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Flow Injection Methods of Sample Introduction for ICP-MS

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To my parents, my sisters

and Sarah

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Abstract.

This thesis is concerned with the application of flow injection techniques for on-line sample preparation for inductively coupled plasma mass spectrometry (ICP-MS).

A comprehensive introduction to ICP-MS and the various sample introduction methods which have been applied for the technique is presented in Chapter 1. The advantages and disdvantages of the different sample introduction procedures are also considered and discussed. Since ICP-MS is a highly sensitive technique for multi-element analysis at trace levels, there are few methods which can compete with it. The methods which are available are compared with ICP-MS and the advantages and disadvantages of each are discussed in the context of the available literature.

The second chapter contains a detailed review of the various reagents which have been used for the selective retention of trace element ions from solutions containing high concentrations of so called 'matrix species' (e.g. Na, Ca, K and Mg). The use of ion-exchange and chelating reagents for trace analyte retention is discussed together with an appraisal of the properties and applications of activated alumina as either a cation or anion exchanger. Most ion-exchange and chelating reagents consist of an organic functional group covalently immobilised onto a support material. The properties of polymer and controlled pore glass support materials are discussed with respect to their behaviour in on-line systems.

In Chapter 3, the use of the chelating reagent 8-hydroxyquinoline, covalently immobilised onto controlled pore glass, for on-line preconcentration and matrix separation with ICP-MS detection, is described. A manually operated manifold, directly coupled to the ICP-MS, was developed for this work. The optimisation and analytical

performance of this manifold is evaluated for Ti^{4+}, V (as VO^{2+}), Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Ag^{+}, Cd^{2+}, Ce^{3+} and Pb^{2+} in saline matrix samples and reference materials.

Following the development of the manual manifold described in Chapter 3, the construction of an automated preconcentration / matrix separation manifold, using a commercially available liquid handling system is described in Chapter 4. For this work, a novel iminodiacetate chelating reagent, immobilised onto a controlled pore glass support, was used for analyte retention. An initial manifold design is considered, followed by a refined version of the system, which is optimised for the retention of Mn^{2+}, V (as VO^{2+}), Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Ag^{+}, Ce^{3+}, Cd^{2+}, Pb^{2+} and U (as UO_2^{+}). The performance of the novel iminodiacetate chelating material is evaluated for the analysis of two saline reference waters and some saline industrial effluent samples.

In Chapter 5, the effects of humic material in the sample on the on-line retention of Ti^{4+}, V (as VO^{2+}), Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Ag^{+}, Cd^{2+}, Ce^{3+}, Pb^{2+} and U (as UO_2^{+}) by the iminodiacetate reagent is explored. Humic material, which arises from soil and decaying vegetable matter, exists in fresh and saline waters at concentrations of typically 1 - 50 ppm. This complex material is composed of a number of different organic molecules which contain a variety of functional groups capable of forming chelates with the analytes of interest in the sample. For this reason, interference effects are expected to occur with increasing levels of humic material in the sample. The effect of increasing levels of elements which have an affinity for the iminodiacetate reagent is examined in terms of the retention efficiency of the analytes of interest.

In Chapter 6, the subject of microwave digestion for sample preparation is studied. This work initially involves assessing the efficiency of a simple nitric acid digestion for the digestion of selected reference materials in a batch system. The study continues with an evaluation of on-line microwave digestion with off-line analysis using ICP-MS and

concludes with an investigation into the feasibility of directly coupling the on-line microwave digestion system to the ICP-MS instrument. Finally, in Chapter 7, the general conclusions to the thesis and suggestions for future research are presented.

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CHAPTER 4. ON-LINE MATRIX SEPARATION AND PRECONCENTRATION USING IMINODIACETIC ACID IMMOBILISED ONTO CONTROLLED PORE GLASS.

CHAPTER 5. INTERFERENCE EFFECTS OF DISSOLVED ORGANIC MATTER AND CONCOMITANT ELEMENTS ON MATRIX SEPARATION.

CHAPTER 6. APPLICATIONS OF MICROWAVE DIGESTION IN COMBINATION WITH ICP-MS.

