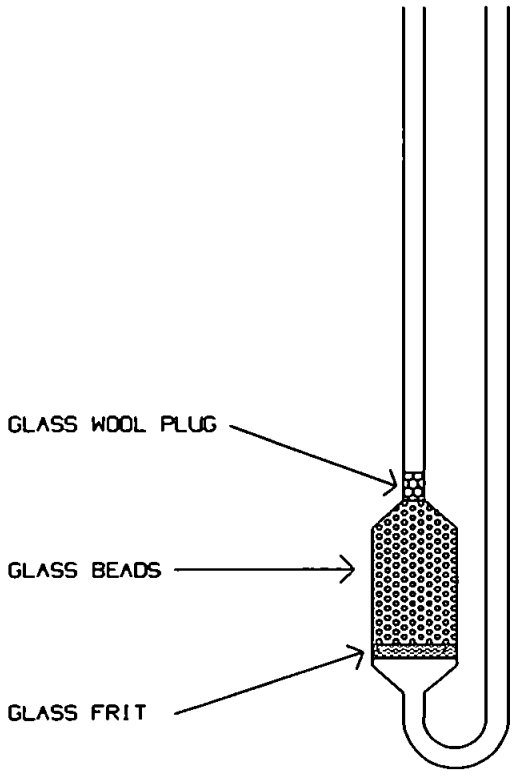
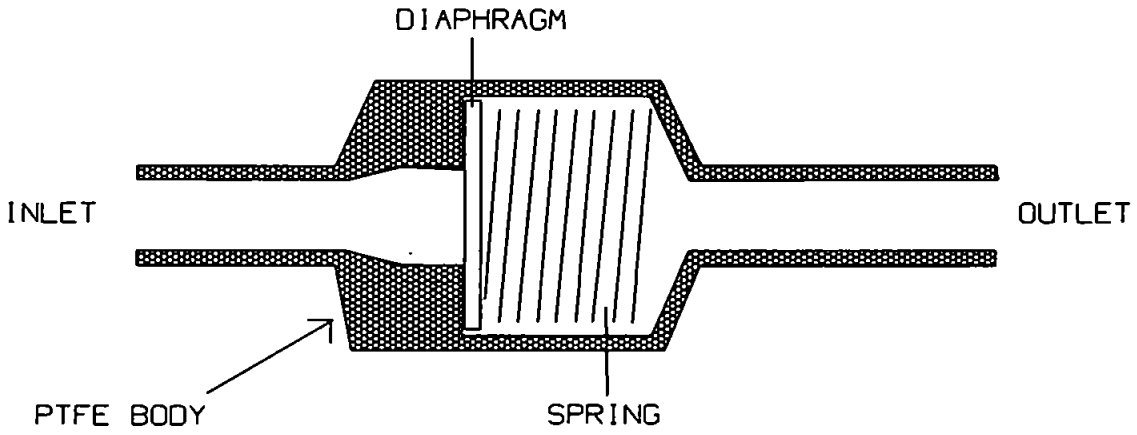


FIGURE 3.4.2b

It was observed that rapid fluctuations occurred in the flow rate of the analyte into the gas-liquid separator, and it was realised that these would cause the base-line instability. An attempt to dampen out these fluctuations using pulse damping tubing was made. This commercially available product consists of a narrow bore tube, which has more lateral elasticity built into the tube material than is normal. The result should be that as the pressure increases due to a pulse, the tube wall dilates to absorb the increase. As the pulse subsides, the reverse takes place, so damping occurs. Attempts to employ this type of product on this system were totally unsuccessful - it is felt that they are almost certainly more suited to liquid only systems in which the pressure increases would be greater.

Pressure check valves are commercially available, but are very expensive, and so one was designed and constructed from available materials, the design of which is shown in Figure 3.4.3. As can be seen, it is simply a non-return valve, the body fabricated from P.T.F.E., as was the diaphragm. The major problem of the valve involved the spring. Clearly, this needed to be fabricated from an inert material, and although a number different plastic materials were tried, none proved able to withstand the hostile chemical environment in which they were required to operate.

Figure 3.4.3 Diagram of a non-return valve



Since the system still suffered from an unacceptably fluctuating baseline, it was decided to investigate whether the system could be operated at a lower temperature, below the boiling point of the analyte mixture, and still give consistent reductions. It was theorised that even if all the selenium(VI) present was not reduced, if a constant proportion was reduced, then the basis of a reduction system could exist. It was thus discovered that "conventional wisdom" had been wrong regarding the kinetics of the reduction reaction, and later work (see seStion 3.7) resulted in providing a more accurate idea of how long the reduction actually takes.

By careful optimisation of the system parameters, it was found that total reduction could be achieved without boiling, and on a time scale which enabled an on-line system to be developed.

First, the effect of differing hydrochloric acid concentrations were investigated since, for economic reasons, it is desirable to use the lowest acid concentration consistent with full reduction. A 25 $\mu g\ l^{-1}$ solution of selenium as sodium selenate was prepared, aliquots taken and acidified to produce equal volumes of sample with acid strengths of 2,4,6,8 and 10 $mol\ l^{-1}$. The resulting absorbances are shown in Table 3.4.1. As may be observed, no increase in absorbance occurred at concentrations above 6 $mol\ l^{-1}$, and so this acid strength was employed for the rest of the work.

Table 3.4.1 Absorbance readings for incresing concentrations of hydrochloric acid

Acid concentration (mol l^{-1})	Absorbance
2	0.005
4	0.011
6	0.160
8	0.160
10	0.159

Since the output of the microwave unit was adjustable in 5% steps, it was decided to set the flow rate of the sample at the maximum permitted using the particular pump and tubing combination employed (9.3ml min^{-1}), and to vary the power input to the sample cell. The power output to achieve maximum reduction efficiency was then optimized. This was achieved at a power setting of 35%, with a diminution occurring above this figure. This reduction in efficiency may be due to the back oxidation of the selenium(IV) by free chlorine.

A number of different designs of heating, reaction and cooling coils were evaluated, before arriving at a system which provided maximum conversion efficiency. The coils were fabricated in house from boro-silicate glass, to the structure shown in Figure 3.4.4 and sizes shown in Table 3.4.2.

Once these parameters had been optimized, the system was evaluated using sodium selenate standard solutions in the range 10 - 50 $\mu g\ l^{-1}$. Reductions were carried out using both the on-line microwave system and the conventional method of heating aliquots of the solution in 6 mol l^{-1} to 70 °C for 30 minutes on a hot plate. The results are shown in Figure 3.4.5. The results indicate that the response of the microwave flow injection system was linear up to a concentration of 30 $\mu g\ l^{-1}$, which represents an absorbance of 0.3, above which the plot becomes non-linear due to self absorption.

Figure 3.4.4 Diagram showing the construction details of the heating, reaction and cooling coils

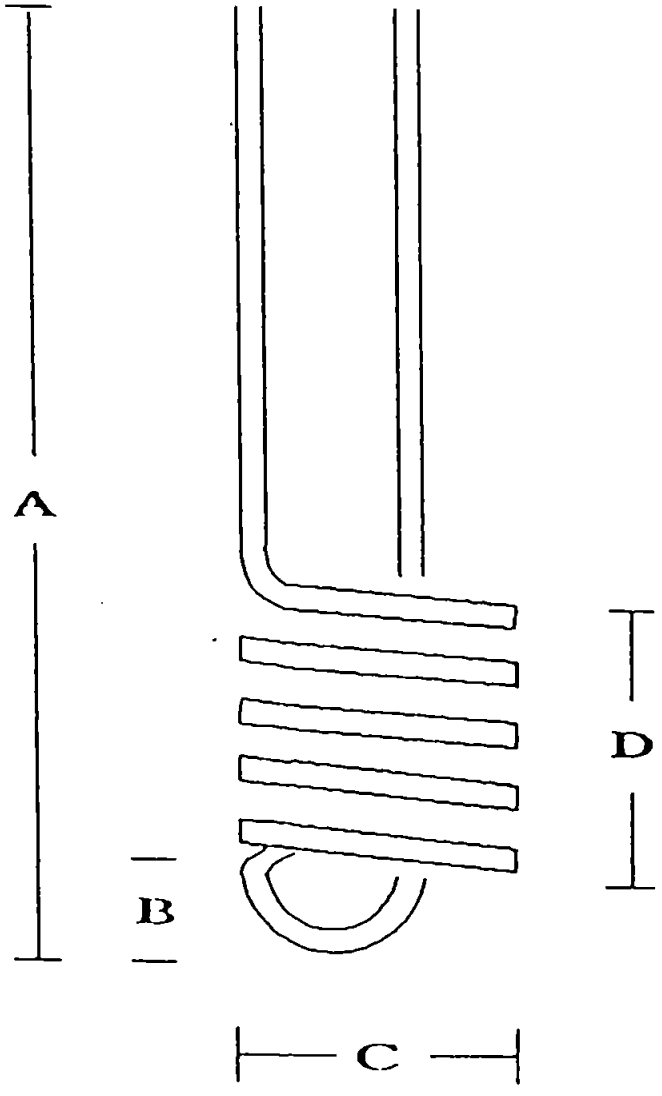
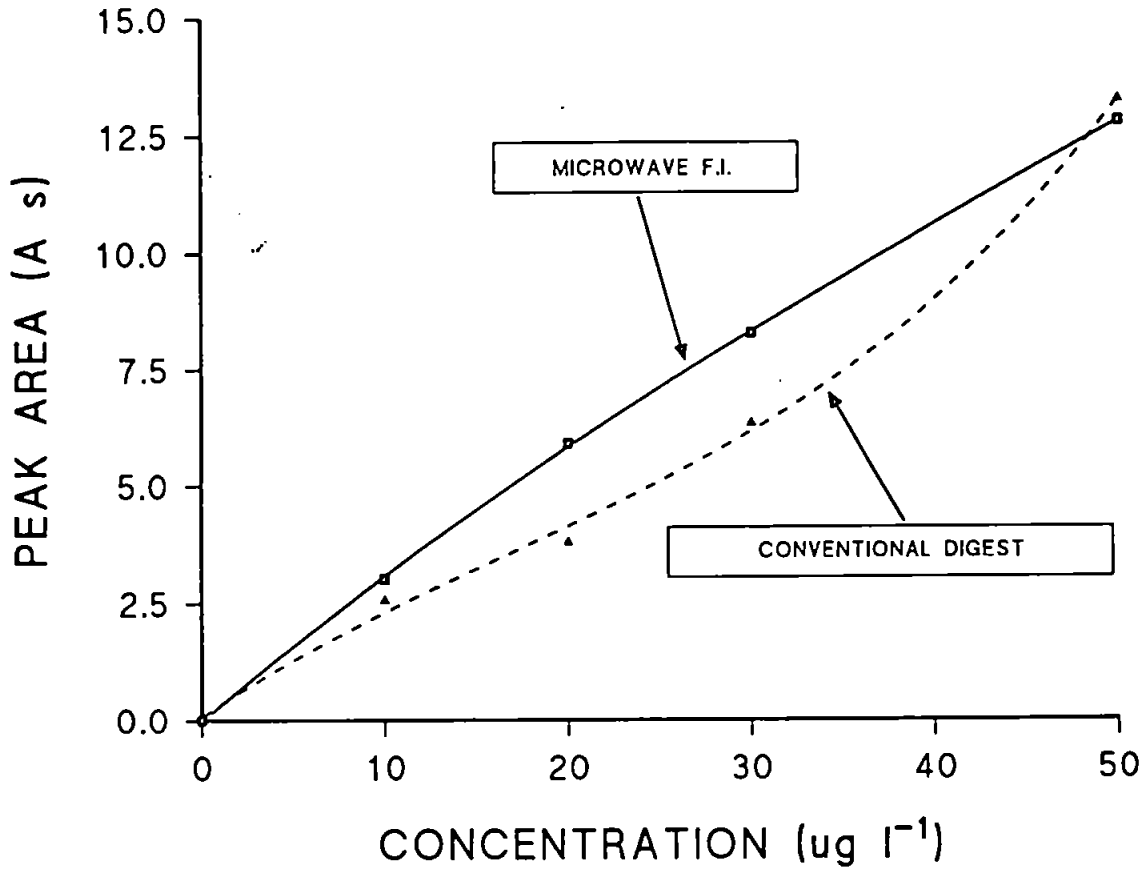


Table 3.4.2 Table of the dimensions of the heating, reaction and cooling coils

	A mm	B mm	C mm	D mm	N turns
HEATING COIL	200	13	20	20	5
REACTION COIL	180	0	20	55	13
COOLING COIL	200	10	20	100	25

Figure 3.4.5 Comparison between microwave flow injection and conventionally digested standards

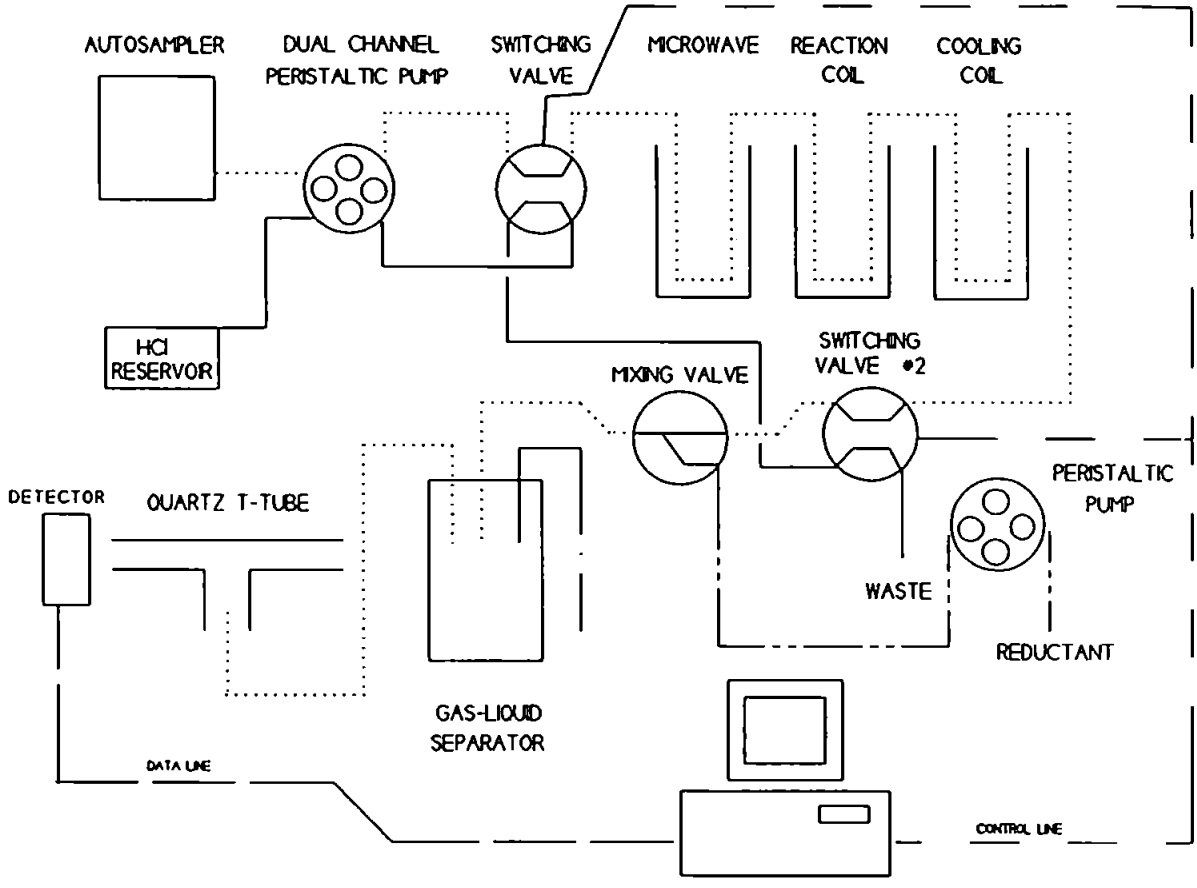


3.5 Incorporation of microwave heating into a fully automated hydride generation system

Since the efficacy of the technique to provide the reduction of selenium(VI) to selenium(IV) had been established, the basic system was developed to provide a totally automated speciation system. The design for this system is shown in Figure 3.5.1. In this system, a dual-channel peristaltic pump introduces both a sample stream and hydrochloric acid stream to a switching valve which is under computer control. In the position shown in the diagram, the sample travels through the microwave route, and is directed by a second computer controlled switching valve through the hydride generator stage and on to the detector. Hydrochloric acid is meanwhile pumped from the reservoir, bypasses the microwave unit, and is directed to waste by the second switching valve.

When switching valve 1 is rotated through 90°, the sample passes to waste, whilst the hydrochloric acid stream passes into the microwave unit. The second switching valve controls which of the two streams passes on to the hydride generation step, and subsequently to the detection system. Should sample volumes be limited, the autosampler may sample from a second hydrochloric acid reservoir, once the sample has been introduced to the system. It is obviously vital that the microwave unit has either a blank acid stream or a sample stream passing through it at all times.

Figure 3.5.1 Computer controlled automated microwave reduction selenium speciation system



The switching arrangements, timings and resultant signal profiles are shown in Figure 3.5.2. Using this system, throughput rates of fifteen samples per hour were achieved. This figure could almost certainly be improved by upon further optimization of purge gas rates and modifications to the design of the mixing valve. These should reduce the signal decay times, and thus increase the speed of the system.

To validate the system, a certified reference material, NIST 1643c Trace Elements in Water was analyzed, and the results obtained are given in Table 3.5.1. As can be seen, good agreement was obtained between the total selenium concentration (12.3 $\mu g\ l^{-1}$) in the samples analyzed, and the certified figure of 12.7 $\pm$ 0.7 $\mu g\ l^{-1}$. It was further found that the presence of small concentrations of nitric acid had no effect on the accuracy of the results, provided that matrix matching of sample and standards was carried out, as is, or should be normal practice.

Figure 3.5.2 Valve switching and timing diagram

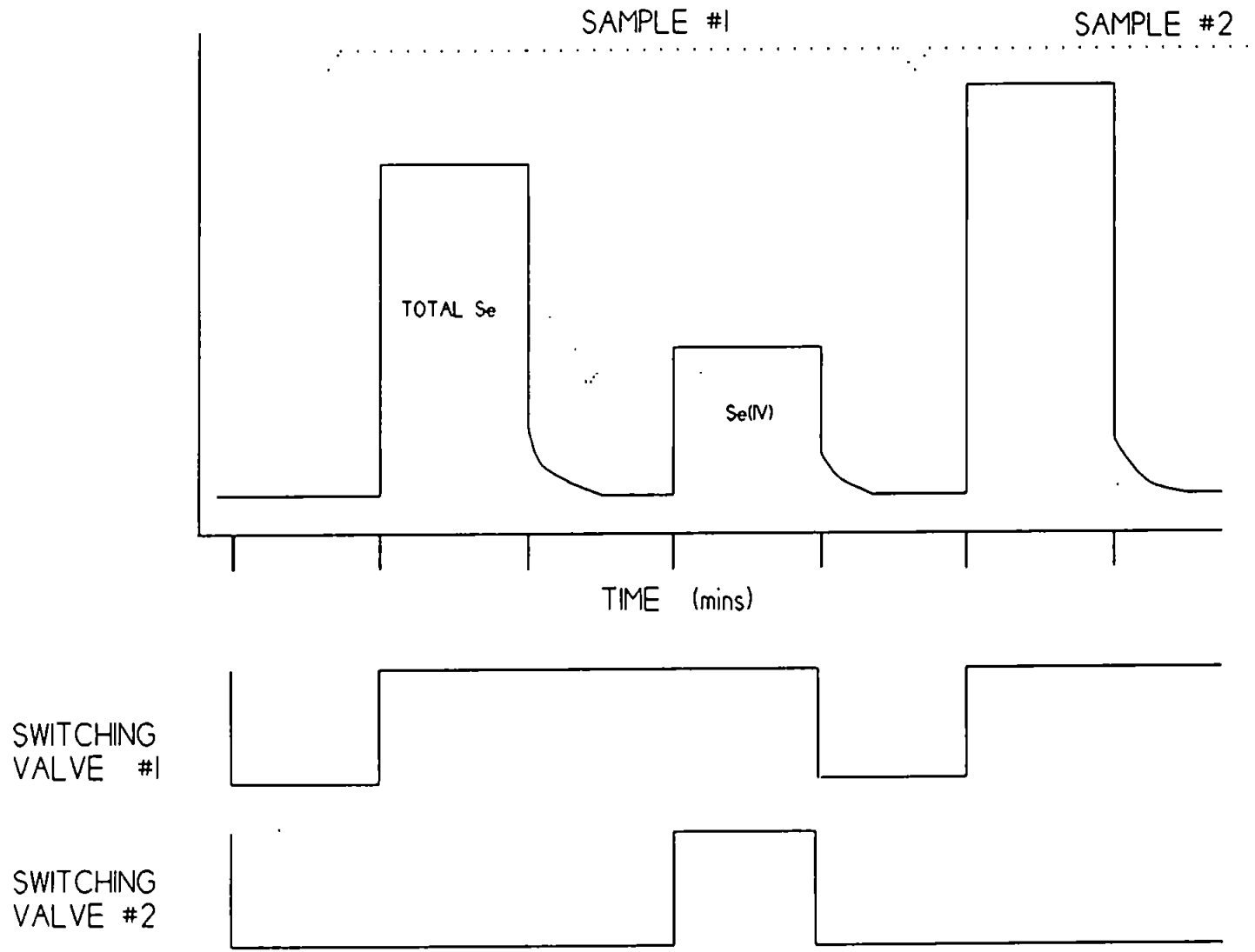


Table 3.5.1 Analysis of Certified Reference Material NIST 1643c - determination of selenium

Analysis no	Se(IV) µg l^{-1}	Se(VI) µg l^{-1}	Tot. Se µg l^{-1}
1	9.8	2.5	12.3
2	9.8	2.5	12.3
3	9.8	2.6	12.4
4	10.0	2.6	12.6
5	9.9	2.4	12.3
Mean	9.8	2.5	12.3

3.5.1 Data handling

When selenium is determined using flow injection-hydride generation, steady state conditions are usually sought in the gas-liquid separator, and a flat topped, plateau shaped peak results. This peak shape lends itself to quantification using peak height measurements. However the Touchstone Software (PS Analytical Ltd., Orpington, Kent) used with the system employed also enabled peak area measurements to be made. Since in practice not all peaks were ideal in shape, a comparison of the results obtained from identical samples was carried out, using both peak area and peak height modes. Both methods of quantification gave almost identical results. However, the peak area mode was adopted for the rest of this study.

The software uses a system of continuous measurement, and performs a box-car integration on the readings from the analogue to digital converter. The programme is written in Turbo Pascal, and the essential elements are illustrated in Appendix 1. This shows the method in which the data is handled, the full programme being more complex due to the inclusion of hardware operating instructions etc.

3.6 Selenium speciation studies

The automated system described in the previous section was used to evaluate the inorganic selenium speciation in a number of aqueous samples. The results compared favourably with those obtained using other techniques. However the basic on-line system relied upon the difference method of calculating the results. A number of criticisms of this approach can be made. The prime reason for not applying a difference calculation is that if an error is made in the determination of one of the species, this result automatically affects the result obtained via the calculation for the other moiety. Thus further development of the system was investigated in order to see if it could be modified or altered to determine each species directly.