CHAPTER 1

SAMPLE INTRODUCTION METHODS FOR ICP-MS

1.1 Research objectives.

The main objective of the work described in this thesis was to explore the application of on-line preconcentration and separation of trace elements from saline matrix samples, before analysis by ICP-MS, using a flow injection approach. This sample introduction approach has begun to be evaluated in recent years, with varying degrees of success. Problems remain with the efficiency in which the sample matrix can be eliminated and the length of time the procedure takes (often up to 10 minutes per sample). Traditionally, polymer based chelating reagents, particularly of the iminodiacetate type, have proved popular for this application because of their high analyte retention capacity and affinity for a wide range of analytes. However, polymer based reagents often suffer from swelling and contraction problems with changing solution composition, which can limit their use in on-line systems. In this work, it is intended that alternative chelating reagents, based on a rigid, controlled pore glass support (CPG) will be used for this application. The performance of the popular CPG-8-hydroxyquinoline reagent for extraction of V (as VO^{2+}), Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Ag^{+}, Cd^{2+}, Ce^{3+} and Pb^{2+} from saline solutions will be examined and efforts made to improve upon the matrix separation efficiency and rate of sample throughput which has been reported for this material in ICP-MS studies. A new controlled pore glass reagent containing iminodiacetate functional groups will also be assessed as a reagent for on-line preconcentration and matrix separation for ICP-MS. The performance of this material for extraction of V (as

VO^{2+}), Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Ag^{+}, Cd^{2+}, Ce^{3+}, Pb^{2+} and U (as UO_2^{+}) from saline solution, using an automated on-line matrix separation manifold, will be explored. The effects of high concentrations of concomitant elements and the presence of humic material (a natural organic chelating substance) in the samples, in terms of the analyte retention efficiency of the system, will be discussed.

Finally, the area of on-line microwave digestion as a means of sample preparation will be evaluated. This method of sample preparation and introduction has not, at present, been reported for ICP-MS, and has received relatively little attention in general. The practical considerations and potential difficulties associated with on-line microwave digestion will be discussed. The performance of a commercially available on-line microwave digestion system for the digestion of sediment and soil reference materials will be compared with that of a batch digestion system. In addition, the feasibility of directly coupling the on-line microwave digestion system to the ICP-MS will be investigated and the practical requirements discussed.

1.2 Historical aspects of Inductively Coupled Plasma Mass Spectrometry (ICP-MS).

The story of ICP-MS began with the work of Alan Gray, at ARL, Luton, in the early 1970's. Gray was investigating new techniques for inorganic analysis of geological samples. At this time ICP-AES, though still in its infancy, was already proving to be far from ideal for this type of analysis, principally because of numerous emission interferences from the major matrix species, notably Ca, and insufficient detection limit capabilities. Mass spectrometry (MS) was recognised as an ideal solution to these problems, but the vacuum requirements of this instrumentation made sample introduction complicated compared to ICP-AES. Gray's background meant he had limited knowledge of MS, so to gain a better insight into how atmospheric sampling for MS

detection might be possible, he attended conferences on the subject. It was in late 1970, at one such conference in Manchester that the idea of ICP-MS was first conceived by Alan Gray. At this conference, work was presented on the subject of sampling atmospheric pressure flames for analysis by MS. Gray made the connection between sampling flames and sampling electrical plasmas and work began on interfacing a capillary arc plasma (CAP) to an MS system. The results of these promising early studies highlighted the relative simplicity of the spectra obtained and also drew attention to the very low detection limits that could be achieved.[1,2,3] However, the CAP operated at an effective temperature of only 3000 K, which was insufficient to quantitatively ionise elements having first ionisation potentials above 8.5 eV. This relatively low temperature also lead to severe matrix effects and incomplete dissociation of molecular species. It was evident that a more efficient, higher temperature atmospheric pressure ion source, such as the ICP, was required and Gray planned to extend the project in this direction. Unfortunately, at this point ARL's research facility at Luton was closed down and work with the CAP ended. However, Gray's early work had sparked interest in the technique in the US, where work started using an ICP ion source, and Canada, where investigations began using a microwave induced plasma[4] but later progressed on to an ICP.[5] A collaboration was established between Gray and the US group, which included Velmer Fassel, renowned for his work with ICP-AES, and Sam Houk, a research student. In 1978, just four years after the initial CAP studies by Gray, the first study using an ICP as the ion source was carried out by Houk *et al.*[6], at the Ames Laboratory in Iowa, USA. In this pioneering study, published in 1980, the authors developed an electrically grounded interface, consisting of two water cooled metal cones, to link the atmospheric pressure ICP to the vacuum system of a quadrupole mass spectrometer. The electrical grounding was necessary to reduce the positive potential (known as the plasma potential) between the plasma and the interface cones and thereby prevent