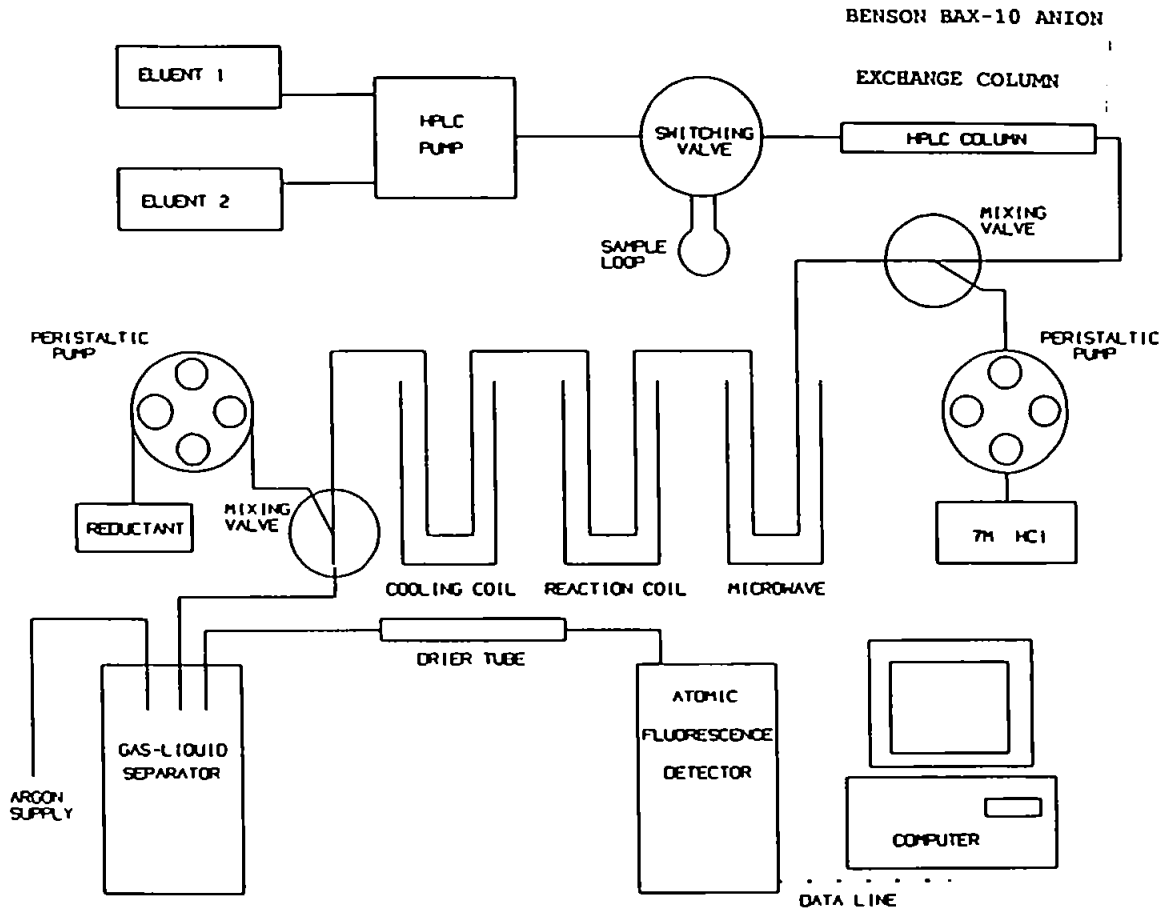
In order to achieve this, it would clearly be necessary to separate the species prior to analysis, and the obvious way in which to attempt this separation was by the use of High Performance Liquid Chromatography (HPLC). The advantage of employing HPLC for the separation is that the species present are analyzed directly without derivitization. The main problem however, in adapting the system described in the previous section to incorporate HPLC separation, became one of detector sensitivity. A typical elution rate for HPLC is 1 - 1.5 ml min^{-1}, the sample introduction rate in the system described in Chapter 3.5 is 8 ml min^{-1}, and this mismatch also had to be overcome. In addition, the previous system used a pre-

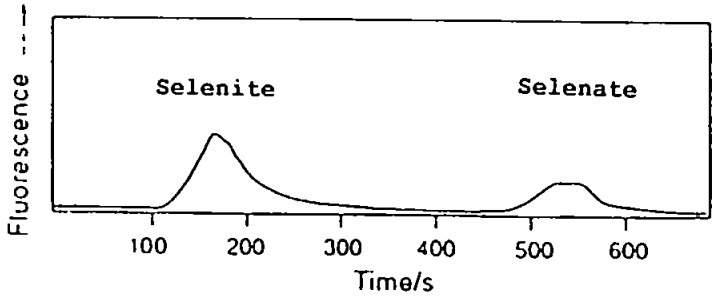
acidified sample with an acid concentration of 6 mol l^{-1} - such an acid concentration could not be employed with an ion exchange column, since it would destroy the column. Therefore post-column acidification would be required, further diluting the eluent. It became obvious that AAS would be insufficiently sensitive to be used as the detector, and the atomic fluorescence detector would have to be employed. A system was therefore set up as shown in Figure 3.6.1.

As may be observed from the diagram, the first block of instrumentation is a simple HPLC set up including the ability to switch between different eluents. A post-column mixing valve was fitted and 7 mol l^{-1} hydrochloric acid was introduced to the analyte stream at the rate of 7 ml min^{-1}, thus achieving the desired flow rate and acid concentration for the microwave reduction step. The remainder of the system was similar to that employed in earlier automated system, up to the gas-liquid separator. The selenium hydride and hydrogen mixture emanating from the separator was passed through a membrane drying tube before entering the atomic fluorescence detector. Drying of the gases are necessary due to the fact that water vapour quenches fluorescence.

The first experiments carried out were to evaluate eluents, to see if separation could be achieved. A mixed standard containing 5ng ml^{-1} of selenium as both sodium selenite and sodium selenate was prepared, and used to determine the performance of the eluents. Initially, low concentrations of

Figure 3.6.1 Diagram of the automated HPLC-microwave reduction-hydride generation-AFS selenium speciation system





Typical chromatogram of 5 ng g^{-1} selenium as selenite and selenate

phthalate (100 mmol l^{-1}) were employed, and whilst excellent separation was achieved, crystals of phthalic acid formed at the post-column mixing valve when the acid stream met the column eluent. These crystals eventually led to a blocking of the tubing, rendering phthalate unsuitable for use. Potassium sulphate was then tried, and this provided base-line separation when a step concentration gradient was employed, i.e. 25 mmol l^{-1} at pH 5 for the first 200 seconds followed by 100 mmol l^{-1} at a similar pH for the remainder of the run. A number of different pHs were evaluated between pH 5 and pH 7, with no discernable differences being detected in either peak shape or peak area. The conditions pertaining to the microwave operation were as described previously in Chapter 3.5.

Once the system had been shown to operate efficiently, five identical injections of the mixed standard were made and the peak areas measured. These results are shown in Table 3.6.1. As can be seen, the precision offered by the system is in the order of 1.5 and 2.0% RSD for selenite and selenate respectively. The limits of detection were then determined for selenium(IV) and selenium(VI) and were found to be 0.2 and 0.3 ng ml^{-1} respectively.

In order to evaluate a real sample, a Certified Reference Material, NIST SRM 1643c - Trace Elements in Water was analyzed. It was found to contain 10.6 ng ml^{-1} of selenium(IV) and 2.8 ng ml^{-1} of selenium(VI), giving a total selenium content of 13.4 ng ml^{-1}. This is in good agreement with the

Table 3.6.1 Analysis of a mixed selenium standard (5 ng ml^{-1} Se(IV) and Se(VI))

	Peak areas	
Analysis	Se(IV)	Se(VI)
1	761	249
2	776	238
3	770	249
4	761	247
5	788	241
Mean	771.2 s	244.8 s
s_{n-1}	11.34 s	5.02 s
LOD (3 x s_{n-1})	0.22 ng ml^{-1}	0.31 ng ml^{-1}

certified value of 12.7 ± 0.7 ng ml^{-1}. No reference material is currently available with certified values for individual selenium species. The values obtained agree closely with the values previously obtained with the earlier automated system. It was found that higher concentrations of selenium(VI) gave rise to an elevated base-line, but this could be overcome by dilution of more concentrated selenium(VI) solutions. The full operating conditions employed for the total system are shown in Table 3.6.2.

The system was then employed to evaluate the concentrations of the selenium species in samples of selenium solutions supplied to this laboratory for analysis under the BCR selenium analysis programme. The concentrations obtained were in good agreement with those obtained by conventional reduction and subsequent analysis via hydride generation coupled to atomic fluorescence detection.

Table 3.6.2 Optimized operating conditions for the complete system

HPLC	
Sample loop	1 ml
Sample pH	7
Mobile phase 1	25 mmol l^{-1} K_2SO_4, pH5
Mobile phase 2	100 mmol l^{-1} K_2SO_4, pH5
Mobile phase switching	200 seconds
Eluent flow rate	2.0 ml min^{-1}
Microwave	
Power	20% continuous
Hydride generation	
Reductant flow rate	4 ml min^{-1}
HCl flow rate	8 ml min^{-1}
Argon purge flow rate	350 ml min^{-1}
Argon drier flow rate	1000 ml min^{-1}
Detector	
Primary lamp current	25 mA
Boost lamp current	25 mA
Gain	1000 x 10

3.7 An investigation into the kinetics of the reduction of selenium (VI) to selenium (IV)

As discussed in Section 3.1, there appears to be conflicting reports in the literature regarding the rate at which selenium(VI) is reduced to selenium(IV). This disagreement is also reflected in the recommended conditions for which the reduction should be carried out, the debate being fuelled by an apparent loss of selenium during the reduction process.

As previously stated, the most commonly employed method for the reduction of selenium(VI) is to heat the sample to 70 °C in the presence of 6M hydrochloric acid for a given period. "Conventional wisdom" suggests that this period is usually thirty minutes. A number of workers have reported that heating above this temperature causes a loss of volatile selenium compounds. It has been reported by Krivan et. al. [68] following work using radiotracers, that apparent losses of selenium were in fact due to the back oxidation of the selenium(IV) produced, by the action of free chlorine. Their work showed that if the selenium(IV) solution produced by the hydrochloric acid reduction was left for one week, 94% of the selenium(IV) was converted back to selenium(VI). This figure remained constant over the next four weeks, and so may be regarded as the equilibrium concentration. Since the generation of the hydride depends upon the selenium being in the selenium(IV) oxidation state, it is clear that the analytical determination should proceed immediately upon completion of the reduction

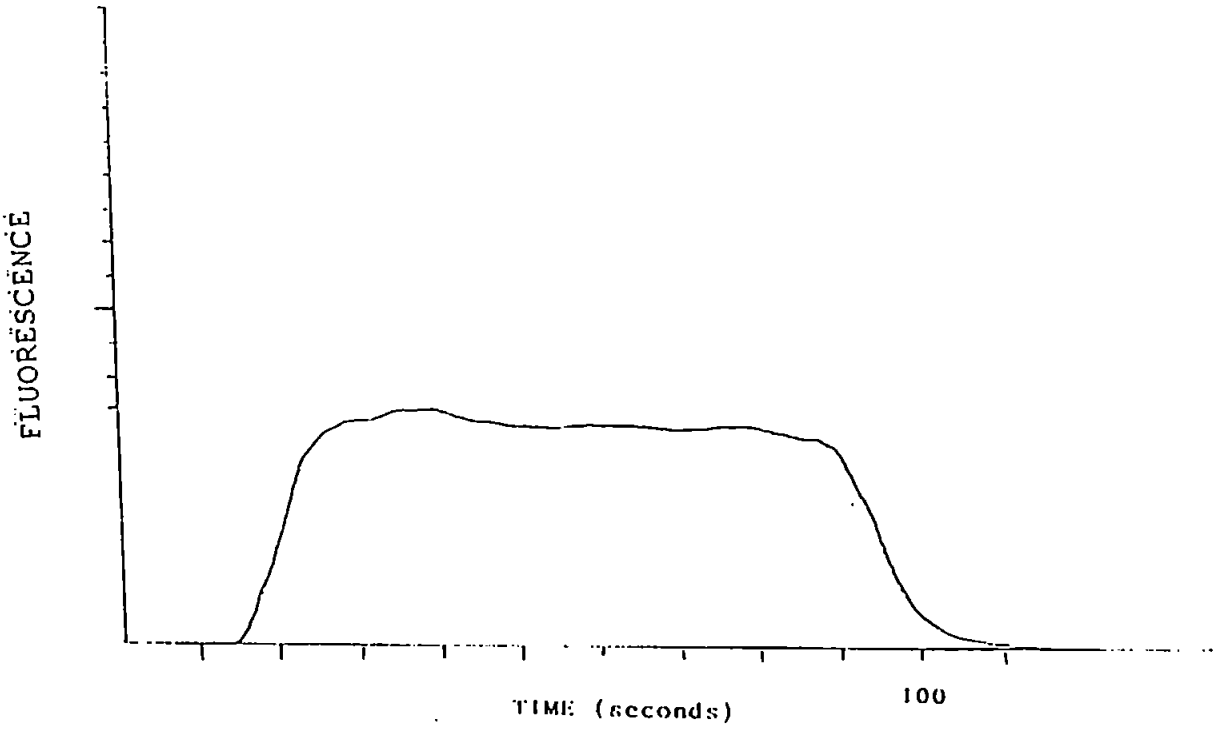
step. It is also easy to understand the early confusion produced by this back oxidation.

During the development of the microwave reduction system (Section 3.4), it had been assumed that if the reduction step took thirty minutes at 70 °C, then in order to achieve reduction in an on-line process, the reduction reaction would have to be carried out at a higher temperature. This would be necessary due to the limited time that the analyte would be in the system. It was anticipated that the reaction would have to be carried out in the gas phase. As discussed above in Section 3.4.1, this was not in fact found to be the case, and so the kinetics of the reduction were studied in more detail.

3.7.1 Experimental

The determination of trace levels of selenium has generally relied upon the technique of hydride generation, coupled to an element specific detector, since it was introduced by Holak. Latterly, the use of a quartz furnace-atomic absorption spectrometer (QFAAS) as a detection system has become increasingly common, since it provides good detection limits, good reproducibility and employs equipment commonly available in most laboratories. Using this technique, with a continuous flow hydride generator similar to that shown in Figure 2.1, a typical analysis may take in the order of 2 minutes, and give a response as shown in Figure 3.7.1. As can be seen,

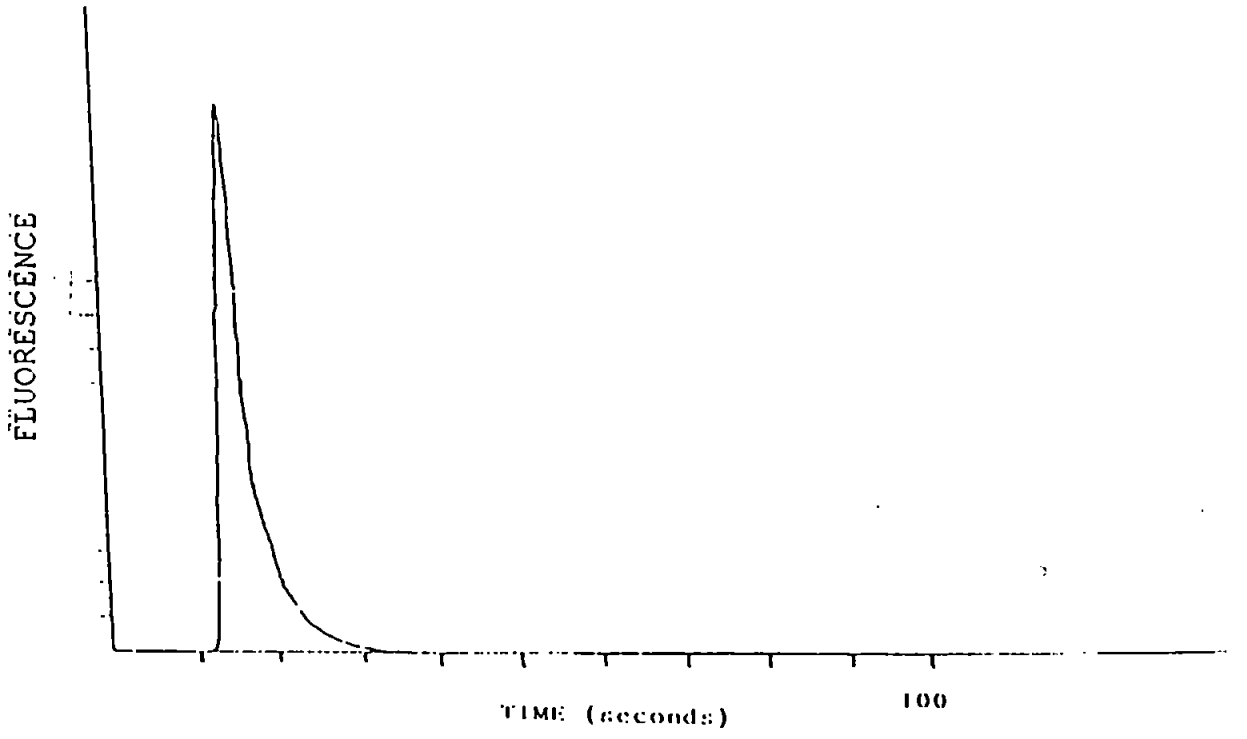
Figure 3.7.1 Typical analysis trace obtained by hydride generation operating under equilibrium conditions



there is an initial delay period of typically 10-15 seconds, during which time the sample reaches the switching valve, immediately prior to sample introduction into the gas-liquid separator. This is followed by a rise time of typically 15-20 seconds in which the detector output heads towards a steady state. An analysis period of around 45-60 seconds follows, during which time the measurement is made. At the end of this period, the switching valve ceases to supply analyte to the gas-liquid separator, and the signal returns to the base line as the gas-liquid separator is cleared of any remaining analyte. The decay time is typically 30-45 seconds. Thus the method relies upon an equilibrium being reached in the gas-liquid separator, and as a consequence of this, it has proved difficult to follow anything but the slowest reactions due to these inbuilt delays.

The use of atomic fluorescence detectors, which are several orders of magnitude more sensitive than QFAAS, offer a rapid response to the presence of an analyte. It is also possible to alter the conditions used for the hydride generation, and under computer control, use very short sample injection times. Under these conditions the resulting peaks resemble flow injection peaks as shown in Figure 3.7.2, and the equilibrium conditions in the gas liquid separator are not upset over the very short period in which the sample is being introduced. As can be seen in Figure 3.7.2, the complete analysis is over in less than 45 seconds. Since the sample injection stage only lasts for 2 seconds, a "snapshot" of the reaction may be

Figure 3.7.2 Trace obtained employing hydride generation coupled to atomic fluorescence detection with a one second injection time



obtained. If this is repeated at one minute intervals, it is then possible to build up a picture of the complete reaction process.

3.7.1.1 Instrumentation

The apparatus used for this study consisted of a hydride generator (model 10.004 from PS Analytical Ltd., Sevenoaks, Kent) fitted with a Perma-pure drier tube (PS Analytical Ltd), and used in conjunction with an atomic fluorescence detector (Excalibur, PS Analytical Ltd.). The instruments were under computer control using Touchstone™ software(PS Analytical Ltd.). Further details of the instrumentation are given in Chapter 2.

3.7.1.2 Reagents

The reagents used for this study were sodium borohydride 98% (Aldrich), sodium selenate 99% (Aldrich), sodium selenite 99% (Aldrich) and hydrochloric acid (Analar-BDH). Water was 18M ohm from a Milli-Q system.

100 µg ml^{-1} stock solutions of sodium selenate and sodium selenite were prepared. Working strength solutions of 50ng ml^{-1} were prepared daily from these. Sodium borohydride solutions 1.3% m/v in 0.1 mol l^{-1} NaOH were also prepared

daily. 6 mol l^{-1} hydrochloric acid solutions were prepared as required.

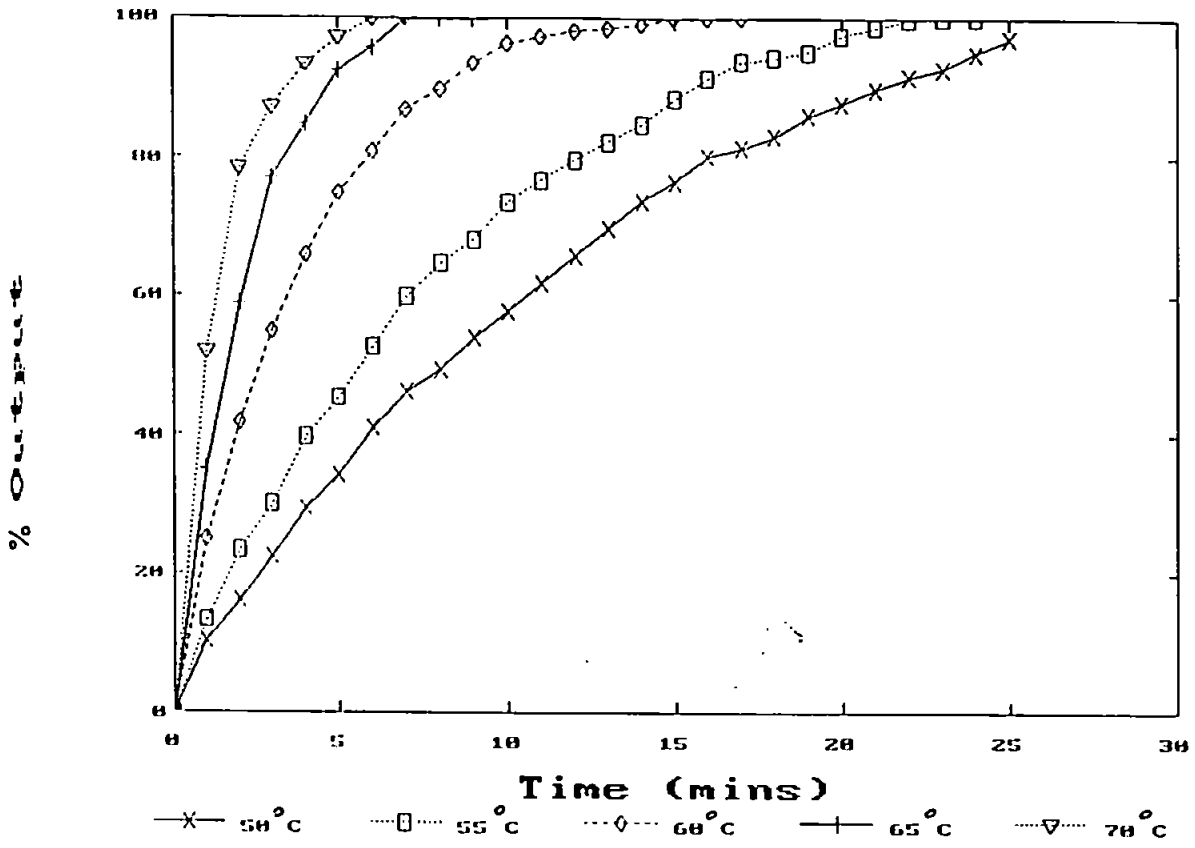
3.7.1.3 Method

A beaker containing 200ml of 6 mol l^{-1} hydrochloric acid was placed in a water bath, and heated to the required temperature (50, 55, 60, 65 and 70 °C). Once thermal equilibrium had been achieved, a small aliquot (100 µl) of the stock solution was introduced whilst maintaining continuous agitation. The hydride system was then operated to sample from the beaker every 60 seconds, and the resulting selenium concentrations calculated and stored by computer. This process was carried out in triplicate at each temperature . The software was operated in the peak area mode, since it was felt that this would provide greater precision than peak height, bearing in mind the very short sampling times used, and the sharp peaks obtained.