electrical discharge occurring at the interface. With this grounding arrangement, the potential at the interface was essentially zero, but a potential gradient across the plasma still remained. This potential was not considered to degrade analytical performance, and the grounded interface design remains in use today. An alternative approach to grounding the plasma relative to the interface was devised by Douglas and French.[7] Their design was based on grounding the coil in the centre and applying the rf voltage from both ends of the coil, in such a way that the magnitude of the voltage at each end was the same but of opposite polarity. In this way, the plasma potential could be reduced to practically zero, because the potential difference between the plasma and the interface was removed. These early studies presented a comprehensive evaluation of the potential of the ICP as an ion source for inorganic mass spectrometry. The work also described some of the inherent limitations of the technique, such as low tolerance to dissolved solids and the formation of polyatomic interferences, that still continue to plague ICP-MS today. Despite these limitations, the technique developed extremely rapidly and just four years after the benchmark study by Houk *et al.*[5], the first commercial instruments became available, courtesy of VG Elemental in the UK (based on the work of Gray and Houk) and Sciex in Canada and the US (based on the work of Douglas and French).

1.3 Principles of operation of ICP-MS.

In the years since its conception, much of the work carried out with ICP-MS has been concerned with sample preparation and presentation to the instrument, rather than with further instrumental development. The considerable, and growing, interest in the technique is reflected in the large number of citations in the literature which, since 1983, total over 1200.[8] The layout of a typical quadrupole ICP-MS instrument, configured for

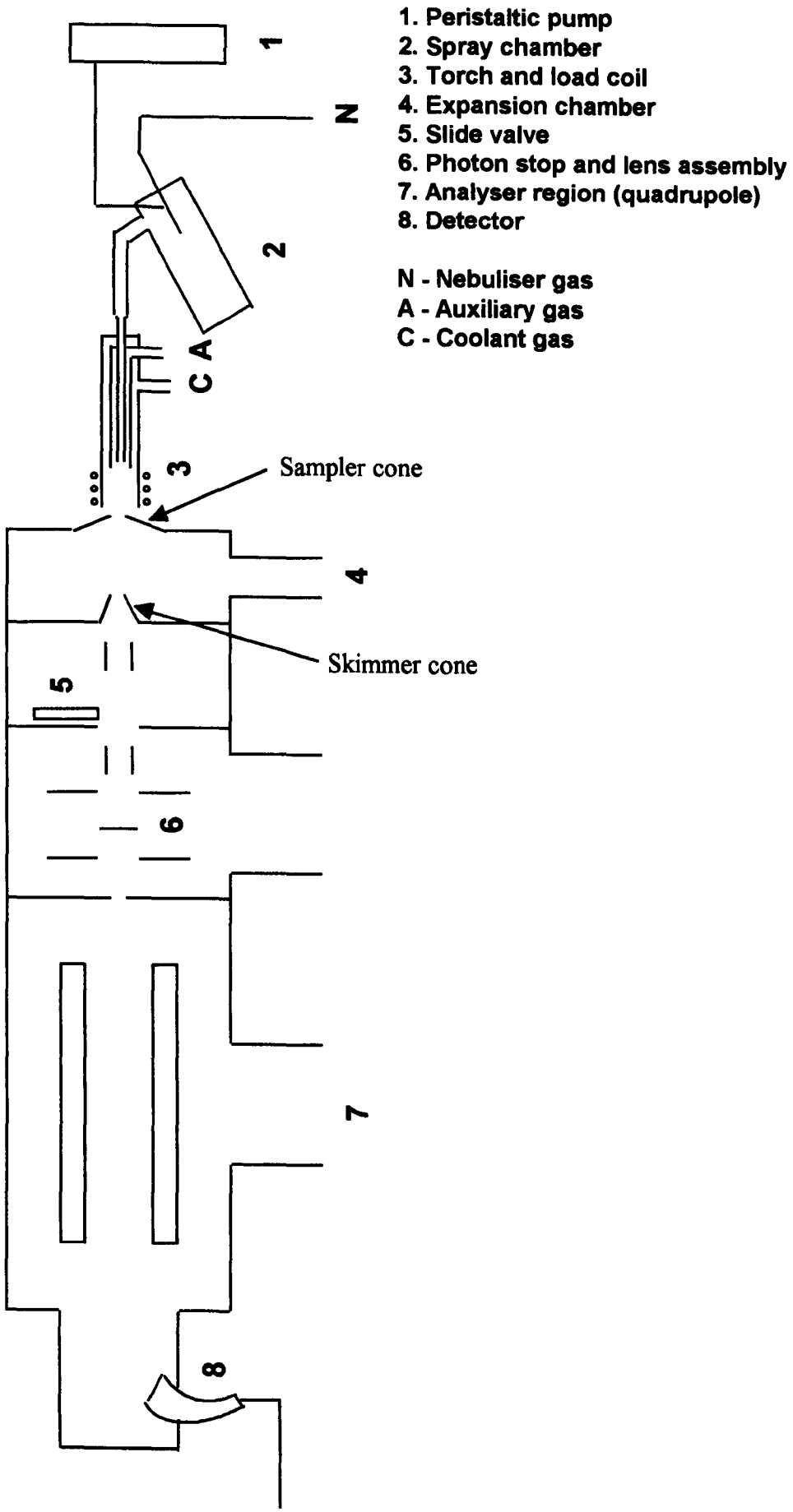


Figure 1.1. Schematic diagram of a quadrupole ICP-MS.

direct sample aspiration, is illustrated in Figure 1.1. This schematic figure illustrates a peristaltic pump (Fig. 1.1, (1)) which is used to pass sample to the nebuliser and spray chamber (Fig. 1.1, (2)). Nebuliser systems used in ICP-MS instruments are generally of the pneumatic or ultrasonic type. The types of pneumatic nebulisers routinely used are the Meinhard glass concentric and De Galan (V - groove) high solids designs. In terms of transmission to the plasma, pneumatic nebulisers have typical efficiencies of only 1 - 3%, because the larger sample droplets (>5 μm diameter), which make up >90% of the sample are lost to waste on the way to the plasma. Ultrasonic nebulisers (USN's), which are currently more rarely used in ICP-MS,[9,10] have transmission efficiencies closer to 35%, because of the greater percentage of small droplets produced by the ultrasonic transducer. USN systems also require the addition of a desolvation unit because of the higher solvent loading in the aerosol. The greater nebulisation efficiency of a USN also increases the level of sample matrix in the plasma which increases matrix related problems. The spread in the droplet size of the nebulised sample has a deleterious influence on analytical precision, so the larger droplets (above 5 μm diameter) are removed by passing the aerosol through a cooled spray chamber prior to entry to the plasma. In this way improved precision is obtained, due to better aerosol reproducibility. In addition, solvent loading in the plasma is reduced which leads to a decrease in the level of solvent related polyatomic interferences. Most ICP-MS instruments are fitted with a double pass spray chamber of the Scott type which is usually drained by pumping. This type of spray chamber is not ideal as it is known to suffer from memory effects due to its large dead volume. This necessitates long wash out times especially for the elements Hg and Li which tenaciously adhere to the quartz spray chamber walls. Although more efficient, lower dead volume spray chambers are available, very little attention has been paid to their use in ICP-MS. The aerosol passes through the base of the torch into the plasma (Fig. 1.1, (3)), where desolvation, atomisation and ionisation of

the analyte species occurs as in optical ICP instruments. The gas which supports the ICP is almost invariably argon alone, although some work has been carried out using mixed gas plasmas. The three separate gas flows which are supplied to the torch in order to sustain the plasma are also illustrated in Figure 1.1. The coolant gas (C) flows at typically 13 to 14 l min^{-1} and serves the two functions of providing the main gas flow for the plasma and cooling the quartz torch, to prevent it from melting. The auxiliary gas (A) flows at between 0.4 and 1.5 l min^{-1} and serves the main function of separating the plasma from the tip of the torch injector, thereby preventing it from melting. The nebuliser gas (N) is typically operated between 0.7 and 1 l min^{-1} and serves the function of nebulising the sample into a fine aerosol and transporting it to the plasma, via the injector located in the centre of the torch. The plasma is initiated by application of a high voltage spark from a Tesla coil. This spark seeds the plasma with electrons and positive argon ions which are accelerated into a rapidly circulating path induced by an oscillating rf electric field supplied via a copper load coil at a power of typically 1350 W. The rapidly moving electrons in the plasma collide with more argon atoms, transferring energy and causing further ionisation. This energy transfer rapidly increases the plasma temperature to around 10000 K, giving a temperature in the central channel of between 5000 and 7000 K. The load coil is grounded to prevent secondary discharge between the plasma and the interface, which has been shown to cause an increase in the levels of both doubly charged ions and ions from the sampling cone material.[7] This discharge also reduces the lifetime of the sampling cone. The load coil is accompanied by an impedance matching network which balances the plasma potential to keep the reflected power to less than 5 W, and further reduces secondary discharge.