3.7.2 Results

The results of the triplicate experiments were averaged, and the detector output (related to Selenium(IV) concentration) plotted against time for each temperature as shown in Figure 3.7.3. It can be seen that the curves obtained are exponential

Figure 3.7.3 Plot of fluorescence detector output against time for various temperatures



in character, and so were replotted using ln(output maximum - output) against time as shown in Figure 3.7.4. The resulting straight lines indicate that the reaction is pseudo-first order in character. It can be seen from the Arrhenius equation,

$$k=A \exp(-E_a/RT)$$

where k = rate constant, A = pre-exponential factor, E_a = activation energy, r = gas constant and T = temperature (K) that the slope of these lines may be calculated to obtain the rate constant. A plot of ln(k) against 1/T also produced a straight line as shown in Figure 3.7.5, the slope of which is equal to E_A/R. The result obtained gave a value for the activation energy for the reaction of 90.39kJ mol^{-1} using 6M hydrochloric acid.

3.7.3 Discussion

The activation energy calculated in this study is higher than that reported by Bye & Lund[64] but lower than the figure reported by Petterson & Olin[70] (90.4kJ mol^{-1} c.f. 83kJ mol^{-1} and 126 kJ mol^{-1} respectively). However, the experimental conditions with regard the chemical composition of the test solutions were not identical to those used by Bye and Lund. They were looking at solutions containing perchloric acid in

Figure 3.7.4 Plot of ln(output maximum - output) against time

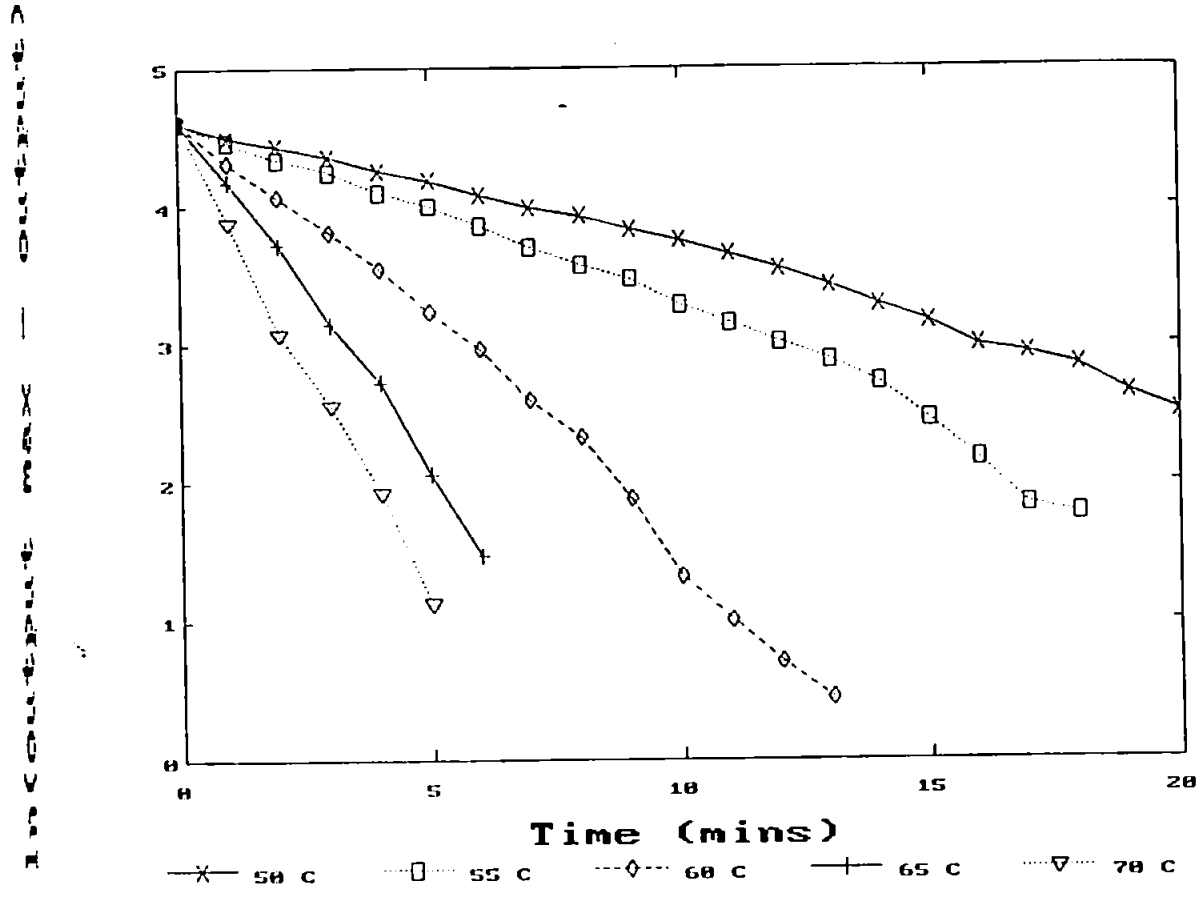
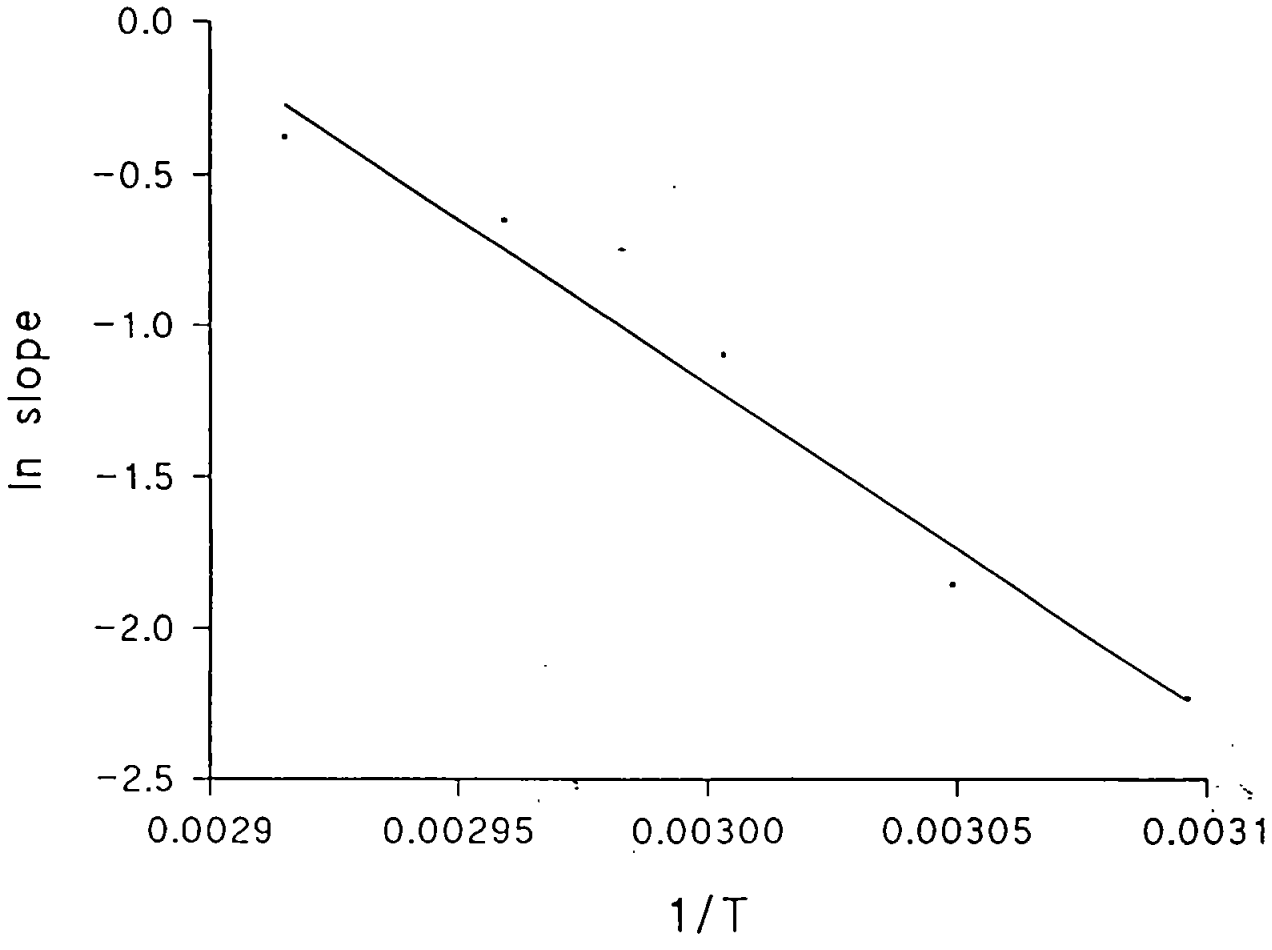


Figure 3.7.5 Plot of ln(k) against 1/T



addition to hydrochloric acid, in an attempt to more closely duplicate conditions applicable to "real" samples. In addition, their value for the activation energy was obtained using 4 mol l^{-1} hydrochloric acid, as opposed to the 6 mol l^{-1} used in this study. The application of the more rapid atomic fluorescence system in this work also enabled many more data points to be obtained, on which to base the calculations.

Petterson and Olin explained their higher value for the activation energy as a function of uncertainties in temperature, since they repeated the conditions used by Bye and Lund with regards the exact matrix used. They indicated that there was a time lag between the introduction of the flask to the water bath and the contents of that flask reaching a constant temperature, and also that the contents of the flask did not reach the temperature of the bath. It is difficult to believe that Bye and Lund did not realise that these factors would affect their results, and it is more likely that the lower figure for the activation energy obtained by Bye and Lund was due to the lack of data points. From their publication, it appears that they obtained only four points at each temperature - clearly this would affect the accuracy of the plots, and hence the slopes of lines produced. Since the calculation of activation energy relies on these gradients, an obvious explanation of the lower figure becomes apparent. In this study, the actual temperature of the test solutions were measured. Petterson and Olin also indicate that full reduction took thirty minutes at 65 °C. As can be

seen from Figure 3.7.3, our results indicate that at this temperature, full reduction was achieved within ten minutes under our experimental conditions.

From the plots of output against time in Figure 3.7.3, it can be seen that the reaction goes to completion after 6 minutes at 70°C. Prolonged heating for periods of 30 minutes or more are therefore unnecessary. Shorter heating times may also reduce the potential for contamination when using open topped containers. As reported by Krivan et. al.[68], the solution may be boiled under reflux, but it is often more practicable when dealing with large numbers of samples to have containers sitting in a water bath, rather than having a large array of refluxing systems in operation.

3.8 Photolysis

Since the development of the HPLC-microwave-HG-AFS system proved successful for the speciation of selenium in it's inorganic forms, organo-selenium compounds were also investigated, to see whether they were rendered detectable by microwave treatment. In order to achieve this detectability, the treatment would need to cleave the selenium-carbon bonds, and convert any selenium released to the selenium(IV) oxidation state. In spite of a number of efforts in this direction, they remained stubbornly unaffected and undetectable.

Organo-selenium compounds are extremely resistant to acidic degradation[71-73], and as a result, severe regimes have had to be employed for their determination in environmental and biological samples e.g. the use of a mixture of nitric, sulphuric and perchloric acids[74]. Newer techniques using GC-MS after derivitization to form the trimethylsilylated compound have been reported[75] for the analysis of seleno-methionine. However, these techniques are primarily employed when a number of organo-selenium compounds have to be quantified individually. In environmental analysis, it is frequently only required to speciate on the basis of organo-selenium compounds, selenium(IV) and selenium(VI), and so a method of releasing the selenium from organo-selenium compounds to render it detectable via hydride generation is of great potential use.

Since microwave energy had no effect in an acidic environment, it was decided to investigate whether photolysis would release the selenium directly by breaking the carbon-selenium bond. This technique has been employed in similar circumstances for the determination of arsenic[76,77] and tin[78].

3.8.1 Experimental

A stock solution of seleno-methionine (Aldrich, Gillingham, Dorset) was prepared, containing approximately 100 mg l^{-1} in 3 mol l^{-1} hydrochloric acid. From this solution, a further dilution was made to give a solution of 100 µg ml^{-1}, and this was divided between the four quartz tubes of the UV exposure unit described in Section 2.1.3.6. The tubes were then placed in the exposure unit, and exposed for 1,10, 30 and 60 minutes. The samples were removed from the exposure unit at the appropriate times, and analyzed using standard hydride generation techniques (see Section 3.3). The results are shown

graphically in Figure 3.8.1, and as can be observed, show that the maximum conversion from organic to inorganic selenium had taken place after 60 minutes. The experiments were then repeated using a more dilute solution (25 μg ml^{-1}), and exposures made at 5, 10, 20, 30, 40, 50, 60 and 70 minutes. These samples were again analyzed, and the results calculated to obtain recovery yields. These are shown in Figure 3.8.2, and show that 100% recovery is reached after 60 minutes.

Since bromide/bromate is employed to convert organo-mercury species to the inorganic form, and in view of the affinity exhibited by selenium for bromine, these reagents were added to the seleno-methionine solutions in varying concentrations, and the exposures repeated in the hope that they may catalyse the photolytic reaction. The bromide/bromate solution strengths were varied from 0.5 to 10%, the solutions being left for 30 minutes before being decolourised with 50 μl of 12% hydroxylamine hydrochloride. In all cases, no improvement in reaction rate was observed.

3.8.2 Discussion

The exact mechanism involved in the breakdown of seleno-methionine by UV radiation in the strongly acidic conditions employed here is not known. It is probable that the selenium released from the seleno-methionine goes directly to the selenium(IV) oxidation state, since the solution conditions do not appear to be likely to give rise to further oxidation. However, the possibility of the reaction proceeding to give selenium(VI) (with free chlorine playing a part as in the back oxidation of selenium(IV) by conventional reduction -see Section 3.4) and this being reduced back to selenium(IV) due to the heating effects of the lamp and the high acid concentration cannot be ruled out on the basis of the results obtained. Further investigations are obviously required to

Figure 3.8.1 Plot of selenium concentration detected against UV exposure time for seleno-methionine

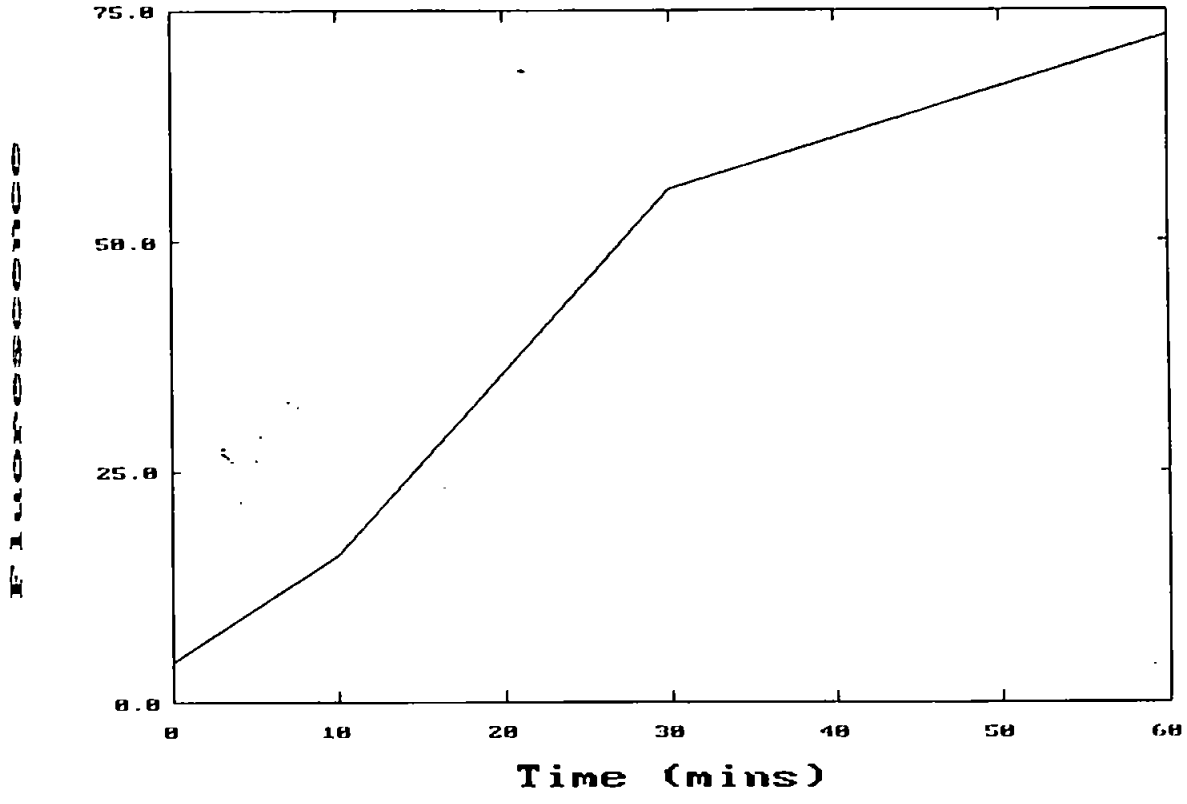
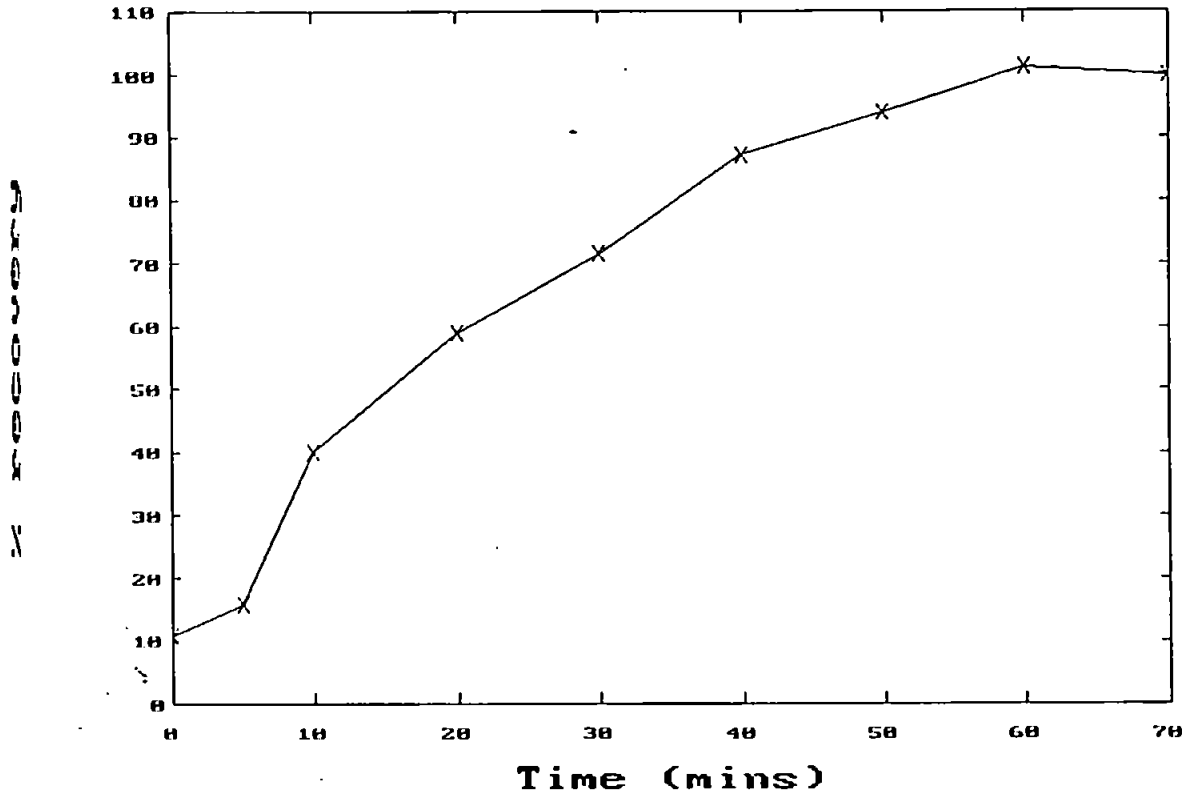


Figure 3.8.2 Plot of selenium concentration detected against UV exposure time for seleno-methionine to illustrate reaction rate



elucidate which of these potential mechanisms are in fact followed.

Whilst the time taken to achieve cleavage of the carbon-selenium bond may at first appear to be too long for this technique to have any application in an on-line system, it should be remembered that in these experiments, large sample volumes were used, and these were placed at some distance from the UV source. If a capillary carrying the sample solution were placed closer to the lamp, a much shorter period for the reaction should be realised. The distance between sample and lamp is critical since the intensity of the radiation diminishes with the square of the distance. Thus, the possibility of incorporating a UV exposure stage within a system similar to that described in Section 3.6 could provide a "universal selenium speciation system", capable of providing determinations for selenium(IV), selenium(VI) and organo-selenium compounds.

3.9 Summary

The studies carried out on selenium have yielded a number of interesting results.

The apparent increase in signal when determining selenium in the presence of traces of nitric acid via hydride generation was found to be a real effect and found to be associated with the type of pump tubing employed. The exact mechanism responsible for the effect was not determined, although two possible explanations were suggested.

The work carried out on the conditioning of the hydride generation systems indicated that at trace and ultra trace

levels, conditioning could take some considerable time if the system is not pre-treated by initial sample injections of a relatively high concentration of selenium(IV). If conditioning is not carried out, then determinations should be carried out using the "bracketing method".

The use of an on-line microwave pre-reduction step was found to be feasible, and this led to the development of an automated system for selenium speciation determinations using hydride generation and then quartz furnace-atomic absorption spectroscopy as a detection system. This development was further refined to achieve direct determinations of selenium species by separation using HPLC, and employing the more sensitive atomic fluorescence detector to compensate for sample dilution.

The investigation into the kinetics of the selenium(VI) reduction process indicated that under our conditions, full reduction took place within six minutes at 70°C, thus obviating the need for longer heating, and thereby reducing the dangers of contamination.

The use of a photolysis stage to free selenium from organo-selenium compounds has been established to be effective. The possibility of incorporating such a stage into a comprehensive selenium speciation system is an obvious future development.

Chapter 4

Applications of on-line pyrolysis

4 Pyrolysis

4.1 Introduction

Pyrolysis may be defined as "the thermal decomposition of organic compounds" [79], although for this study inorganic materials have also been included. The study has also concentrated exclusively on mercury as the metal under investigation.

Mercury is an unusual element, being the only metal which is liquid at room temperature. It forms amalgams with most other metals (except iron and platinum) and these take the form of solids or liquids, depending on the proportion of mercury present. This property is employed widely in dentistry, and also provides the basis for a simple and extremely sensitive detector for traces of mercury. The change in electrical properties of a gold wire due to amalgamation are employed to provide quantitative measurements for the element. After exposure and measurement, the wire is simply heated to drive off the mercury and render it suitable for the next sample. Both the metal and it's compounds are highly toxic, and there are very many reports in the literature of toxicological studies on flora and fauna[80]. Since environmental mercury exists from both natural and anthropogenic sources, and in view of the severe effects trace quantities of the element may have on both industrial processes and on human health, much

work is currently being undertaken to fully elucidate the biogenic pathways of the species involved.

Mercury contamination incidents such as the much quoted Minamata Bay disaster[81] have alerted scientists to some of the problems concerning mercury, but in various areas of the world, mercury is still widely used for reclaiming gold from muds and gravels, and hence large quantities escape into rivers and subsequently reach the sea. There is current concern in the United States over high levels of mercury found in fish and the mammals which feed on them in the Everglades. These high levels are thought to come from mercury used by gold prospectors in the Amazon and it's tributaries, which is washed by ocean currents around the bay of Mexico, before being deposited in the Everglades area[82].

Concern is also growing regarding the use of mercury amalgams in dentistry. A number of studies have illustrated the harmful effects which may arise from having dental cavities filled with mercury amalgams[83,84], and concern is growing for the health of dentists and their staff who have been subjected to continuously high levels of mercury in their work. Mercury, once ingested, concentrates in the kidneys and also passes through the brain membrane barrier. Once in the brain, it blocks various nerve functions. Mercury is also able to pass through the placenta into the foetus[85], and development of the brain and nervous system may be impaired. There is increasing evidence of a link between mercury and Alzheimers disease[86,87],

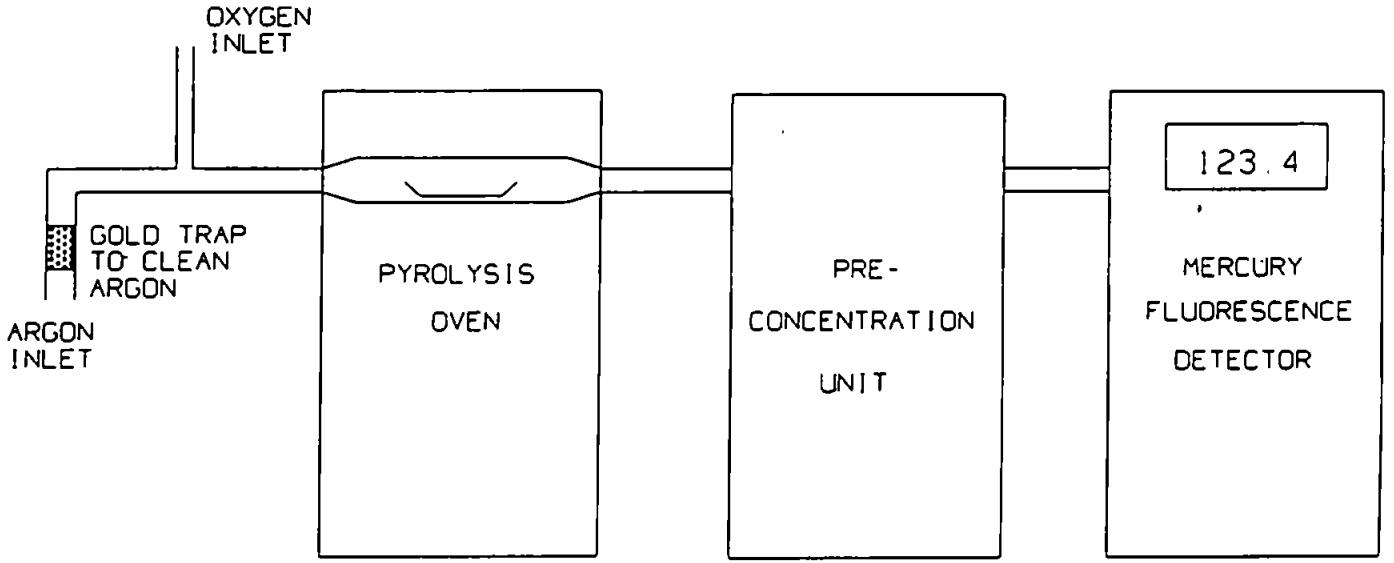
and also of it's detrimental effects upon the immune system[88].

There is clearly a need for methods for the determination of mercury which are both selective and very sensitive. Early techniques were based on colourimetric methods, but suffered from a lack of sensitivity, and also problems of losses and contamination due to the long preparation steps involved. In 1963, the use of cold vapour generation - atomic absorption spectrometry was reported[89], and since then, this has become the most widely employed technique for the determination of mercury in biological samples[90]. The later introduction of fluorescence detection instruments coupled to cold vapour generation has improved still further the limits of detection, and this technique is fast gaining acceptance as a simple, sensitive and reliable method for the determination of mercury. A mercury fluorescence detector was used throughout this study and this is described in Chapter 2.

4.2 Experimental

For all the investigations in this section, the system employed was configured as shown in Figure 4.1. As may be observed from the diagram, the apparatus consisted of a gas mixing valve at the inlet of the pyrolysis oven. The carrier gas employed was argon, and the mixing valve allowed the addition of oxygen when required. The gases from the pyrolysis oven then passed into a pre-concentration unit,

Figure 4.1 Basic pyrolysis system



which trapped the mercury liberated from the sample in the oven on a gold sand trap. The outlet of the pre-concentration unit was connected directly to the fluorescence detector.

Mercury has a vapour pressure of 0.0016 mbar at 22° C which corresponds to a concentration of approximately 14 mg m^{-3}. The vapour therefore provides a source of contamination which affects everything in the vicinity[90]. Assuming that the analyst has amalgam fillings, contact between the sample and exhaled breath must be avoided, since this contains easily detectable mercury vapour. Rigorous cleaning of all equipment concerned with the analysis of this element is vital. This cleaning extends to the silica sample tubes used in the pyrolysis oven. This was accomplished by subjecting the tubes to an initial heating at 800° C for 30 minutes, after which they were stored in a 30% solution of Aristar nitric acid. Before the samples were introduced, the tubes were dried externally with paper towelling, then argon which had been purified using an in-line gold trap was used to dry the inside of the tube. The tube was then subjected to a further ten minutes at 800° C, with purified argon flowing through it after which it was allowed to cool before the sample was loaded into it. This cleaning regime was found to reduce the background levels of mercury to a level which did not affect the determination of the element in any of the sample types listed below.

Using the system outlined above, a number of investigations were carried out. These were :

a) the determination of mercury in sediments
b) an attempt to determine mercury in silver nitrate
c) an attempt to use the technique for the determination of mercury in hair and finally
d) an attempt to determine mercury in oil

4.2.1 The determination of mercury in sediments

Since the more commonly employed methods of determining mercury in sediments involve much sample preparation, with the consequential risks of losses and contamination, any technique which is able to reduce these steps is of interest. Some workers have reported the use of capillary gas chromatography (GC) for organo- mercury studies[91], but these methods still require considerable sample pre-treatment. A simple and robust technique for determining total mercury in a sediment is therefore desirable.

Using the apparatus outlined above in Chapter 5.2, weighed samples of sediment (approximately 30 mg) were carefully positioned in the heating zone of the silica sample tubes used in the pyrolysis oven.

After running a number of different heating regimes, it was found that heating the sediments to 550° C for five minutes produced the results shown in Table 4.1. Heating at lower temperatures resulted in inconsistent results, whilst heating at higher temperatures had no beneficial effect, neither did heating for longer periods. The results show good agreement between the certified values and those obtained experimentally (64.2 ng g^{-1} c.f. 63 ± 12 ng g^{-1} in the case of NIST 1646).

4.2.2 The attempted determination of mercury in silver nitrate

Silver nitrate is used extensively in the photographic industry as a precursor in the preparation of photosensitive emulsions in both films and printing papers. Mercury contamination of silver nitrate is a serious problem for the film manufacturers even at low ng g^{-1}. Various methods for the analysis of mercury in silver nitrate at the nanogram level have been reported, but most involve the conversion of the silver nitrate into a halide salt, followed by cold vapour methods applied to the filtrate[92]. As discussed above, problems from mercury losses and contamination are of paramount importance when analysing for this element, and thus efforts were made to employ pyrolysis.

The apparatus was set up as described previously, and samples of silver nitrate (approximately 30-40 mg) were loaded into

Table 4.1 **Determination of mercury in certified reference sediments**

Analysis	NIST 1646 Estuarine sediment	NIST 8406 Tenessee River Sediment
1	67.2	58.1
2	61.8	59.3
3	66.0	58.1
4	64.2	61.2
5	61.4	59.4
Mean	64.2	59.2
Certified value	63 ± 12 ng g^{-1}	(60) ng g-1

the silica sample tubes. Various heating cycles were tried, from very gentle heating at 100° C up to more intense heating at 500° C. Various heating times were also employed. Absolutely no mercury was detected at any temperature. A sample was then spiked with mercury vapour by drawing various volumes of the vapour above a reservoir of the element into a gas syringe, and then injecting this into the silver nitrate sample. Again no mercury was detected. The instrumentation was checked and found to be operating correctly, and so the conclusion was reached that at low temperatures, the affinity of mercury for silver was so great that the mercury was not released. At higher temperatures, the silver nitrate sublimes and presumably the mercury is temporarily released, only to recombine with the silver nitrate as soon as it condenses. It was therefore concluded that the technique was unsuitable for this particular problem.

4.2.3 The attempted determination of mercury in human hair

The determination of mercury in human hair may be used as a screening technique if mercury exposure is suspected. Normal mercury levels in hair[90] are around 1 μg g^{-1}, and so sensitive analytical procedures are required. The conventional methods employed for this analysis involve the digestion of the hair sample, and subsequent analysis by cold vapour generation.

A number of experiments were performed along the lines

illustrated previously, but with sample weights of less than 10 mg. These low weights of sample were due to the fact that the material has extremely low density, and sample volume is limited by the size of the heating zone and the bore of the silica sample tubes. Heating of the sample to a temperature of 300 °C in argon alone resulted in the production of smoke particles which flooded the fluorescence detector. Experiments were performed to investigate whether mercury would be released at temperatures below the charring point by heating samples from 60 °C to 150 °C in 10 °C increments, and whilst this did occur, results were very inconsistent.

The addition of varying amounts of oxygen to the argon carrier gas was then made so that the organic matter was converted into carbon dioxide. Slowly increasing the sample temperature at a rate of 10 °C min^{-1} was required to avoid any smoke production, but again the results were inconsistent, often by at least one order of magnitude.

In spite of the fact that these experiments were repeated on a number of occasions, no consistency of results were obtained. This may be due to limitations in weighing equipment, particularly when such small weights are being employed, difficulties in handling the material or simply the fact that such small sample weights are not sufficiently representative of the main bulk of material.

4.2.4 The attempted determination of mercury in oil

A similar technique was applied to oil samples, to that given above. In this case, the use of a silica crucible spoon, shaped and constructed to fit into the silica sample tube was employed to contain the oil samples.

A similar lack in the reproducibility of the results was obtained, and the same conclusions reached as to the possible causes i.e. that the low density of the sample results in problems in weighing the very small samples with sufficient precision, and that the size of such samples may now sufficiently represent the main bulk of the sample.

4.3 Mercury determinations employing gas chromatography coupled to the pyrolysis system with atomic fluorescence detection

The use of capillary gas chromatography as a means of separating volatile organo-mercury compounds has already been referred to[90]. This is commonly followed by an electron capture detector when determining halogenated organo-mercury compounds. A derivitization step is therefore required to process non-halogenated compounds, and this requires considerable care to ensure that no contamination occurs, since the sensitivity of the detector is so high.

Contamination of solvents is often a particular problem.

Using the apparatus shown in Figure 4.2, it was possible to pyrolize the organo-mercury compounds directly after separation by the column, and thus detect the mercury directly using atomic fluorescence. The GC was a Pye Unicam model 104, fitted with an ultrabore inlet adaptor of 0.53mm internal diameter (S.G.E., Australia). The column was 3m x 0.53mm internal diameter fused silica, coated with a 5 micron non-polar BP-1 stationary phase (S.G.E., Australia). A 350mm x 0.53mm internal diameter fused silica post-column was also employed. This column, employed as a resistively heated transfer line and held at 150 °C via a Variac transformer, fed the eluents from the column directly into the heated zone of the pyrolysis oven, which was held at 800 °C.

A number of experiments were performed to determine the effect of column temperature, carrier gas flow rate, sample gas flow rate and shield gas flow rate, and these are shown in Figures 4.3 - 4.6. Using the conditions shown in Table 4.2, experiments were performed to determine the response of ethyl mercury chloride and diethyl mercury, and these are shown in Figure 4.7.

Figure 4.2 Coupled GC-pyrolysis-AFS system

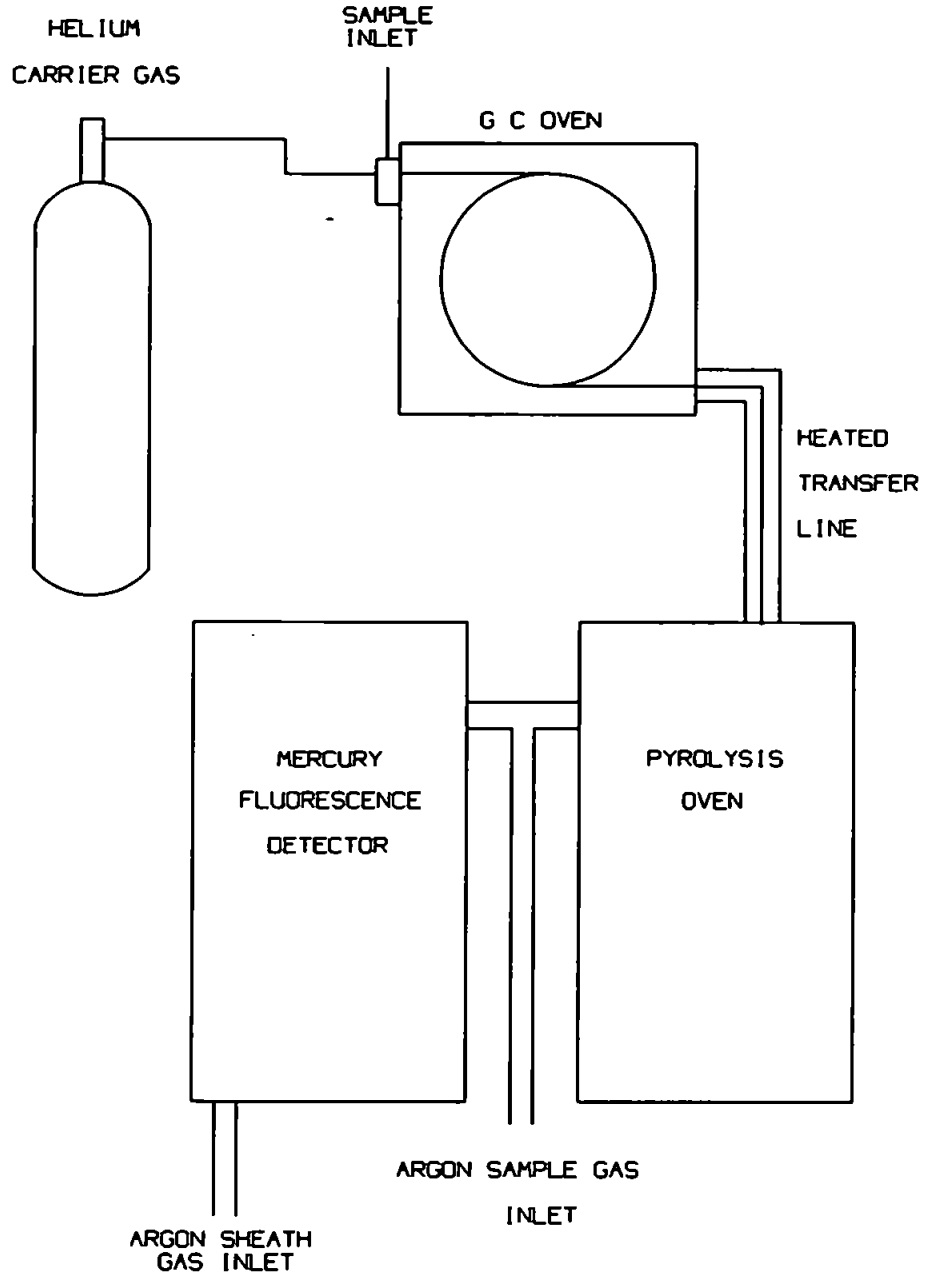
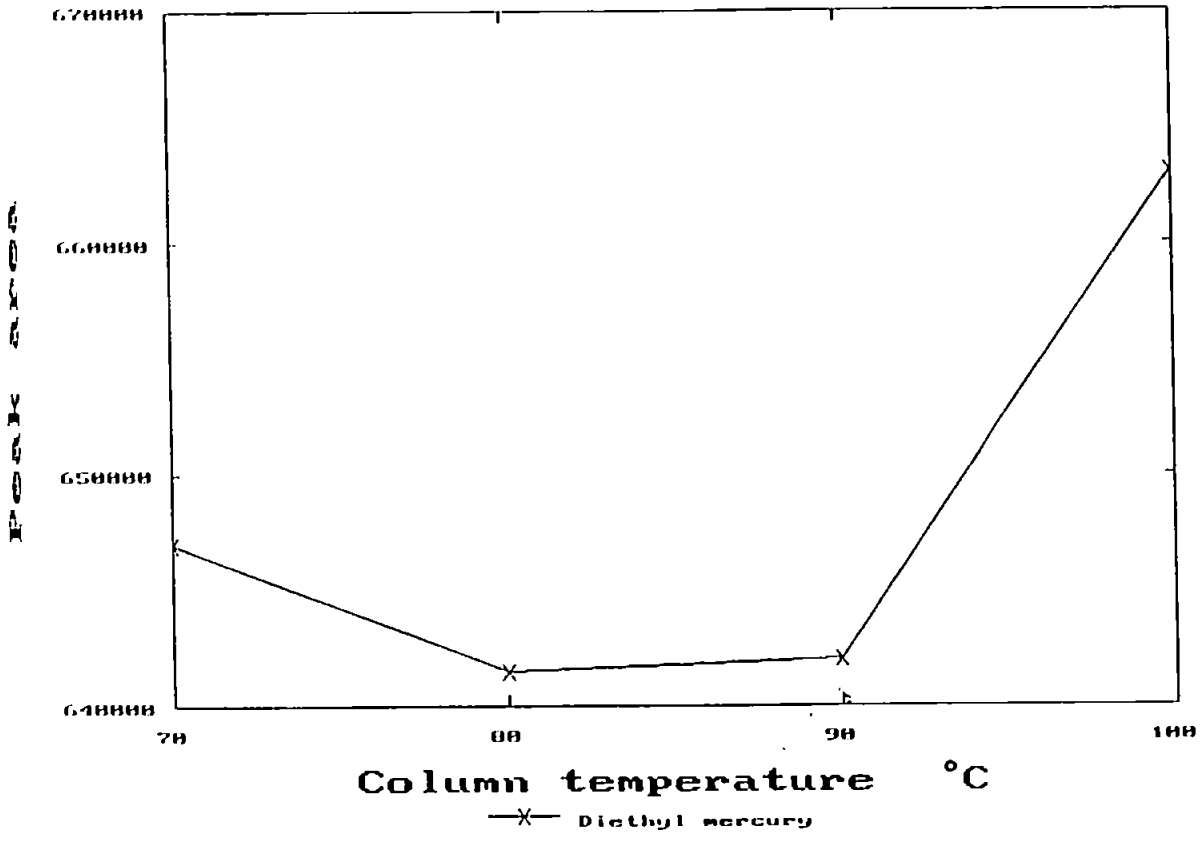
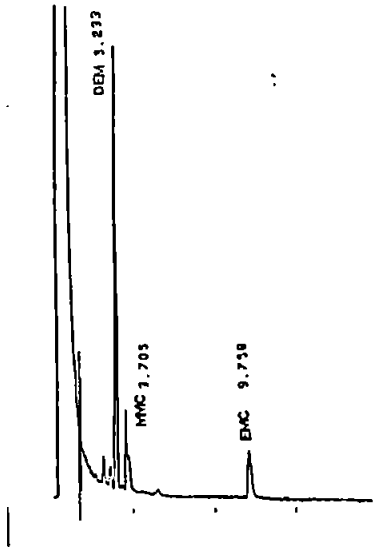


Figure 4.3 Effect of column temperature





GC-FID chromatogram of 1 µl of a standard mixture of 10 µg/ml of DEM, MMC and EMC as mercury in n-hexane.