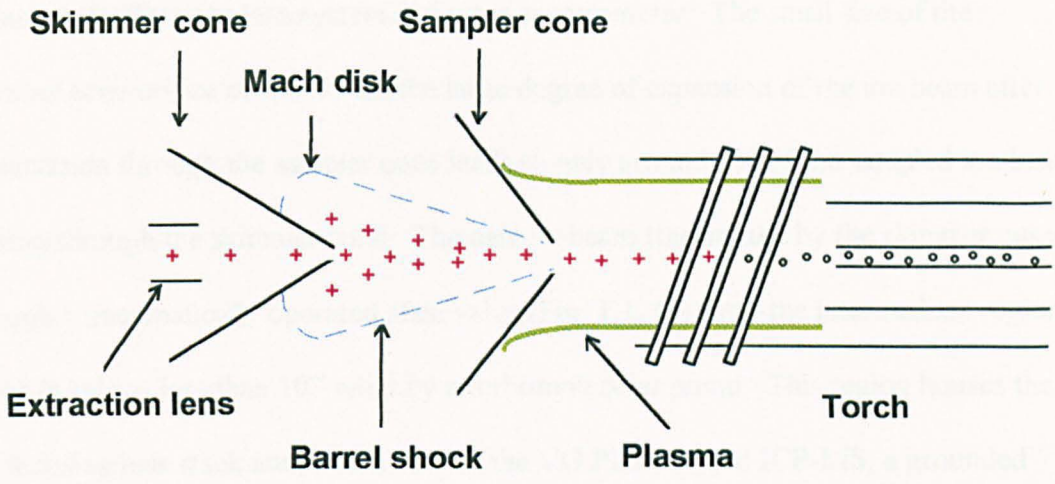
At the temperatures achieved in the plasma, application of the Saha equation (equation 1.1) shows that many elements yield over 90% of singly charged, positive ions[11].

The Saha Equation

$$M^+/M^o = 1/n_e\,(2\pi m_e kT/h^2)^{3/2}\,Q^+/Q^o\,e^{-IP/kT} \quad \text{(equation 1.1)}$$

where M^+/M^o is the ratio of the population of singly charged ions to neutral species, n_e is the electron density, m_e is the mass of the electron (9.11×10^{-31} kg), k is the Boltzmann constant (1.38×10^{-23} J K^{-1}), T is the temperature (in Kelvin), h is the Planck constant (6.63×10^{-34} J s), Q^+/Q^o is the ratio of the partition functions of the singly charged ion and the neutral atom and IP is the first ionisation potential of the element. In this equation, the partition functions are used to express the populations of the charged and neutral species in terms of temperature. The Saha equation is a simplified view of what is occurring within the plasma, because it is derived on the assumption that the plasma is in local thermal equilibrium. Although this is not strictly the case, the concept of local thermal equilibrium has been shown to give a sufficiently clear picture of the velocity and energy distributions within the plasma, in terms of the Maxwell-Boltzmann equations, for an effective model of the ICP to be made.[12] The ionisation efficiency values obtained from the Saha equation are therefore not grossly incorrect and may even be greater than 90%.[13] The plasma is sampled through an interface (Fig. 1.1, (4)), held at a pressure of approximately 2 mbar by a rotary pump. A detailed schematic of the sampling interface is shown below (Figure 1.2).

Figure 1.2. The ICP-MS sampling interface.



This interface consists of a pair of cones termed the sampler cone and the skimmer cone, which have small diameter holes cut into them. These interface cones need to be cooled, usually with water, to prevent them from melting. The sampler cone, which is most often constructed from nickel (as is the skimmer cone), is in direct contact with the plasma and samples the gas jet through an orifice which is typically 1 mm in diameter. The position of the sampling interface relative to the torch is optimised so that the central channel of the plasma is sampled near to the tip of the initial radiation zone of the ICP, at the point where the ion population is at a maximum. This first stage of sampling is efficient with nearly 100% of the sample ion beam passing through into the low pressure zone behind the sampling cone (Figure 1.2). In this low pressure region, the sampled gas forms a supersonic jet, which undergoes rapid expansion and cooling thus essentially 'freezing' the ion populations at the same level as they were in the plasma. The rapidly expanding gas jet is surrounded by a shock wave arising from contact of the jet with residual gas species in the interface region. At the front of this so-called 'barrel shock' another shock wave called the 'Mach disc' forms. The skimmer cone is located at the front of this disc in order to achieve representative sampling of the ion beam before