Figure 4.4 Effect of carrier gas flow rate

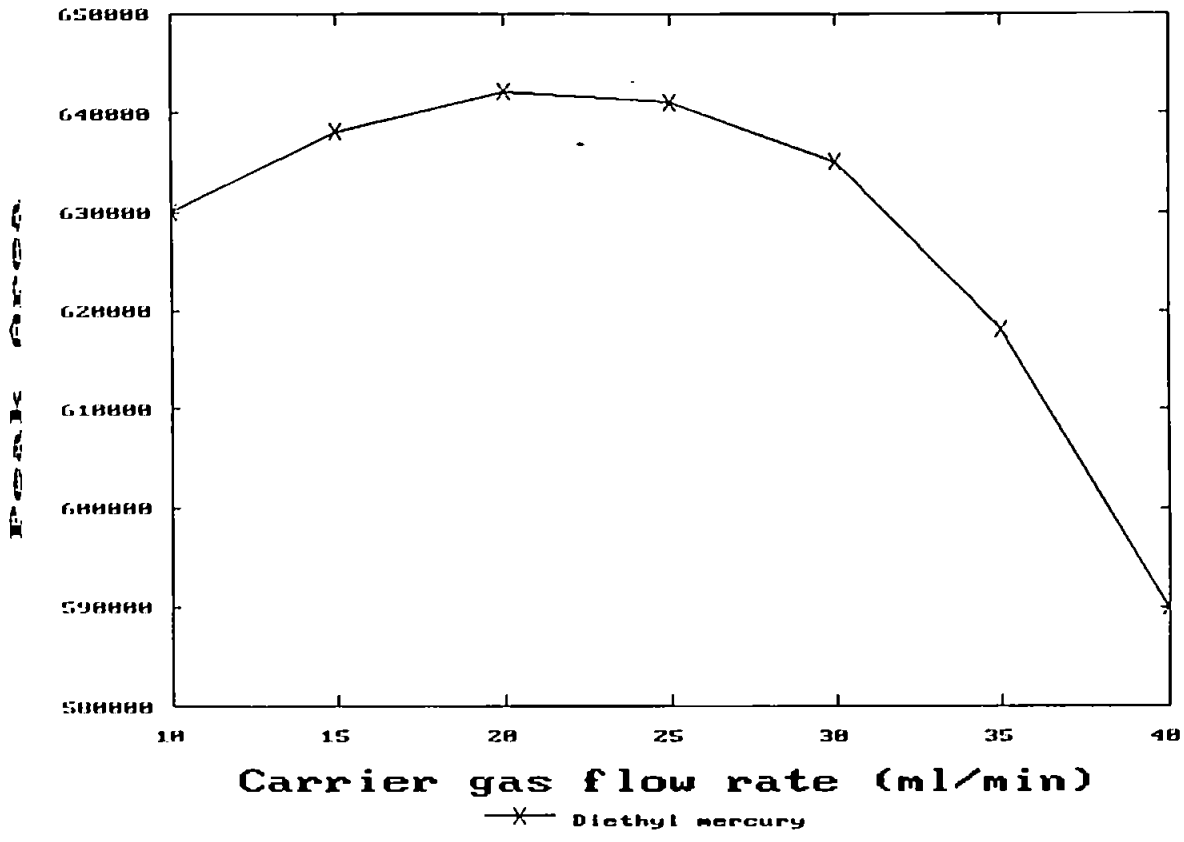


Figure 4.5 Effect of sample gas flow rate

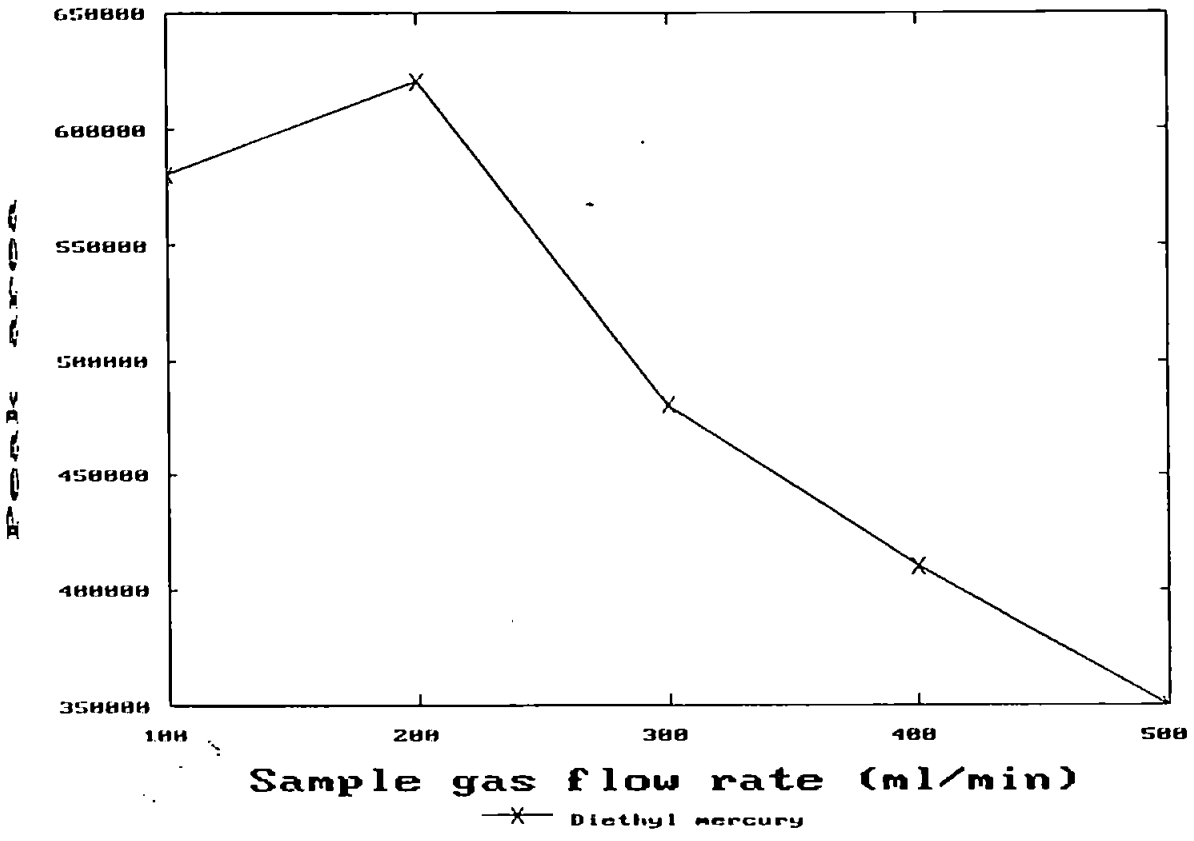


Figure 4.6 Effect of shield gas flow rate

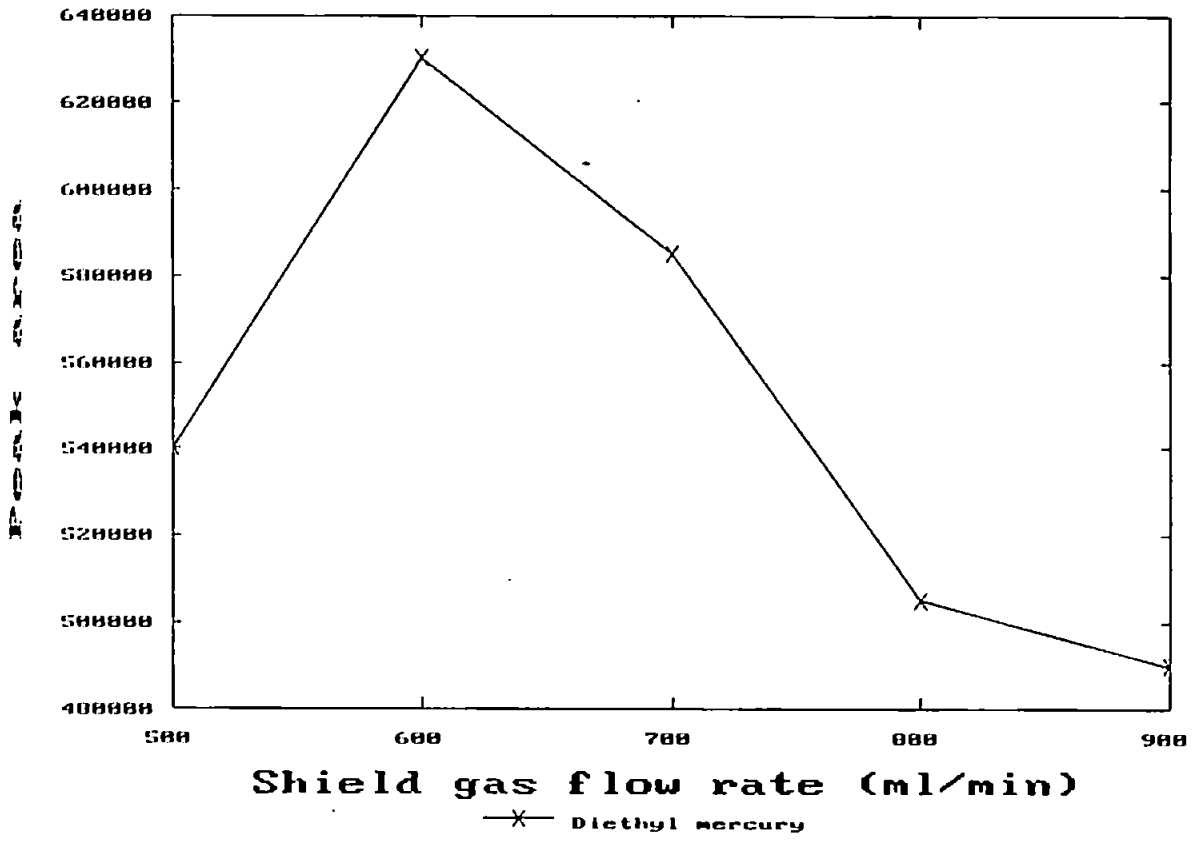
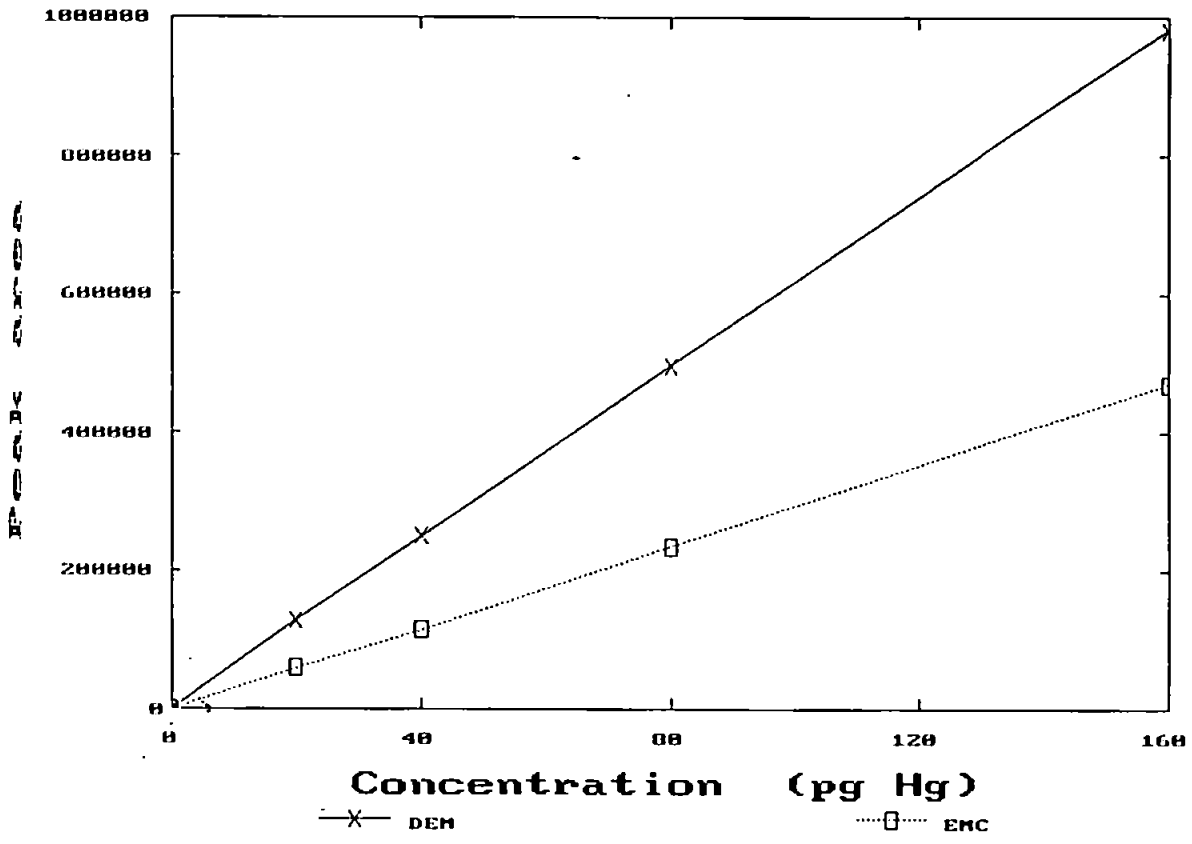


Table 4.2 Instrument conditions for GC-pyrolysis-atomic fluorescence

Gas chromatograph oven temperature	100 °C
Column type BP-5 5% phenyldimethylsiloxane	600 x 0.53 (id) mm
Carrier gas	Helium (7.5 ml min^{-1})
Transfer line temperature	150 °C
Pyrolysis oven temperature	800 °C
Sheath gas	Argon (600 ml min^{-1})
Sample gas	Argon (250 ml min^{-1})

Figure 4.7 Response of ethyl mercury and dimethyl mercury



4.4 Summary

The use of pyrolysis as a method of determining total mercury in sediment samples was found to work satisfactorily with good agreement being obtained with sample concentrations of approximately 60 ng g^{-1}. When applied to lower density organic rich samples, the method did not prove reliable. It is strongly suspected that this is due to the unrepresentativeness of the sample size, rather than a defect in the equipment or the technique.

The use of the coupled technique GC-Pyrolysis-AF produced some encouraging results, and warrants further investigation.

Chapter 5

Automated pH adjusting system

5 Development of an automated pH adjusting system

5.1 Introduction

In many analytical determinations, control of the pH of a sample is critical if optimal performance of the technique employed is to be maintained. One example of this has already been mentioned in Chapter- 3, i.e. the determination of selenium species by HPLC-microwave reduction-HG-AFS, however many other examples do exist. In many cases, samples are acidified upon collection in order to stabilize the sample and prevent "plate out", the loss of cations from the solution due to their adsorption onto the surface of glass containers[93]. In such cases, it is often necessary to adjust the pH of the sample just prior to analysis. This process is usually carried out manually, which is extremely tedious for the practitioner, and costly in terms of labour.

When trace and ultra-trace determinations are required, the pH adjustment also requires that the reagents used do not add contaminants to the sample. At such levels, this may be problematic since many commonly used reagents are unavailable in the purity necessary to avoid contamination. In addition, when working at these levels, dilution of the sample is undesirable for obvious reasons. The need for an automated pH adjusting system, which addresses the aforementioned problems is therefore apparent.

Previous work by Jackson et al[94] describes a system for the neutralization of samples obtained from the digestion of food by sulphuric acid. In this process, the strongly acidic sample was neutralized using gaseous ammonia. Since the use of this reagent avoids affecting the volume of the sample solution by dilution, and also avoids contamination of the sample, it was selected as the reagent for this work. The original work employed batch processing, using electromechanical timers etc. This approach clearly has limitations, and so the design of an automated on-line system was initiated.

5.1. Theory

In a sample of pure water, it's electrical conductivity never falls to zero, due to self-ionization. The process may be represented by the equations :

$$2H_2O\ (l) \rightleftharpoons H_3O^+\ (aq) + OH^-\ (aq)$$ [95]

or more simply

$$H_2O\ (l) \rightleftharpoons H^+\ (aq) + OH^-\ (aq)$$

Using the latter equation, the equilibrium constant K_c may be defined :

$$K_c = \frac{[H^+]\ [OH^-]}{[H_2O]}$$

Since only a few molecules of water actually ionise, $[H_2O]$ may be regarded as a constant at constant temperature. The ionic

product of water K_w is :

$$K_w = K_c \times \text{constant} = [H^+]\,[OH^-]$$

At a temperature of 25°C, $K_w = 1 \times 10^{-14}$ mol^2 dm^{-6} and since the concentrations of hydrogen and hydroxyl ions are equal, their concentrations are 1×10^{-7} mol dm^{-3}.

In any aqueous solution, the degree of acidity or alkalinity is determined by the relative concentrations of the hydrogen and hydroxyl ions.

If $[H^+] > [OH^-]$ the solution will be acidic

and if $[OH^-] > [H^+]$ the solution will be alkaline

A neutral solution contains equal concentrations of both ions

$$[H^+] = [OH^-] = 10^{-7} \text{mol dm}^{-3}$$

The pH of a solution is defined as $-\log_{10}[H^+]$, and effectively removes the need for awkward negative indices.

Measurement of pH may be carried out using a hydrogen electrode in conjunction with a standard reference electrode. The connection between the e.m.f of the cell (E^θ_{cell}) and pH is given by the Nernst equation :

$$E_{cell} = E^\theta_{cell} + (2.3\ RT/F)\ \log\ [H^+(aq)]$$

In order to measure pH, two half cells may be considered, the

left-hand cell typically being a saturated calomel reference electrode and has a potential $E_{(cal)}$, whilst the right-hand electrode is a hydrogen electrode who's potential is given by the equation :

$$E(H^+/H_2) = -59.16mV \times pH$$

At 25°C, the potential of the cell thus becomes :

$$E = -59.16mV \times pH - E_{(cal)}$$

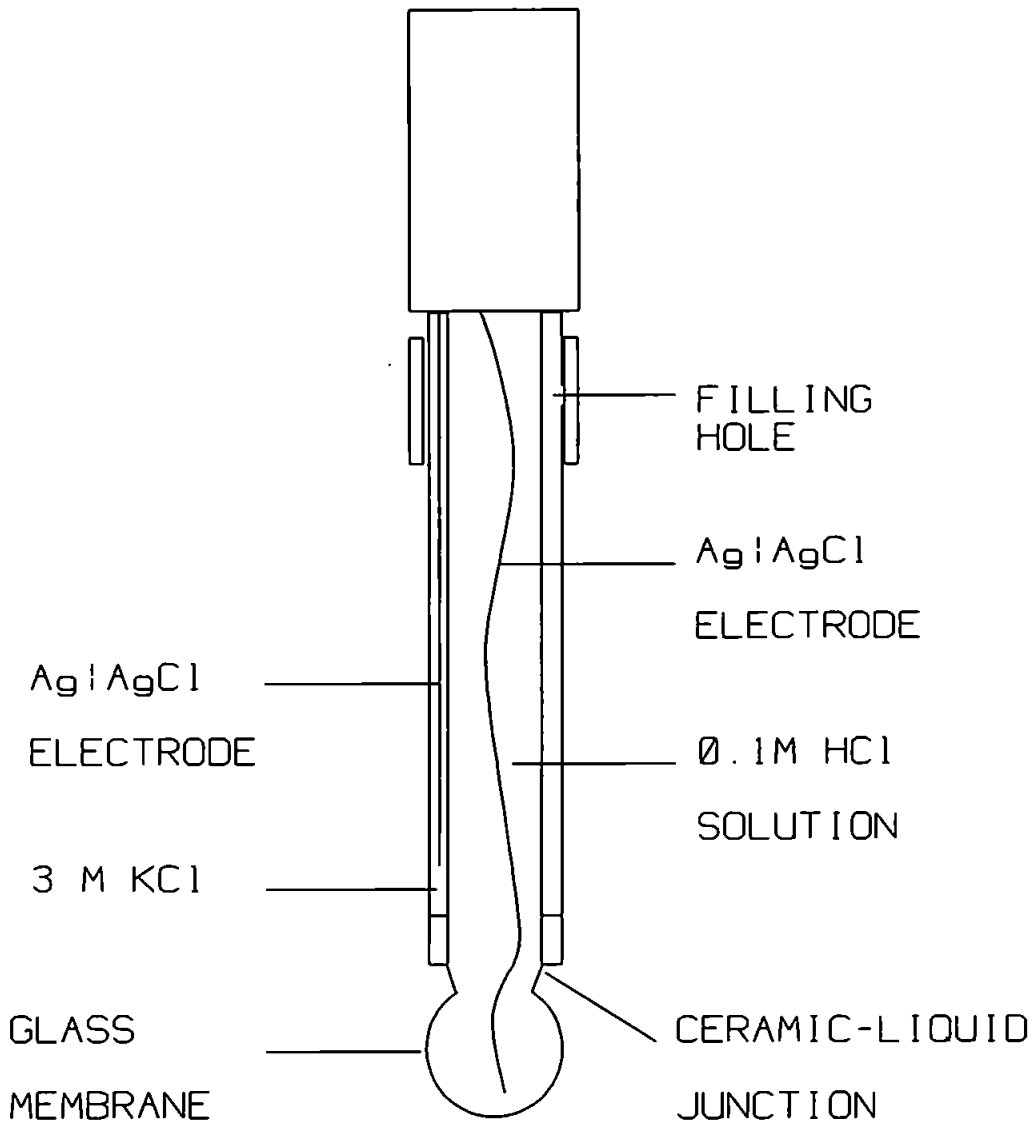
Thus it may be seen that for an increase in pH of 1 unit, the voltage from the electrode will decrease by 59.16 mV. This change is what is measured by "pH meters" which are in fact high input impedance voltmeters. By convention, pH 7 is defined as being at 0 volts, and therefore acidic solutions give a positive output, and alkaline solutions a negative one.

In practice, glass or membrane electrodes are employed in place of the glass electrode, due to greater ease of use, and combination electrodes are now generally employed. One such combination electrode is illustrated in Figure 5.1.1.

5.2 Development of the system

The overall design criteria identified for the system involved the transfer of the sample from an autosampler to a vessel in which treatment would occur, i.e. adjustment of the sample's

Figure 5.1.1 Typical combination pH electrode

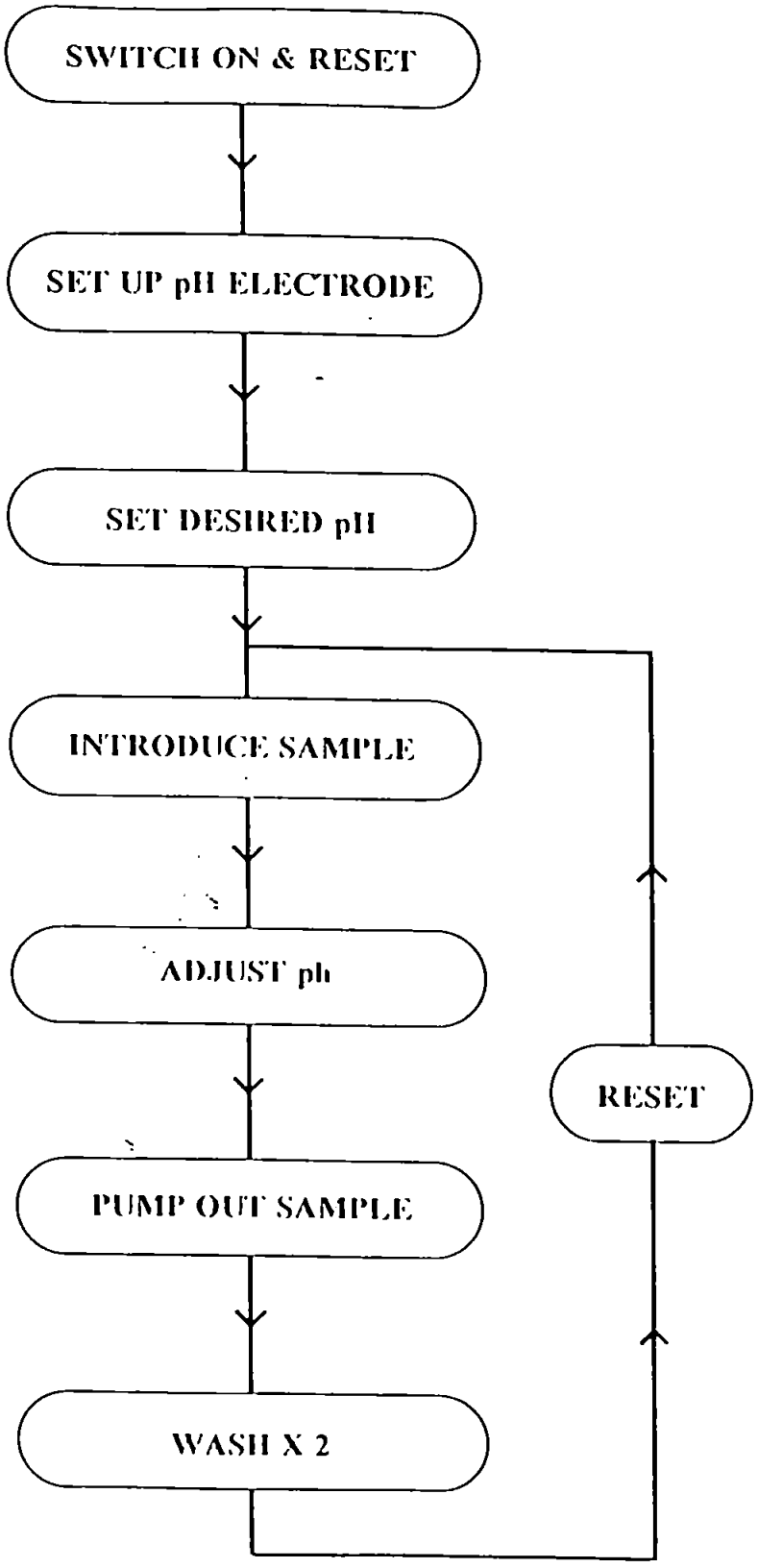


pH to a pre-determined value. The sample would then be returned to a fresh container positioned on the autosampler, or passed directly to the analytical instrument which was to carry out the determination. At this stage, the reaction vessel and connecting tubing can be subjected to a cleaning cycle, before the process is repeated.

An overview of this process is shown as a flow diagram in Figure 5.2.1. From the diagram, it can be seen that the first process step is to reset the system at switch on. The next step is to set up the pH electrode in the normal way using two buffer solutions of known pH via the buffer and slope controls. The desired pH of the treated sample solutions is then set.

From this point, the system enters a loop which consists of sample introduction, the adjustment of it's pH and subsequent removal of the sample from the system. Following this, the tubing and entry valves are washed to eradicate all traces of the sample, and the reaction vessel and exit system are washed out twice. At the end of the second wash cycle, the system is reset, and the next sample is introduced. In the unlikely event that each sample required adjusting to a different pH, the setting of the pH could be included in the control loop.

Figure 5.2.1 Process overview



5.2.1 Design of the valving

From the specification identified above, it was possible to design the valving arrangements for the flow of sample, wash solution and gases, and these are shown in Figure 5.2.2. The valve switching conditions for each process were evaluated, and these are shown in Table 5.2.1. Figure 5.2.2 shows the system in the reset condition - all valves are as shown and the pump is off. An increment signal is sent to the autosampler to enable selection of a sample. After a preset delay the pump and valve 2 are switched on and sample introduction commences. Valve 2 is a vent, and prevents the vessel becoming pressurised during filling. Sample introduction terminates upon a signal from the level sensor, whereupon an increment signal is sent to the autosampler for it to select a wash solution, adjustment of the pH and washing of the entry tubing commences. This is facilitated by valve 1 switching on, so as to pass the wash solution to waste, and valve 3 also switching on, to allow ammonia laden argon to enter the reaction vessel. Neutralisation of the sample solution now occurs. When the desired pH of the sample has been achieved as determined by the pH probe, valve 3 switches off to prevent further ammonia from reaching the sample. Valves 4,5 and 6 switch on, valves 5 and 6 to select the route taken by the treated solution, and valve 4 to allow argon to enter the vessel. Valve 2, the vent, is now closed and thus the vessel becomes pressurised by the argon, and this effectively pumps out the solution. The solution may be directed to a new container on

Figure 5.2.2 Valving diagram

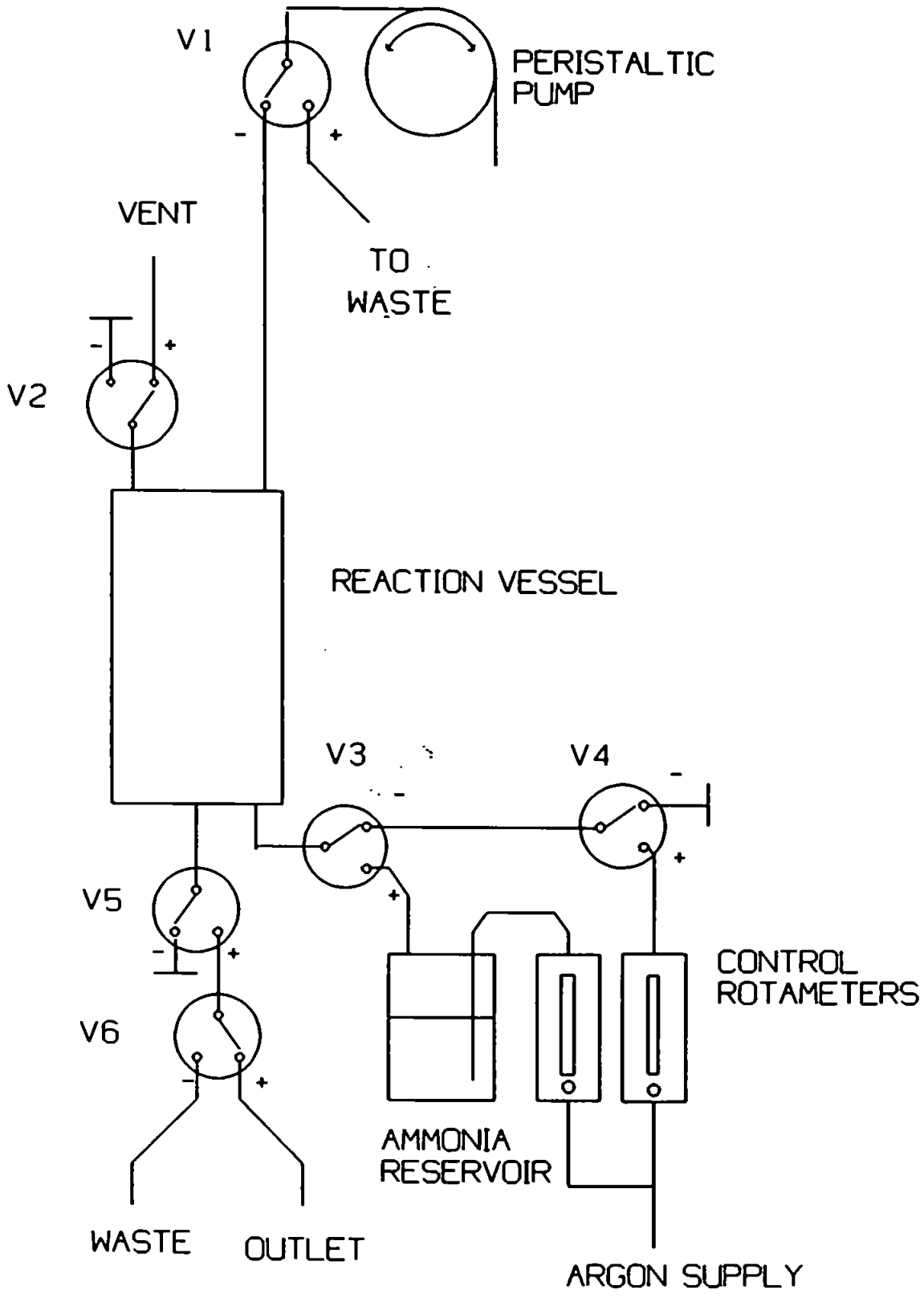


Table 5.2.1 Initial valve switching regime

	VALVE 1	VALVE 2	VALVE 3	VALVE 4	VALVE 5	VALVE 6	AUTO-SAMPLER	PUMP
RESET	-	-	-	-	-	-	S	-
SAMPLE INTRO	-	+	-	-	-	-	S	+
ADJUST pH	-	+	+	-	-	-	W	-
EJECT SAMPLE	+	-	-	+	+	+	W	+
WASH INTRO	-	+	-	-	-	-	W	+
WASH CYCLE	-	+	-	+	-	-	W	-
EJECT WASH	-	-	-	+	+	-	W	-
WASH INTRO	-	+	-	-	-	-	W	+
WASH CYCLE	-	+	-	+	-	-	W	-
EJECT WASH	-	-	-	+	+	-	W	-

S signifies sample - W signifies wash

the original autosampler, to another autosampler altogether or directly into a measuring system.

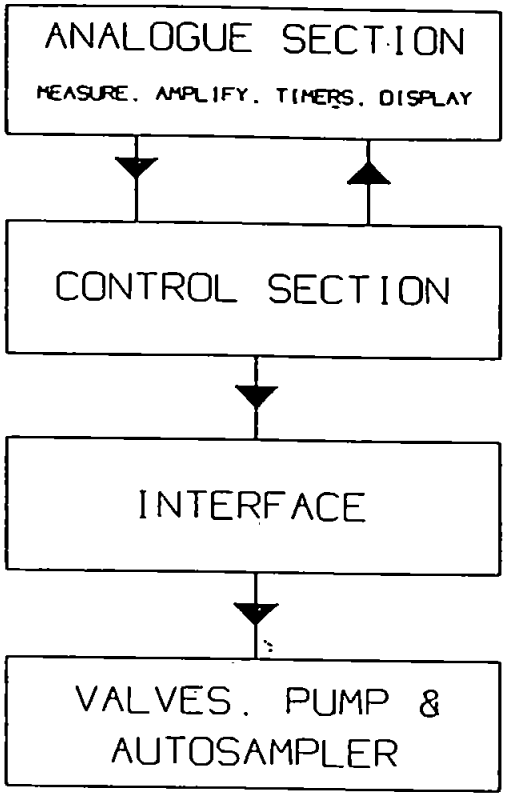
Washing of the reaction vessel now commences - all valves switch off, with the exception of valve 2, and thus the vessel is filled with wash solution. Once the level sensor detects that the vessel is full, the pump is switched off and valve 4 switched on to allow argon to bubble through the solution and thus provide agitation. After a preset wash period, the wash solution is ejected to waste by switching on valve 5 and valve 2 off. This whole wash procedure is then repeated, before the system advances to the reset position.

With the operation of the valve system established, the design of the electronics was initiated. All the components used in the design of the different sections are readily available from most suppliers.

5.2.2 Development of the electronics system

The electronics employed in this system has three distinct sections and the overview is shown in Figure 5.2.3.. The primarily analogue section which carries out the measurement and comparisons of values, a digital section for control and command, and an interface which provides switching of the valves.

Figure 5.2.3 Electronics overview



5.2.2.1 The analogue system

An overview of the analogue system is shown in Figure 5.2.4, with the full circuit diagram shown in Figure 5.2.5. As can be seen, the output signal from the probe first enters a CMOS operational amplifier. This device, a CA 3140E, has a very high input impedance (10^{12} ohms) which is required to avoid loading the probe. The amplifier is configured in the non-inverting mode, which adds to the overall input impedance of the input circuit. Due to manufacturing variations in the production of pH probes, some adjustments must be available to compensate, and these are provided by the slope and buffer controls. Since pH is also temperature dependant, an adjustment is also required to compensate for variations in solution temperature. These adjustments are all incorporated between the output of the input amplifier and the final amplifier AIC3. The output from this section is measured via an analogue to digital converter and displayed on a liquid crystal readout, these devices being incorporated in one package. The processed signal is also compared with a value which has been pre-set by the operator, and when the voltage from the pH probe reaches the pre-set desired pH level, the output from the comparator AIC4 will go high, and this signal will be sent to the logic circuitry. Since the system has been designed to neutralize acidified solutions, the voltage equivalent to the desired pH will always exceed the voltage from the probe, until adjustment of the solution's pH has been achieved.

Figure 5.2.4 Overview of the analogue system

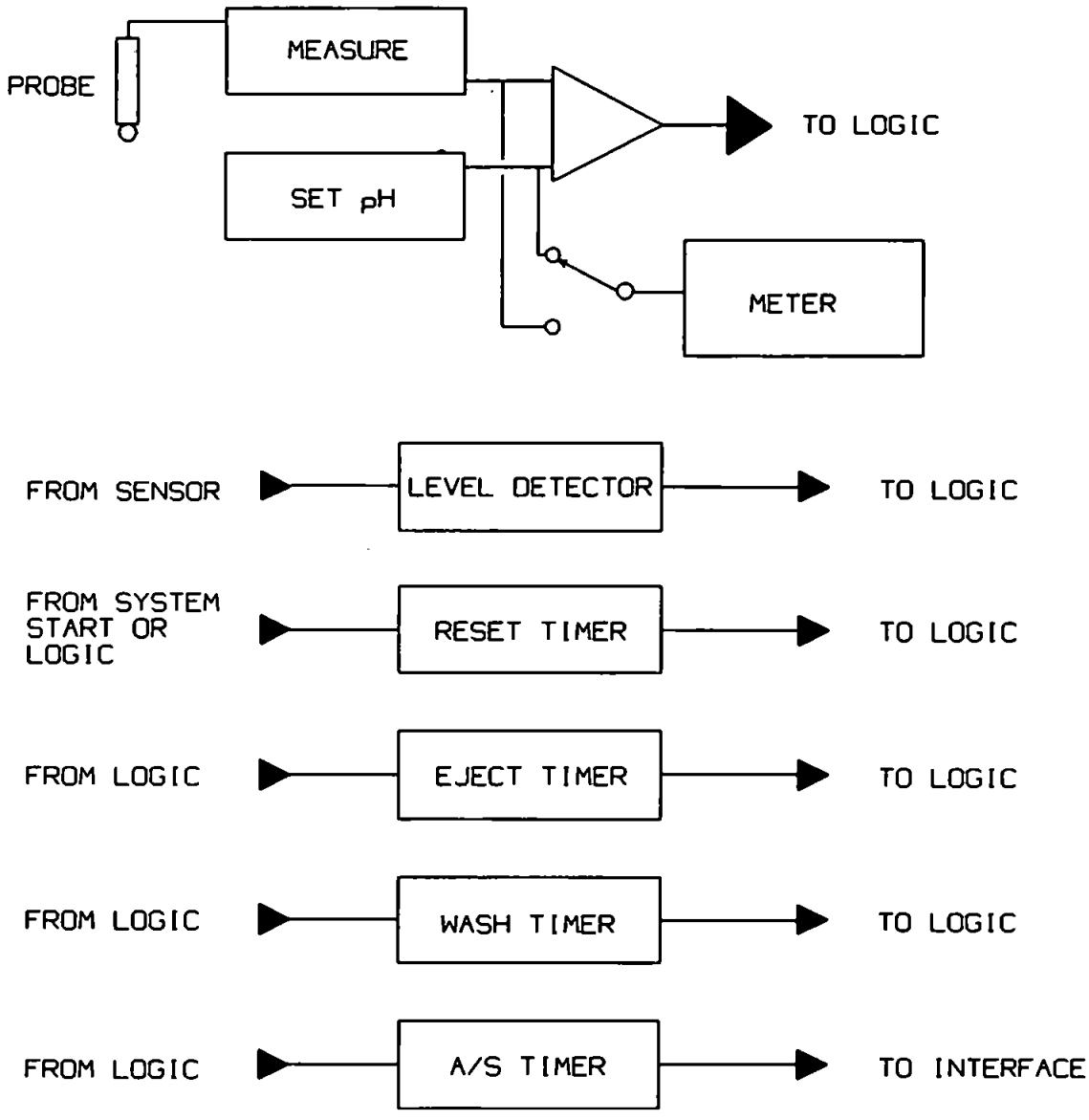
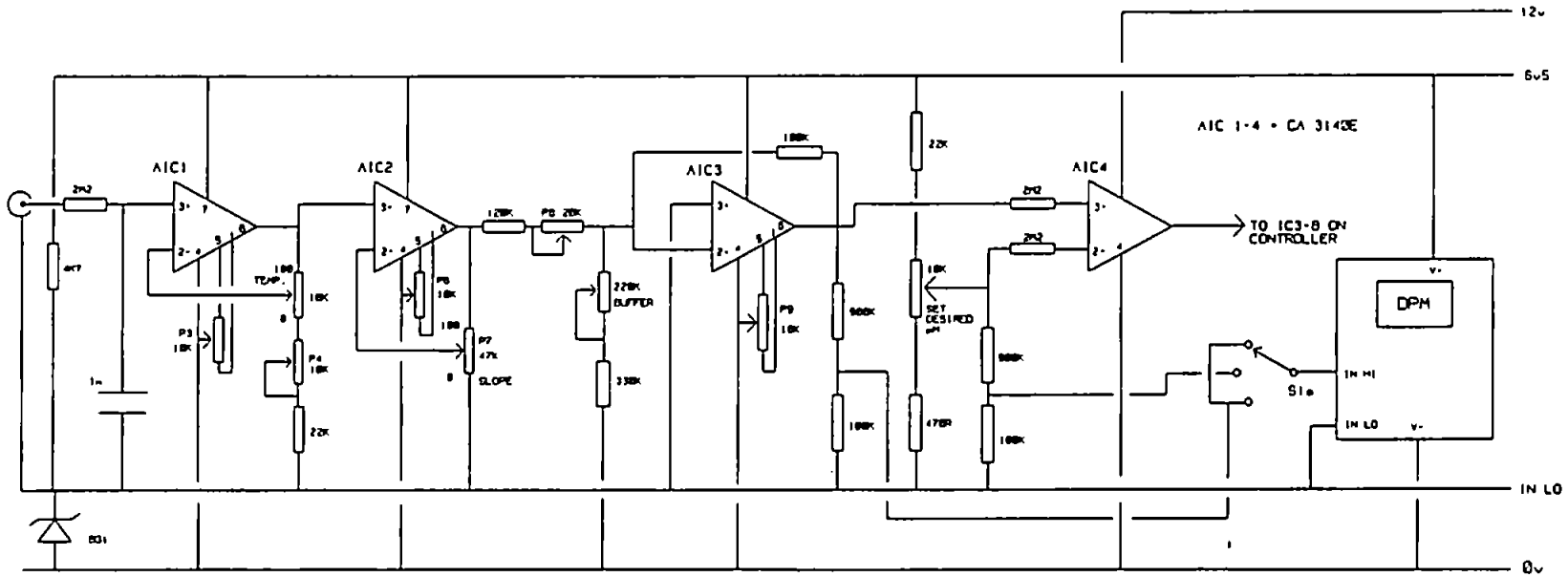
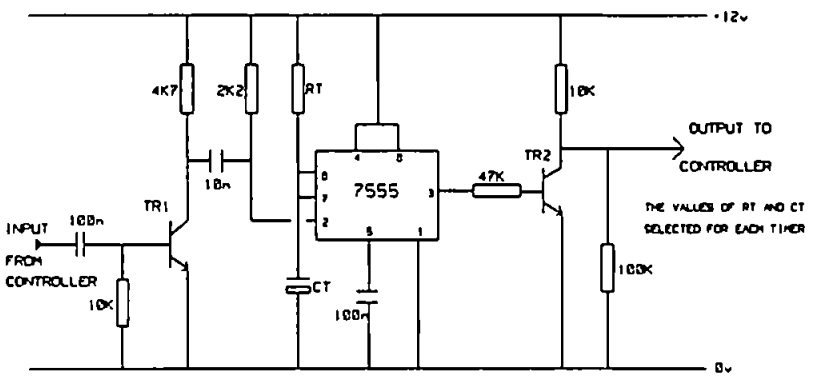


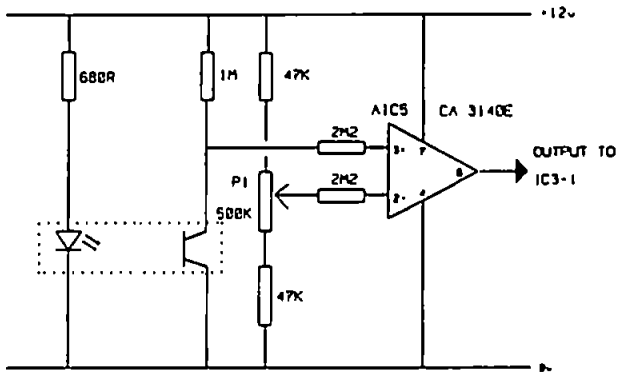
Figure 5.2.5 Analogue, timers and level detector circuit diagram



MAIN ANALOGUE CIRCUIT



TIMER CIRCUIT DIAGRAM



LEVEL DETECTOR CIRCUIT

This section of the circuitry also incorporates a level detection circuit. Since the system is designed to neutralize samples containing trace quantities of analyte, the use of invasive probes to detect the level in the reaction vessel was thought undesirable. The use of an infra red detection system was developed which transmits a signal to the logic circuitry when the desired volume has entered the vessel. The is described more fully later.

Three timers are incorporated into the system, one providing a reset delay, one providing a wash period and another timing the ejection of the analyte. The input to the reset timer comes from either from a system start up circuit or from the logic control. The inputs to both the wash and eject timers both originate from the logic control. All timer outputs are returned to the logic control system. There is also a timer to operate the autosampler with an input from the logic circuitry, and an output going to the interface unit. The period of the reset timer was arbitrarily set at about ten seconds. The wash period was set to approximately twenty seconds, and the eject period was adjusted to approximately thirty seconds, after measuring the time taken for the system to empty, and then allowing a further 15% for error.

Whilst the final instrument will be under computer control, and will therefore be adapted to receive it's timing from the computer, timers were required for the development of the system. The CMOS version of the ubiquitous 555 timer was

selected for use. The 555 timers are simple to use and are very inexpensive - the CMOS version used here have similar benefits, and also consume less power, and do not suffer from spike problems found in their bipolar counterparts. Since the timers are required to be triggered by a positive going transition, and the IC requires a negative going signal, the circuitry around TR1 was designed to achieve this. The circuitry around TR2 inverts the output signal from the timer, making it compatible with the requirements of the rest of the logic system.

5.2.2.2 Setting up procedure for the analogue system

Once the system had been constructed as per the circuit diagram, the system required setting up. Before the integrated circuits were fitted into their sockets, initial continuity checks were carried to check that the pcb layout was correct. Once these checks had been made, the system was powered up, and the operation of the 12 volt regulator checked. Then the 6.5 volt supply was established, by monitoring the output of regulator R2, and adjusting potentiometer P2 until 6.5 volts was measured relative to the 0 volts rail. The mains supply was then disconnected, and the integrated circuits fitted. One lead was disconnected from the buffer control so as to effectively remove it from the circuit. The rest of the setting up procedure is given in Table 5.2.2.

Table 5.2.2 Setting up protocol for the analogue system

ACTION	CONTROL SETTINGS	MONITOR	ADJUST	TO READ
SHORT INPUT	TEMP - 100 SLOPE - MAX	AIC1 pin 4 with DVM	P3	0.0mV
		AIC2 pin 4 with DVM	P6	0.0mV
		PANEL METER	P9	0.00
INPUT +379mV	TEMP - 0 SLOPE - MAX	AIC1 pin 4 with DVM	P4	+516 mV
Refit lead to buffer control - short input	TEMP - 0 SLOPE - MAX	PANEL METER	BUFFER CONTROL	7.00
INPUT -175mV	TEMP - 25 SLOPE - 50%	PANEL METER	P8	10.50
INPUT -70mV	TEMP - 25 SLOPE - MAX	PANEL METER		13.99
INPUT +70mV	TEMP - 25 SLOPE - MAX	PANEL METER		0.01

Once the setting up procedure had been completed, the analogue section was checked for accuracy using a pH meter calibrator (Model 5657-00, Cole-Parmer, of Chicago, Illinois, USA). For this check, the ambient temperature was measured, and that reading set on the temperature control of the analogue system. The calibrator was adjusted to this temperature. The calibrator was set to pH 7, i.e. 0 output, and the buffer control set to give 7.00 on the panel meter display. The calibrator was then set to pH 3, and the slope control adjusted to give a reading of 3.00. The calibrator was then reset to pH 7, and the reading again checked. The calibrator was then set to all values on it's range ie pH 1,3,5,7,9,11,13, and the corresponding values shown on the panel meter display noted. In all cases, the readings were correct to both places of decimals, indicating that the analogue measuring system was functioning correctly and accurately.