collisions and scattering attenuate the beam intensity. The skimmer cone orifice has a diameter of typically only 0.7 mm (smaller than the sampler cone) to restrict the volume of gas passing into the lens system and mass spectrometer. The small size of the skimmer cone orifice coupled with the large degree of expansion of the ion beam after transmission through the sampler cone leads to only around 1% of the sampled ion beam passing through the skimmer cone. The narrow beam transmitted by the skimmer passes through a pneumatically operated slide valve (Fig. 1.1, (5)) into the intermediate region, which is held at less than 10^{-4} mbar by a turbomolecular pump. This region houses the ion focusing lens stack and, in the case of the VG PlasmaQuad ICP-MS, a grounded photon stop as well (Fig. 1.1, (6)). The photon stop prevents vacuum UV radiation from the plasma reaching and activating the detector and also stops a percentage of the ion beam, leading to a further loss in sensitivity. On exiting the intermediate region the ion beam passes into the mass analyser region (Fig. 1.1, (7)) where mass separation is performed. This region of the instrument is typically held at approximately 3×10^{-6} mbar by a second turbomolecular pump. In the majority of routinely used ICP-MS systems, a quadrupole mass analyser is installed. In commercial instruments, this type of mass analyser is generally only capable of unit mass resolution, which means that while resolution of $^{114}Cd^+$ from $^{115}In^+$ can be easily achieved with a quadrupole, resolution of $^{58}Fe^+$ from $^{58}Ni^+$ is not possible. In recent years, the use of high resolution, magnetic sector ICP-MS instruments has been reported for a variety of applications.[14,15,16,17] Magnetic sector mass analysers are capable of clearly resolving $^{56}Fe^+$ from the notorious $^{40}Ar^{16}O^+$ interference, which requires a resolution of nearly 3000, and have been reported[18] to be able to resolve $^{76}Ge^+$ from $^{40}Ar^{36}Ar^+$, which requires a resolution of 10000. Of course, such high resolution can only be achieved at the cost of sensitivity, but in magnetic sector ICP-MS, this is partly offset by the absence of a photon stop in the ion beam path. The photon stop is unnecessary because although the ion beam is

curved by the magnetic sector on its way to the detector, stray photons from the ICP source are not. The absence of stray radiation at the detector means that background counts with magnetic sector ICP-MS are also extremely low, often being as few as 5 - 10 counts per minute. This improves detection limits because it leads to very large signal to noise ratios. Despite the advantages of magnetic sector instruments, they have disadvantages in that they are more expensive, more complex to operate and are larger than their quadrupole counterparts. Nonetheless, such instruments are proving popular for highly specialised applications such as trace element contamination of materials for the semiconductor industry.[15] In addition to quadrupole and magnetic sector ICP-MS, some interesting work has been described by Hieftje *et al.*[19] on the subject of coupling an ICP source to a time of flight mass spectrometer (TOFMS). This robust, simple and inexpensive type of mass analyser has been reported by Hieftje *et al.*[20] to be capable of relatively high resolution (currently around 500). The application of this instrument to quantitative analysis of fast transient peaks (300 ms duration) arising from pulsed laser ablation of cast iron and lava samples has also been described.[21] Detailed analysis of such short duration peaks was possible because of the rapid, 10 kHz scan repetition rate of the TOFMS.

Following the mass separation, ions are detected using either a continuous or discrete dynode electron multiplier (Fig. 1.1, (8)). In quadrupole instruments, this device is mounted off axis from the central line of the plasma to further reduce counts from stray photons to levels below 20 counts per second. The impact of an ion on the first dynode of the detector releases an electron which is accelerated by the applied voltage into the next dynode. On striking this dynode, the electron releases more electrons which, in turn, are accelerated into the next dynode. This process continues down the detector until, at the final dynode, a cascade of electrons emerges to be detected as a single pulse. For a single incident ion, between 10^6 - 10^7 electrons are released, giving rise to a large