5.2.3 The control system

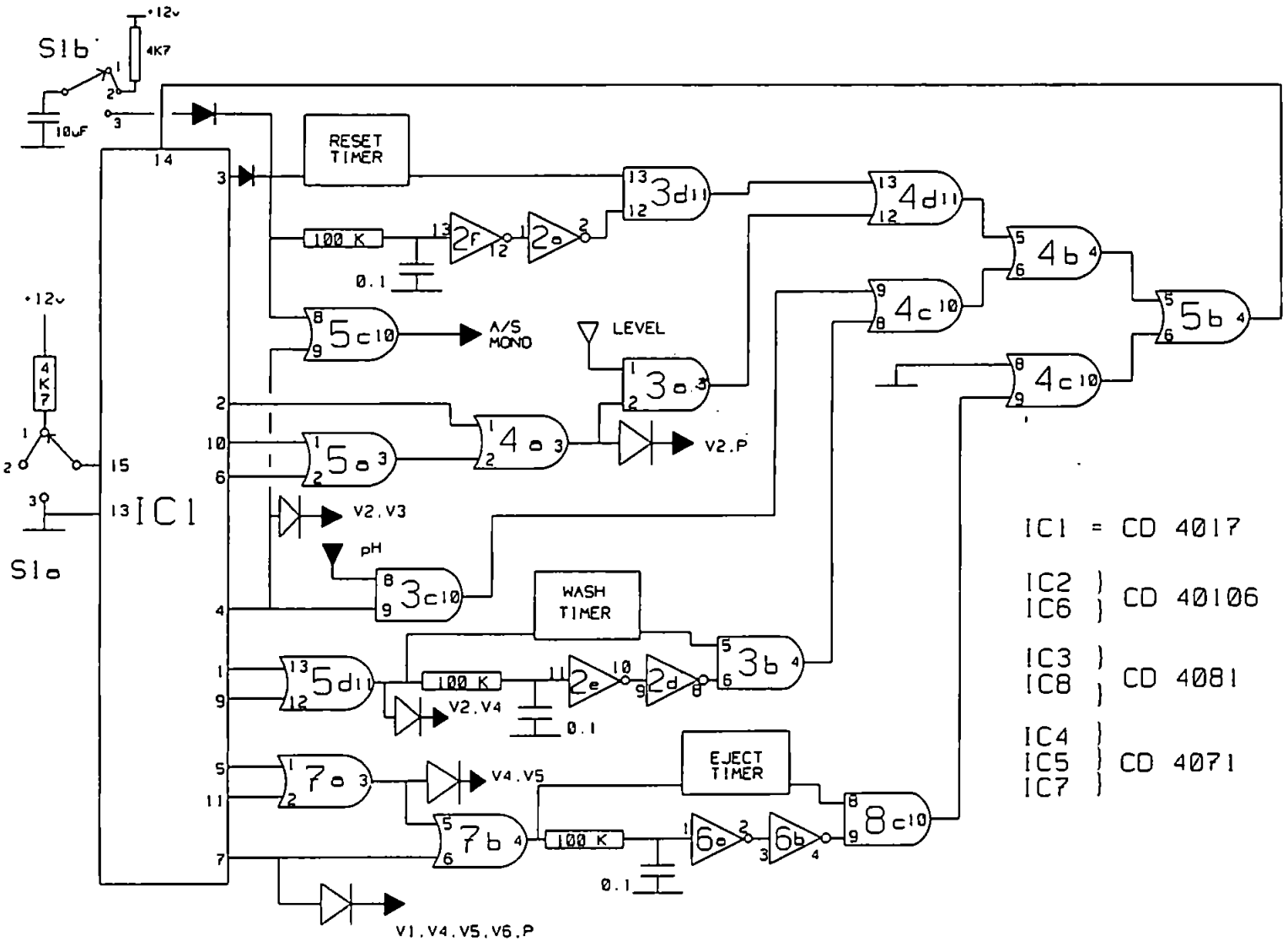
The design of the logic system was then developed. The initial system is shown in Figure 5.2.6. Initially, the use of a microprocessor was considered as the main control device, but as will be observed from Table 5.2.1, there are ten steps overall in the full cycle for neutralization of a sample. This suggested the novel use of a decade counter as the main

controlling device, and this was pursued since these devices are both very inexpensive (around 50p) and reliable. The design problem was therefore to configure the circuitry in such a way as to make each stage trigger the decade counter clock and thereby advance the process to the subsequent stage.

The CD 4017 is a CMOS decade counter, which has ten spike-free outputs, all bar one of which are held low. A positive going pulse on the clock input causes the counter to advance, taking the next output high, and returning the former to the low state, providing the master reset and clock enable pins are held low. It was therefore selected as the main control device, and the rest of the logic built around it.

Selector switch S1 has three positions, 1 - 'probe set up', 2 - 'set desired pH' and 3 - 'start'. During the 'setting up of the pH probe' and the 'setting of the desired pH' potentiometer, switch S1c causes pin 15 (the master reset)

Figure 5.2.6 Logic control circuit diagram



on the decade counter, to go high. This causes output 0 to go high, and all other outputs to go low. In these switch positions, a capacitor is charged via a resistor from the 12 volt line. Moving selector switch S1 to the start position allows this charge to flow through a diode into the reset timer's trigger circuit, and thereby the reset period commences. This pulse also drives pin 8 on IC5c high, sending this gate's output high, which initiates a monostable timer to signal the autosampler to advance to the first sample position. Pin 12 of IC3d is held high by the high output on IC1 pin 3, and at the end of the reset period, IC3d pin 13 also goes high. This causes a high to transfer through three further OR-gates, IC4d, IC4b and IC5b with the output of the latter gate being connected to the clock input of the controller. This positive going signal advances the clock, thereby causing output 1 to go high, and output 0 low.

The high on output 1 causes a high on IC4a pin 3, and this signal is used to switch on valve V2 and the pump. It also acts as a gating signal on IC3a pin 2, the other input to this gate being the level detector output. When the desired level of solution in the reaction vessel is reached, this output goes high, sending a positive going signal through three OR-gates onto the clock input, thereby advancing the counter again. Since output 1 now goes low, the pump and valve V2 are switched off.

The high level on output 2 switches on valves V2 and V3 which

allows the ammonia laden argon to enter the sample solution, and enables AND-gate IC3c. It also puts a high on pin 9 of IC5c, and thence onto the input to the autosampler monostable, so that it is advanced to a wash reservoir. The other input to this gate is routed from the output of the pH comparator, and when the desired sample pH is achieved this goes high. This provides a high level on the output of IC3c, which then passes through IC4c, IC4b and IC5b, thus advancing the counter once again. Since output now goes low, the ammonia supply ceases.

Output 3 now goes high, which provides switching for valves V1, V4, V5, V6 and the pump, and also causes the output of gate IC7b to go high. This initiates the eject timer, and passes through a 100K resistor, via a 100nF capacitor to two inverter gates, IC6a and IC6b. The purpose of the resistor - capacitor combination is to provide a time delay in the high level signal reaching the AND-gate IC8c. This is necessary because the eject timer takes a finite time to switch to it's low (timer on) condition. If the delay were not present, the gate IC8c would have high levels on both input pins before the timer had started, and as a result, the output from gate IC8c would go high immediately, instead of at the end of the timing period. IC6 and its counterpart IC2 are both CD40106's, and these are Schmitt input inverters, which are able to accept the slowly rising input signal produced by the resistor-capacitor filter. Once the eject period is over, pin 10 of IC8c goes high, causing the outputs of IC7c and IC5b to

follow, resulting in the advance of the counter again.

Output 4 of IC1 now goes high, and since it is one of two outputs which allow the vessel to fill with wash solution, the two outputs are fed to the OR-gate IC5a. Either input to this gate causes a high on the output, which then passes to IC4a, another OR-gate. The high output from this gate switches on valve V2 and the pump and gates IC3a. This gate awaits a high from the level detector and when this is received, sends a signal through IC4d, IC4b and IC5b to advance the counter.

The resulting high level on output 5 is again one of two outputs which have identical functions (enabling the wash function), and so are fed to an OR-gate IC5d. The output from this gate triggers the wash timer and switches on valves V2 and V4, and also passes through a resistor-capacitor network before entering IC2e and IC2d, inverting Schmitt gates. The function of these devices are the same as those described above. Once the wash timer period is over, a high level appears on pin 5 of IC3b, which is transmitted on through IC4c, IC4b, IC5b and thus to the clock input of IC1, thus initiating a further change in the counter output.

With output 6 now high, the eject cycle is initiated with a high on the input to IC7a progressing through to switch on valves V4 and V5, and thence through IC7b to start the eject timer. Once this timing period is over, gate IC8c goes high, and this signal progresses through IC4c and IC5b to trigger

another change in state of the CD 4017's output.

The whole wash cycle is then repeated, with outputs 7, 8 and 9 going high in turn, these having similar results to outputs 4,5 and 6 going high. The effects are as described above. At the end of this second wash cycle, output 0 goes high, initiating the reset timer, and the whole process repeats. Switching selector switch S1 back from the start position forces output 0 high, and prevents the process from continuing.

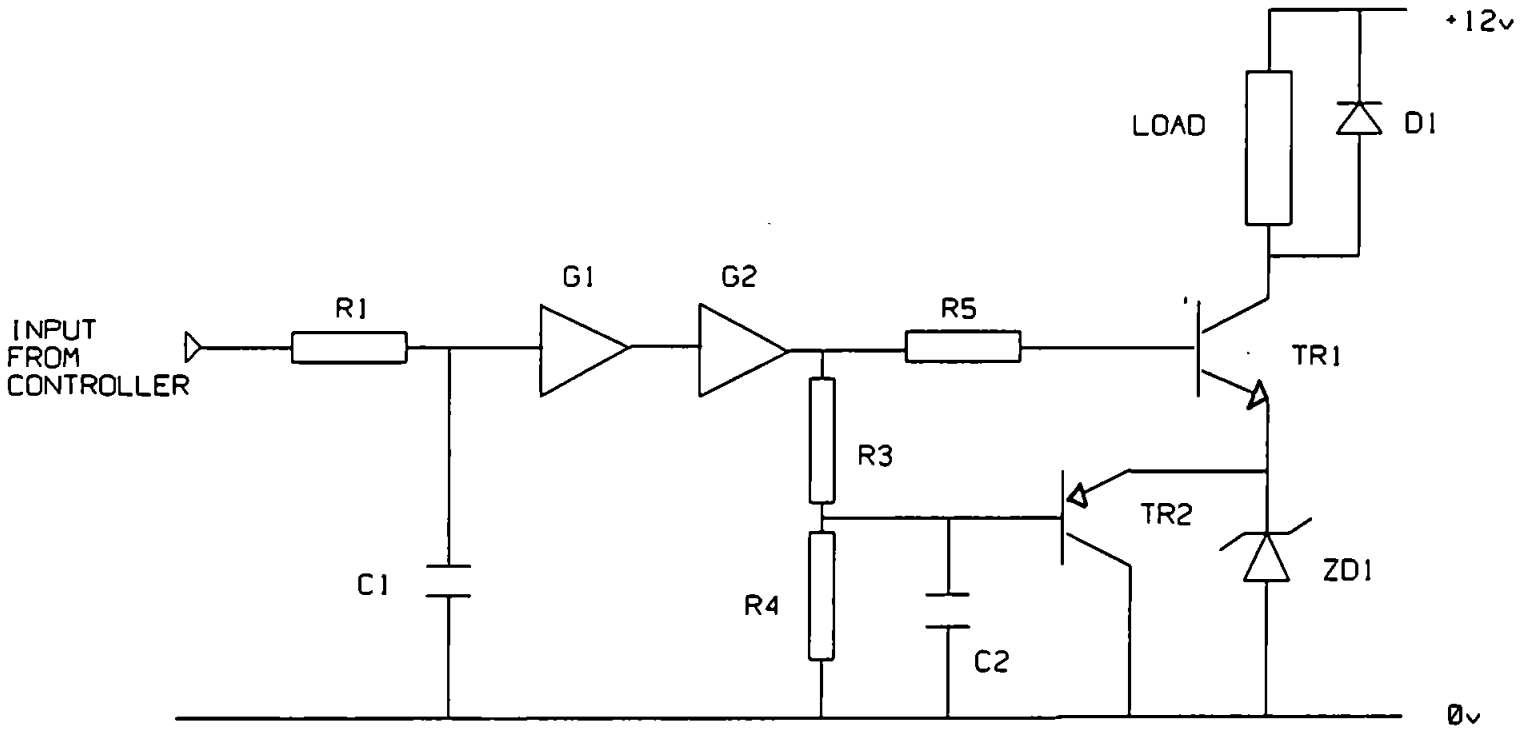
5.2.4 The interface electronics

When any electro-mechanical devices are designed, the rated current of the device is a compromise between that required to cause the device to change state, and the current required to hold that device in the on position. In general, the hold current is much less than the switching current, so if the device is driven continuously with the full switching current, much energy is wasted, and premature failure of the device will occur, due to the effects of heating on the coil and the rest of the mechanism concerned. With these facts in mind, the switching valves were evaluated to determine the hold current. The valves used were all rated at 12 volts, and at this voltage passed approximately 200 mA of current. By lowering the voltage, it was found that the valves still held their 'on' position down to about 4.5 volts. It was decided to allow

a 50% safety margin on this figure, and design the interface in such a way that the valve received the full 12 volts for a second or two, so that efficient switching could be accomplished, and then to lower the voltage to approximately 6 volts to provide the hold current.

The design used is shown in Figure 5.2.7. The input signal (a +12 volts) from the logic control unit first passes through a resistor-capacitor network, which filters out any high frequency noise which may be present, and thereby prevents false switching. The signal is then processed by two Schmitt inverter gates, which have the effect of providing a rapid rise and fall in output, even though they receive a slowly changing signal from the resistor-capacitor network R1-C1. The second inverter, G2, simply alters the signal back from low to high. This +12 volt signal then feeds two resistors, R3 and R5. R5 is a current limiter to protect the base of TR1, and this transistor which is an NPN power type switches on, allowing current to pass through the load valve 1. At the moment of 'switch on', the base of transistor TR2 is at 0 volts, and since TR2 is a PNP transistor, this means that this transistor is fully conducting. Thus the current flowing through the load and TR1 flows through TR2 to 0 volts, and thus the load is subject to the full 12 volts (less $V_{ce(sat)}$ which is approximately 1 volt) . The +12 volts input signal from G2 causes current to flow through R3, and capacitor C2 begins to charge, causing the voltage on the base of TR2 to rise, thereby switching this transistor off. The current is

Figure 5.2.7 Interface electronics circuit diagram



now forced to flow through the zener diode ZD1, which is a 5.1 volt device. The combined voltage drop of ZD1 and TR1 is therefore approximately 6 volts, and this is voltage used to hold the valve in the 'on' state. Diode D1 provides 'back emf protection' to TR1, this being necessary to due reverse induced voltages generated by the collapsing magnetic field in the coil of the valve.

When the input from the controller returns to 0 volts, TR1 loses base drive, and therefore switches off, and the valve immediately closes. C2 discharges through R4, and thus the voltage on the base of TR2 returns to 0 volts. This discharge takes about three seconds, but since none of the valves are required to switch off and then turn back on again immediately, this is of no consequence.

Using this interface, no valve failure was encountered during the months of development and evaluation of the unit. Other users of these valves at this University report that when used at the nominal rating, the valves are prone to a high failure rate, often within weeks of first usage.

The interface unit also contains two relays which are used to supply switching for the autosampler and the pump.

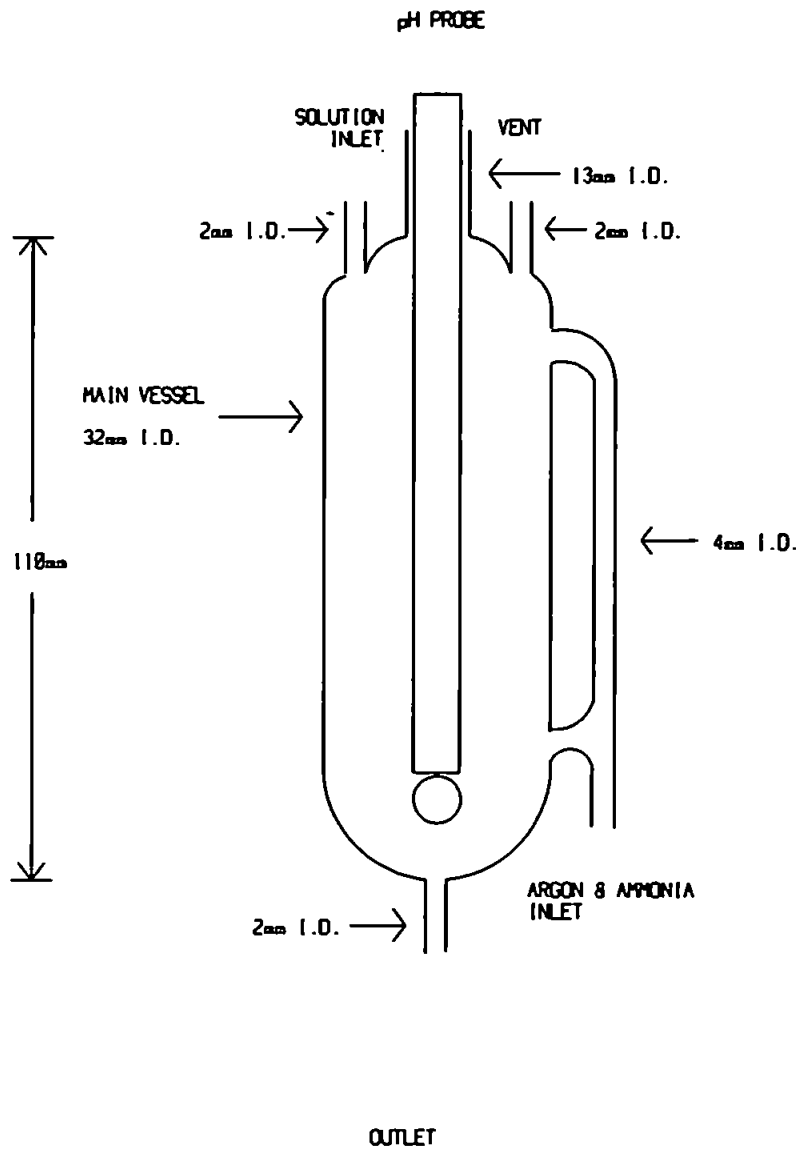
5.2.5 Design of the reaction vessel

The proposed use of gaseous ammonia as the neutralizing reagent dictated the design and construction of a reaction vessel which would permit the dissolution of the ammonia in an efficient manner, and which would avoid the gas coming into direct contact with the pH probe. Such contact would obviously have unwanted effects upon the response of the probe.

It was also considered vital that some kind of agitation or stirring be incorporated, so as to assist in the dissolution of the ammonia, and provide a more uniform solution for the probe to measure. A number of different designs were initially considered, some of which were ruled out as either very difficult to fabricate, likely to suffer from 'memory effects', or simply inefficient.

The idea of a 'bubble pump' system was considered, and this clearly had a number of advantages. An initial design was fabricated in house, and evaluated. The design for this unit is shown in Figure 5.2.8. It worked well when the volume of sample was large enough to give a level above the top of the bubble tube, but did not perform well with small volumes. In these cases, the gas bubbles went up the inside of the main vessel, instead of forcing a slug of the liquid up the bubble tube. It was also realised that the design did suffer from a small dead volume in the outlet tube, and although this was felt to be negligible, the modified design obviated these

Figure 5.2.8 First bubble-pump reaction vessel design



problems. This is shown in Figure 5.2.9. This vessel has no dead volume, and was found to capable of a pumping action with extremely small volumes of sample.

5.3 Evaluation

The complete system was first assembled, the argon supply attached, and the reservoir filled with 0.880 ammonia solution. The analogue system was set up as described in Section 5.2.2.1. Before the probe was fitted, the buffer, slope and temperature controls were set up in the normal manner. The desired pH was then selected. Since the initial requirement had been one of taking 'real' samples and processing them, a test solution of 1% nitric acid was employed in tests to determine the performance of the system.

25ml of this solution were introduced to the vessel, and the level detector adjusted on the bubble tube to this height. The solution was then emptied from the vessel, and a number of sample introduction runs carried out, to evaluate the accuracy of the level detection system. The results are shown in Table 5.2.3.

Figure 5.2.9 Final reaction vessel design

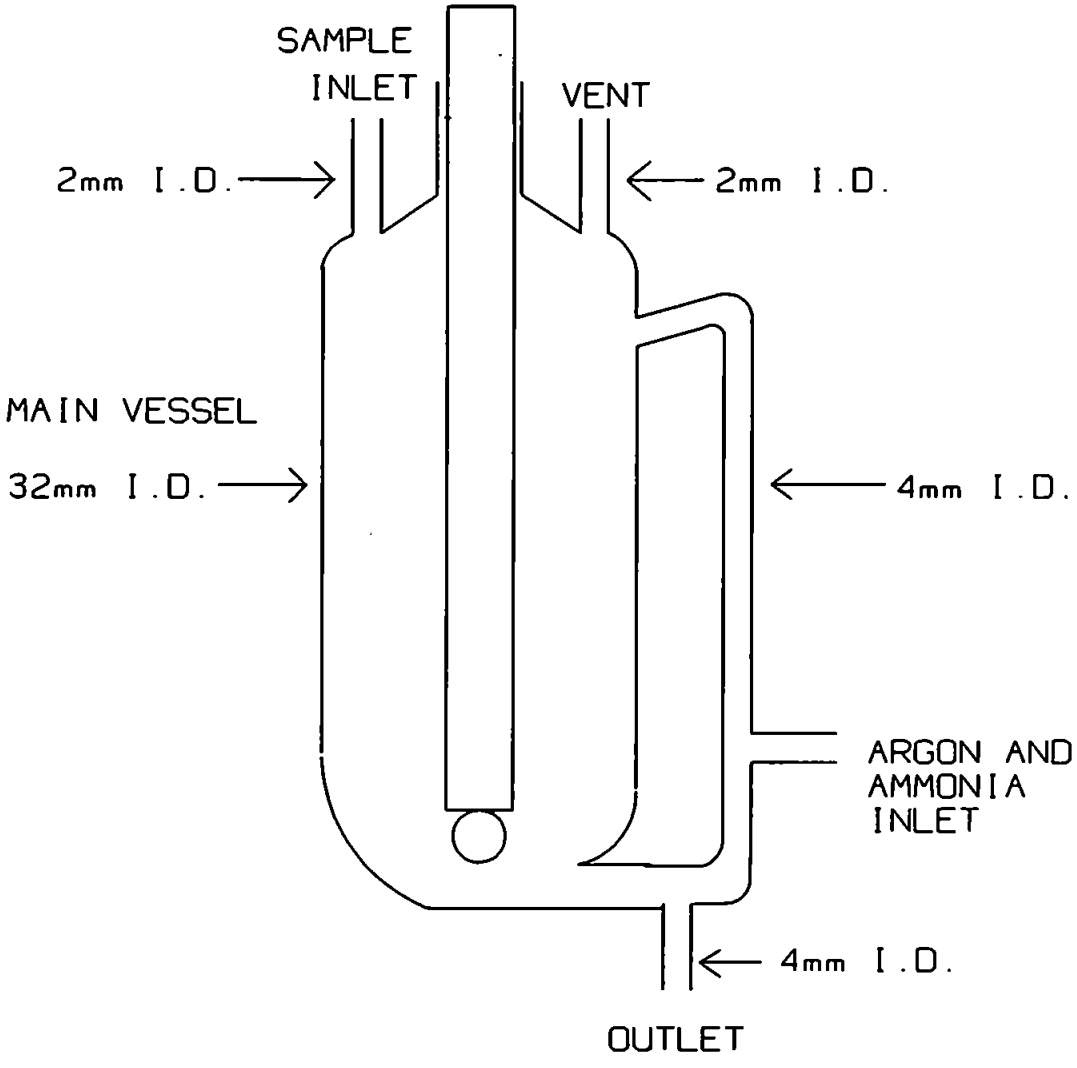


Table 5.2.3 **Evaluation of the reproducibility of the level detection system showing mass of water admitted**

SAMPLE	WEIGHT (g)
1	25.01
2	25.14
3	24.96
4	25.31
5	25.17
6	25.22
7	25.09
8	24.92
9	25.06
10	24.91
MEAN	25.08
σ_{n-1}	0.1326

As can be seen, the level detection system proved reproducible with good precision.