amplification in the initial ion current. This current amplification is referred to as the gain of the multiplier. The rate at which pulses emerge from the multiplier corresponds with the rate at which ions strike the first dynode, which in turn relates to the number of ions present and hence the analyte concentration in the original sample. Hence, by counting the pulses, analyte concentrations as low as 1 - 2 ppt can be determined with a standard ICP-MS instrument, equipped with a conventional pneumatic nebuliser. However, as the concentration increases a point is reached where the multiplier cannot respond quickly enough to detect all of the incident ions. This effect results from the fact that during the time one pulse is cascading down the multiplier it is insensitive to incoming ions. Under these conditions, the multiplier is saturated and the pulse counting (PC) data produced are incorrect. Therefore, in order to extend the dynamic range of the detector, electron multipliers can also be operated in a low gain, analog mode, which allows detection of analyte concentrations up to 50 - 100 ppm, depending on the instrument sensitivity. In analog mode, unlike for PC, the incident ion signal is measured as a voltage close to the first dynode of the multiplier, where the gain is small. By measuring the ratio of the analog response to the PC response for a given analyte concentration a cross calibration factor is generated across the mass range which is used to numerically scale the analog signal, after collection, up to what the equivalent PC signal would have been. In this way, a linear dynamic range of 8 orders of magnitude can be achieved. Traditionally, PC signals and analog signals have had to be collected in two separate acquisitions, using two separate voltage settings on the detector. This has the distinct disadvantage that high and low analyte concentrations cannot be measured simultaneously, which is essential if analog and PC data are required from a transient signal passing through the instrument. By performing two separate data acquisitions, the analysis time is also increased. Recent developments in detector hardware have tackled this problem and it is now possible to use a simultaneous multiplier which allows analog and PC data to be

measured at the same time. This detector uses two fixed voltage settings to control the gain at a point halfway down the multiplier (analog) and at the end of the device (PC). An electronic gate located just after the analog detection point monitors the incoming signal and if it gets too high for the PC part of the multiplier, the gate rapidly 'closes' thereby shutting the signal off before it damages the PC section. While this occurs the analog data continue to be collected. When the signal falls to a safe level, the gate re-opens and the PC signal returns.

1.4 Disadvantages of ICP-MS.

The ICP-MS technique has the practical advantages of very low detection limits, multi-element analysis and relatively high sample throughput, with minimal sample preparation. The ICP source is also sufficiently energetic to almost quantitatively ionise elements whose first ionisation potentials are less than that of argon (15.76 eV).[22] This criteria applies to more than 80 elements in the Periodic Table, so the technique has a wide analytical range. However, ICP-MS does have its limitations. In the initial studies of ICP-MS, in which direct analysis of aqueous solutions was carried out, numerous peaks were observed in the spectra of blank samples. These peaks were found to be caused by polyatomic ions arising from the combination of plasma gases with matrix species and solvent atoms, in the sampling interface region.[3] Subsequent ICP-MS studies were therefore aimed at reducing polyatomic ion interferences and successful solutions to the problem have been developed using both chemical and physical approaches. The physical approach of using high resolution magnetic sector ICP-MS instruments to allow mass resolution of the interference from the analyte of interest has been explored,[23] but this can be an expensive solution to the problem. More recently, use of the shielded low power (620 W) "cool" plasma has been shown to substantially decrease both argon based

interferences (notably $^{40}Ar^{16}O$ on ^{56}Fe) and the Ar^+ concentration, by eliminating secondary discharges between the plasma and the sampling interface.[24] This effect may also reduce matrix related argon interferences such as $^{40}Ar^{23}Na$ and $^{40}Ar^{35}Cl$, thereby permitting accurate measurements of ^{63}Cu and ^{75}As without the need for matrix separation. The disadvantage here however is that cool plasma conditions reduce the ionisation efficiency of elements with ionisation potentials greater than 7 eV, leading to lower sensitivity. Furthermore, cool plasma conditions favour the formation of some molecular species, such as refractory element oxides, and suffer more severe matrix effects in place of argon based interferences.[25]

The chemical approaches which have been adopted have principally been based on preconcentration and matrix separation (to reduce $^{42}Ca^{16}O^1H^+$ interference on $^{59}Co^+$, for example), reduction of the water load reaching the plasma (to reduce $^{40}Ar^{16}O^+$ interference on $^{56}Fe^+$)[26] and addition of small amounts of other gases to the plasma, such as nitrogen (to reduce $ArCl^+$, through preferential formation of ArN^+).[27,28] The area of preconcentration and matrix separation for ICP-MS is explored in more detail in Chapter 2 of this thesis. Apart from the well known polyatomic interferences, so-called isobaric interferences also arise from overlap between elements possessing isotopes of the same nominal mass.[29] Examples include ^{115}Cd / ^{115}In, ^{87}Rb / ^{87}Sr and several overlaps between the rare earth elements. However, with the exception of In, which overlaps with Sn (at m/z 113) and Cd (at m/z 115), selection of another isotope of the element enables this type of interference to be avoided. This is notable in the case of the rare earth elements, as each one of them has at least one isotope free from isobaric overlap. A second major limitation in ICP-MS which became apparent was the low tolerance of the instrumentation to total dissolved solids (TDS) (typically 0.2% m/v or less, for sustained operation). At levels higher than this, the interface cones and torch injector eventually become blocked and require removal and cleaning before the analysis can be continued.