The system was then tested for it's effectiveness in neutralizing 25ml samples of 1% nitric acid. The system was set in run mode, and the gas flow adjusted to provide a rapid steady stream of bubbles into the bubble tube. In this mode, the meter monitors the pH of the solution, and it was observed that for some minutes, no change occurred in the reading. The pH remained below 1 for about four minutes, before starting to climb. From pH 2, the readings increased rapidly, and reached the preset value of 8.5. At this point the valve supplying the ammonia switched and argon alone entered the vessel, although The pH continued to climb, finally reaching a value of 10.2. This process was repeated a further three times, and gave readings in the range 10.1 to 10.4. Clearly the system worked, but required fine tuning to obtain closer control of the pH of the resulting solution.

The next step was to reduce the gas flow through the system, in the hope that this would provide a more manageable change, whilst still facilitating neutralization within a reasonable time period. The time taken to achieve the preset pH value increased slightly, and the final pH values were all approximately one pH unit above the set value.

It was then found that by setting the desired pH to 7.4, five test solutions all ended with pH readings of 8.5 ± 0.16.

Further adjustment of the carrier flow rate resulted in closer agreement between the preset value and that obtained, but at the cost of longer neutralization times. With a neutralization time of approximately 25 minutes, the final pH of the sample was within 0.1 pH units of the desired value on each of the five test solutions.

Whilst these results were encouraging, the time taken to achieve accurate control of the pH appeared excessive, even though the automated system required no operator input. One possible method of reducing the time taken would be to introduce the ammonia rapidly in the initial stages, and then to reduce this flow proportionately as the desired pH is approached. Unfortunately proportional control is difficult and expensive to implement - it was decided therefore to further investigate digital control (i.e. valves either being on or off).

After further observations of the way in which the pH changed, it was confirmed that the rate of change increases rapidly above pH 2. It was therefore decided to apply a two step control system, admitting the ammonia rapidly until pH 2 was reached, and then more slowly in the form of short pulses. A longer time interval between these pulses was also used to allow further agitation of the solution by the argon flow, and sufficient time for the probe to respond..

To achieve the above the analogue system was modified

slightly, and a simple stage was added to the control system. The modifications are shown in Figure 5.3.1. As may be seen, the alteration to the analogue circuitry involves an additional operational amplifier configured to perform as a comparator, with it's inverting input held at 200mV via a potential divider between the +6.5 volt and 'in lo' rails. This input effectively represents a pH value of 2. The non-inverting input is fed from the output of the probe signal amplifying and processing system. The probe output system is therefore monitored by two level detecting circuits, one set to trip at pH 2, and the other operating at the set pH.

The control circuit was also modified by the addition of a timing system which switched the ammonia on for one second bursts every 10 seconds, and a pair of inverter gates. The first inverter gate is fed from the output of the new comparator. When the pH of the sample is less than 2, this comparator's output is low, and thus the output from the inverter is high. This is used to drive the interface circuit, so that the valve remains open, continuously admitting ammonia to the bubble tube. When the pH of the sample reaches 2, the output from the comparator goes high, that of the first inverter gate goes low, and the valve closes. The output from the second gate however is also inverted, and this positive signal is used to operate the timing circuit. This circuit turns the valve on for 1 second and then off for 10 seconds. This technique provided a much closer control of the ultimate pH of the solution, as may be observed in Table 5.3.1. The

Figure 5.3.1 Additional circuitry to improve control of pH and sample throughput

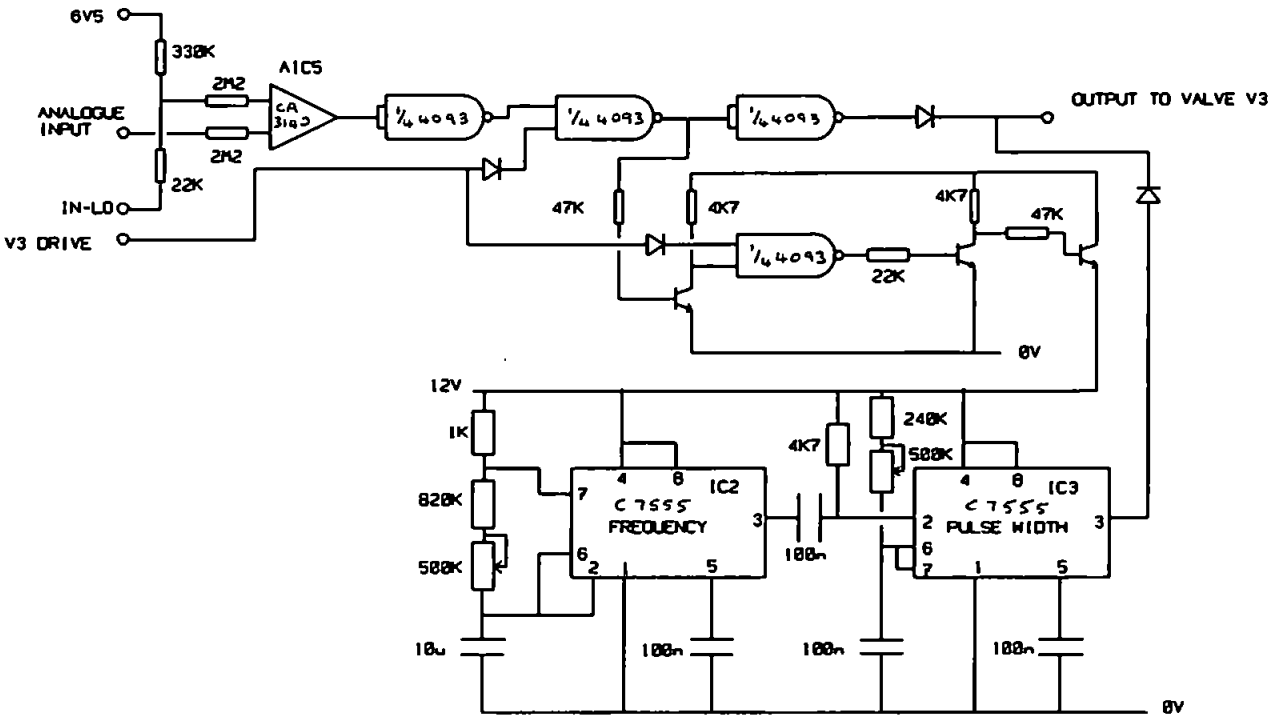


Table 5.3.1 **1% nitric acid samples neutralized to a target setting of pH 8**

SAMPLE	FINAL pH
1	8.06
2	8.09
3	8,11
4	8.10
5	8.08
MEAN	8.08

modified system also provided a more acceptable time for the adjustment of the pH. The average time taken for the adjustment of a 1% nitric acid solution to pH 8 was approximately 8 minutes, giving a throughput of about 5 to 6 samples per hour. Further modifications to the design of the reaction vessel may be possible to increase this figure.

5.4 Discussion

There are very many applications for this system since the majority of aqueous samples are acidified upon collection to prevent "plate out". For many analytical determinations, these samples require neutralization before a particular technique may be employed.

In the selenium speciation system described in Chapter 3, an HPLC column is employed to separate the selenium species present. Since the column is unable to tolerate highly acidic samples, the pH adjusting system could easily be linked to the speciation system to adjust the samples prior to the analysis being made.

The atomic fluorescence cadmium analysis system developed at the University of Plymouth[96], employs sodium tetraethyl borate

to ethylate the cadmium species present, following the equation shown below[97]:

$$Cd^{2+} + 2BEt_4^- \rightarrow CdEt_2 + 2BEt_3$$

High acidity and the presence of transition metals tend to decompose the ethylating agent; with a resultant decrease in the fluorescence signal. The system therefore requires close control of sample pH. Such an analytical system is another obvious application for the neutralizing unit described above.

5.5 Summary

This study has resulted in the development of an automated on-line system for the adjustment of a sample's pH. The use of a gaseous neutralizing reagent results in almost negligible contamination of the sample. Careful design of the electronics associated with the measurement and control of the system has resulted in a simple, reliable. accurate and inexpensive unit, whilst maintaining computer control compatibility. The use of an interface unit to drive the switching valves has resulted in a considerable extension in valve life and reliability.

Chapter 6

Conclusions and further work

6 Conclusions and further work

6.1 Conclusions

The need for more automation in analytical laboratories is self-evident when the number of samples requiring analysis is considered. The demands for increased testing come from many sources - industry, governments, hospitals etc., and this demand is only likely to continue increasing. Ever more sensitive instrumentation means that chemical processes in the environment and in medicine may be studied in ever more detail. As more is understood of these processes, the roles played elements and compounds at trace levels become apparent, and thus the need to analyse for these traces becomes routine. Developments in the research laboratory pass to the analytical laboratory with astonishing speed.

Within this thesis, a number of differing techniques have been studied, but one underlying thread has been common throughout, that of automation. Much work has been carried out on selenium, and this element illustrates well the points made above. Earlier workers were content to merely obtain total concentrations of the element in a sample, but as knowledge of selenium chemistry has continued to increase, it has become apparent that the form of the element in the sample is also of critical importance, and hence the need for speciation has emerged. In addition the levels of detection required both for total concentration and individual species, continues to place

further demands on the analyst.

Until very recently, it was only possible to determine the inorganic species of selenium in a given sample by difference when using hydride generation, since only selenium(IV) reacts with the reductant to form the hydride. Using this technique, the sample is split in two and selenium(IV) is first determined. The other sample is then subjected to a reduction process to convert selenium(VI) to selenium(IV), and the analysis repeated to give a total selenium concentration. Selenium(VI) is thus determined by subtracting the former concentration from the total. This method has been employed for many years as the most convenient method available for trace determinations of selenium. The first of the automated systems described in this thesis relied upon this technique. This system employed a microwave pre-reduction step as part of a computer controlled on-line system, coupled to a hydride system with a upon quartz furnace-atomic absorption spectroscopy detection system. Since such instruments are widely available in most laboratories, the system described represents a way in which automation may be achieved at minimum additional cost. The drawbacks of this technique are that the determination of selenium(VI) is carried out indirectly, and that detection limits are limited to the low ng ml^{-1} range due to the lack of sensitivity of the detector.

These problems were then addressed by the development of the second system, which employed HPLC to achieve the separation

of the inorganic selenium species present. Since the column employed for the study would be destroyed by a highly acidic sample, post column acidification was employed. This was followed by microwave heating which left the selenium(IV) unaffected but reduced the selenium(VI). Normal hydride generation techniques then followed, with detection of the hydride being carried out by atomic fluorescence. This more sensitive detection system was necessary due to the limited sample volume (1 ml) injected onto the HPLC column, which was subsequently diluted by the addition of the acid. Detection limits of 0.2 and 0.3 ng ml^{-1} were obtained for selenium(IV) and selenium(VI) respectively.

Other work conducted on the determination of selenium included the study of signal enhancement due to the presence of traces of nitric acid in the matrix, when hydride generation was employed. This enhancement was shown to be linked to the type of pump tubing employed, but since the order in which the samples were prepared also had an effect, it was felt that the possibility of the formation of a nitro-selenium complex could not be ruled out, although further work to elucidate the mechanism was outside the scope of this thesis. This study does illustrate however, just how much care has to be paid to all parameters in on-line systems, even the selection of such mundane items as pump tubing, when carrying out determinations at trace and ultra trace levels.

The investigation into the conditioning of hydride generation

apparatus when determining selenium at ultra trace levels also demonstrated an important aspect of instrument design, if automated data handling methods are to be subsequently employed. Conditioning in this case was achieved by the injection of two 1 mg ml^{-1} samples of selenium(IV), and then allowing the system to run for at least fifteen minutes before starting to analyse samples. This had the effect of filling the active sites in the system, and resulted in the removal of the drift which had previously been observed.

Clearly, fundamental aspects of the chemistry also need to be considered in some detail if optimal performance is to be obtained with on-line systems. This is well demonstrated by the investigation into the kinetics of the reduction of selenium(VI). This study indicated that the process occured within six minutes at 70° C under our laboratory conditions i.e. much less than the thirty minutes commonly employed to achieve reduction. Thus it was possible to decrease the reduction period, minimising possible losses, and reducing the chances of contamination. However, it is important to understand that the solution has to be at 70°C for six minutes - the total heating time will be dependant upon the method of heating employed. The activation energy for the reaction under our conditions was found to be 90.4 kJ mol^{-1}.

Organo-selenium compounds represent a challenge to the atomic spectroscopist, since it is difficult to break their carbon-selenium bonds. This is required if hydride generation is to

be employed in their subsequent determination, and since this represents one of the most sensitive techniques currently available, the breaking of these bonds is necessary. Microwave heating of seleno-methionine in hydrochloric acid appeared to have no effect. The action of UV from a high intensity mercury discharge lamp was shown to break the carbon-selenium bonds, and thus enable the selenium to be determined by hydride generation coupled to atomic fluorescence detection. Although the time taken under the experimental conditions prevailing was excessive, at sixty minutes, for incorporation into an on-line system, sample volumes were large, as was the diameter of the sample tubes and the distance from the tubes to the source. If this distance were reduced, and a capillary used to carry the sample, then on-line operation would be feasible.

Pyrolysis has been a technique which has generally been used for the destruction of organic samples. In this study, pyrolysis has been employed in the determination of mercury. The technique was successfully employed for the determination of dense inorganic samples such as sediments. It was not successful however when applied to low density organic samples. This could be due simply to the low sample volume employed in the oven used in the study. The difficulty in accurately weighing samples of a few milligrams is obvious, and whether such small samples are representative of the main batch is also highly questionable.

Finally, the development of a pH adjusting system which could

be employed on samples which required trace and ultra trace determinations to be subsequently carried out on them was achieved. This work resulted in a low cost, dedicated system which could be easily interfaced to a computer. Problems concerning the overall reliability of the switching valves were overcome by the design of a novel interface which reduced the drive voltage applied once the valve had switched. The use of a bubble pump system enabled the ammonia to be added to the solution away from the main body of the liquid, thus avoiding contact between the gas and the pH electrode, whilst it also caused agitation of the sample solution. This apparatus was employed successfully in conjunction with the HPLC-microwave selenium speciation system developed as part of this thesis.

This thesis therefore describes a range of techniques employing hydride generation, photolysis, pyrolysis, microwave reduction, all of which have been operated on-line, and all of which have been, or could easily be automated. The operation of the systems have all been checked by the use of real samples, as well as prepared standards.

7.1 Further work

Although much work has been carried out on hydride generation, there are still many areas in which further work would be beneficial. Any study of the literature on this subject will quickly establish that there is still disagreement upon many

fundamental aspects of the technique. Thus, since the use of hydride generation lends itself to on-line systems, further study of the fundamentals would clearly aid the development of such systems.

Further investigation of the signal enhancement effect due to the presence of traces of nitric acid when determining selenium, to fully elucidate the mechanisms involved would be beneficial. Whilst the nature of the tubing was found to be a major cause of the enhancement, the reasons for the variations in the enhancements depending upon the order in which the reagents were added should be determined.

Other work of interest started in this study but not reported in this thesis includes the generation of hydrides directly from the sample solution by electrolytic methods. This technique would seen to offer the potential to obviate contamination of the sample by impurities in the reagents which are currently added, and could provide a cheaper and probably a more consistent method of hydride generation.

Further development of the automated HPLC-microwave-HG-AFS system should result in shorter analysis times, along with lower detection limits. Other eluents may provide a quicker separation in the HPLC stage, and further refinement of the rest of the system should be capable of improving the overall sensitivity.

The photolytic breaking of the selenium-carbon bond in seleno-methionine was very encouraging, and it would appear to be a simple task to produce an on-line system incorporating such a step. The production of a speciation system capable of determining organic selenium, selenium(IV) and selenium(VI) becomes a reality, providing the photolysis step is able to deal with the trimethyl selonium ion, which is more difficult to destroy than the seleno-methionine bonds.

Further development of the pH adjusting system could be carried out - no difficulty should be encountered in modifying the electronics to deal with alkaline samples as well as acidic ones, and so a comprehensive pH adjusting system could be constructed. It should be possible for example to use hydrogen chloride as the neutralizing agent for alkaline samples. In spite of the desire to design the existing system using the simplest and most inexpensive circuitry available, a new design employing a PIC microprocessor would offer increased flexibility (if required), a reduced component count, and programmable setting up procedures. The microprocessor could also be used to provide a log of samples etc., which although not required for the system described within the text, could have possible applications in other systems.

Finally, development of computer software dedicated to on-line systems could be carried out. Although the software employed throughout this study has proved reliable, it was developed

as a general system. The problems observed in the drift of the standards when determining selenium at ultra trace concentrations could be overcome by suitable design of software. If such a programme allowed the running of a standard whenever required by the analyst, and calculated the concentrations of samples between standards on the basis of a time related drift, it could effectively compensate for the drift. In addition, the facility to integrate traces containing two or more plateau type peaks individually with either a computer derived baseline, or one selected by the analyst would be of great value. Such software facilities exist in part in commercially available programmes, but do not appear to have been gathered together into one programme as yet.

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TouchStone Instrument Signal Peak Data Acquisition

The Borland Pascal code below is a simplified extract from TouchStone, showing the approach to peak reading of AA hydride generator signals. The actual program caters for many other techniques and instruments. Comments are in italics beteen braces. *{Comment}*

```
procedure ADPoint;{This procedure is called repeatedly to read and plot each
point}
var ADSum,ReadsDone:integer;

begin
  for ReadsDone:=1 to ADReadings do {Normally ADReadings=4}
    ADSum:=ADSum:=ADRead;
  {ADRead returns a signed integer the mean of 16 12 bit A/D readings}
  {it also synchronises to the computer clock at 18.2Hz}

  ADSum:=ADSum div ADReadings;
  ADSum:=filter(ADSum);{Running Mean Filter, number set in Method-Filter}
  AbsRead:=ADSum / ADFactor * AdMult;{Convert to Absorbance for this AA}
  inc(x);{next X axis pixel position}
  ShowSignal;{Plot Point and show actual reading}
end;{Procedure ADPoint}

Procedure PlotAD;{Reads baseline then peak data using ADPoint to plot on
screen}
begin
  XAxisMax:=546;{Maximum allowed X axis value in pixels}
  TimeEntCalc;{Set up ZeroPlot and ADReadings to Vapour Gen timers}
  x:=0;{X Axis Pixel Counter}
  PeakArea:=0;
  PeakHeight:=-1;
  WriteBarGR('READING BASELINE SIGNAL _Esc-Abort');
  OK:=FALSE;{Set TRUE only if a valid result measured}
  BaseLine:=0;

  repeat                                    {Get BaseLine during delay}
    ADPoint;                                {Read A/D, filter, and plot}
    BaseLine:=BaseLine+AbsRead;             {Add result to BaseLine}
  until keypressed or (x > ZeroPlot);       {Until Delay Time Done}
  BaseLine:=BaseLine / (ZeroPlot+1);        {Calculate Mean}
  drawstring('BaseLine= '+RealToString(BaseLine)+' Abs');
```

```
{Valve Switches Here}
  WriteBarGR('Measuring Signal Peak');
  PeakArea:=0;      {Set Peak Initial values}
  PeakHeight:=-1;  {Ensure invalid result unless A/D read}
  PACount:=0;

  repeat
    ADPoint;                    {Read A/D, filter and plot on screen}
    AbsRead:=AbsRead-BaseLine;  {Subtract BaseLine}
    if AbsRead > PeakHeight then PeakHeight:=AbsRead;{Update PkHeight}
    PeakArea:=PeakArea+AbsRead; {Add to Peak Area running total}
    Inc(PACount); {Increment count of readings summed in PeakArea}
  until keypressed or (x > XAxisMax) or (not hydrunning);
  {Normally stops when vapour generator cycle complete}

  if PeakArea < 0 then PeakArea:=0; {Negative value meaningless}

  if not keypressed then begin {Scale and display the peak results}
    PeakArea:=100 * PeakArea / PACount;{Adjust to timescale selected}
    GotoXYGR (51,3); DrawString(' Peak Area: '+RealToString(PeakArea));
    GotoXYGR (51,3); DrawString(' Peak Height: '+RealToString(PeakHeight)+'Abs');
    ok:=TRUE;
  end;{if not keypressed}
end;{Procedure PlotAD}
```

CONFERENCES AND COURSES ATTENDED

Sixth Biennial National Atomic Spectroscopy Symposium, University of Plymouth, July, 1992.

XXVIII Colloquium Spectroscopicum Internationale, University of York, June, 1993.

Research and Development Topics, University of Bradford, July, 1993.

Seventh Biennial National Atomic Spectroscopy Symposium, University of Hull, July, 1994.

Research and Development Topics, University of Hertfordshire, July, 1994.

Atomic Spectrometry Updates - Atomic Spectroscopy Group, University of Bristol, March, 1995.

Erasmus Eurocourse - Frontiers in Analytical Chemistry : Trace Environmental Analysis, University of Plymouth, September,

1995.

Royal Society of Chemistry lectures and lectures by invited speakers at the University of Plymouth

Various weekly research lectures at the University of Plymouth

PUBLICATIONS

Selenium speciation - a flow injection approach employing on-line microwave reduction followed by hydride generation-quartz furnace atomic absorption spectrometry

Pitts L.J., Worsfold P.J. & Hill S.J.

Analyst, 1994, **119**, 2785.

Investigation into the kinetics of selenium(VI) reduction using hydride generation atomic fluorescence detection

Hill S.J., Pitts L.J. & Worsfold P.J.

Journal of Analytical Atomic Spectrometry, 1995, **10**, 409.

Selenium speciation using high performance liquid chromatography-hydride generation atomic fluorescence with on-line microwave reduction

Pitts L.J., Fisher A., Worsfold P.J. & Hill S.J.

Journal of Analytical Atomic Spectrometry, 1995, **10**, 519.

PRESENTATIONS

An investigation of signal enhancement effects due to the presence of nitric acid on the determination of selenium using hydride generation

XXVIII Colloquium Spectroscopicum Internationale, York, June, 1993.

The determination of selenium using hydride generation - an investigation of signal enhancement effects due to the presence of nitric acid

Research & Development Topics, Bradford, July, 1993.

Selenium speciation in aqueous samples - an on-line technique employing microwave pre-treatment followed by hydride generation-quartz furnace atomic absorption spectrometry

7th. Biennial National Atomic Spectroscopy Symposium, Hull, July, 1994.

The determination of mercury in sediments using on-line pyrolysis, pre-concentration and atomic fluorescence detection

7th. Biennial National Atomic Spectroscopy Symposium, Hull, July, 1994.

The development of an on-line system employing microwave reduction for selenium speciation

Research & Development Topics, Watford, July, 1994.

Selenium speciation - a flow injection approach employing on-line microwave reduction followed by hydride generation-quartz furnace atomic absorption spectroscopy

FACSS XXI, St. Louis, USA, October, 1994.

The determination of mercury in sediments using on-line pyrolysis, pre-concentration and atomic fluorescence detection

FACSS XXI, St. Louis, USA, October, 1994.