This problem can be overcome either by dilution of the sample or by application of flow injection techniques. Dilution of the sample is only possible if the analyte concentrations are sufficiently high enough to remain above the detection limit of the instrument after dilution. Flow injection techniques allow a small volume of the sample to be aspirated into the ICP-MS in the form of a transient band. In this way, the sampler cone only has to sustain a high solids loading for a short period of time and so tends not to become blocked.[30] However, repeated injections of high salt samples still ultimately leads to gradual deposition on the interface cones, and although complete blockage may not occur, polyatomic interferences arising from the combination of deposited material with plasma species will become progressively worse. Other problems with ICP-MS are also related to the sample matrix. For example, direct analysis of organic solvents is not possible because of the rapid deposition of carbon on the interface cones. Introduction of a small quantity of oxygen into the plasma is known to burn off the deposited carbon. However, addition of too much oxygen (above about 6% of the nebuliser flow) will damage the cones. In some cases, the sample may also be flammable, so direct undiluted analysis is inadvisable. To address this problem, methods based on removing the solvent prior to analysis have been developed.[31]

1.5 Analytical alternatives to ICP-MS for trace element determination.

Despite the difficulties outlined above and later in this Chapter, analytical methods that can compete with ICP-MS are somewhat restricted. Analysis of solids by X-ray fluorescence (XRF) yields improved precision and is easier to achieve than by ICP-MS.[32] However, this method lacks the necessary sensitivity, since its detection limits are in the $\mu g\ g^{-1}$ region, and it is generally restricted to solids analysis as liquid analysis is difficult. Furthermore, unlike ICP-MS, valuable isotopic ratio information cannot be obtained

using XRF. Total reflection XRF (TXRF) has detection limits in the sub ng g^{-1} range, so this method has comparable sensitivity to ICP-MS. However, to achieve this high sensitivity, counting times for data acquisition of 1000 to 5000 s are necessary, which leads to very long analysis times and low sample throughput.[33] For TXRF analysis, samples must be prepared as thin films or residues on a quartz carrier plate which means that digestion of solid samples and evaporation of liquid samples is required prior to analysis. Since the carrier plate is only 2-3 cm in diameter the volume of sample which can be evaporated on it is of the order of microlitres. For this reason, ensuring that the evaporation step is reproducible is inherently difficult. Sample preparation is therefore considerably more laborious and complicated than for ICP-MS. TXRF is also plagued by matrix interferences so matrix separation of the target analytes before analysis is essential.[34] Neutron activation analysis (NAA) rivals the detection limits of ICP-MS, but is considerably more expensive, specialised and complex to operate.[35] This method also has the inherent dangers of radiation associated with it which limits its widespread application. Furthermore, in contrast to ICP-MS, NAA has a relatively low sample throughput, because of the long counting times required.

The development of axially viewed ICP-AES systems has significantly improved the limits of detection that can be achieved using this technique.[36,37] With the axial configuration, the detector faces directly into the hottest part of the plasma (the normal analytical zone). For a given concentration, the signal intensity is greater than with radially viewed plasmas, because with the latter design, signal attenuation arises as the emission passes from the central channel of the plasma, through the outer layers and on to the detector. However, even with these improvements in the sensitivity of ICP-AES, the detection limits possible are still not as low as can be achieved with ICP-MS.

Another alternative approach to trace metal analysis is atomic fluorescence spectrometry (AFS). The applications of this technique, together with the theory behind it, have been

reviewed recently in a detailed article by Greenfield.[38] In terms of sensitivity, AFS is better than ICP-AES, with detection limits that are similar to ICP-MS. The technique has the potential for analysing a large number of elements, especially if an ICP source is used, but has only really been exploited for specific applications such as determination of Hg at ultra trace levels[39] and selenium speciation, after chromatographic separation and on-line reduction.[40] Despite the release of a multi-element ICP-AFS instrument by Baird in the early 1980's, this instrument did not prove to be commercially viable and was discontinued just 10 years after its release. At present, no multi-element AFS instruments are commercially available.

In addition to the techniques described above, electrochemical methods have been applied to trace element analysis.[41] Anodic stripping voltammetry (ASV)[42] and adsorptive cathodic stripping voltammetry (ACSV)[43] both have the necessary detection limit capabilities but are subject to severe matrix effects in the presence of saline samples. These electrochemical methods are also susceptible to electrode fouling, which reduces the sensitivity and introduces the need for regular cleaning of the electrode, thereby increasing the analysis time. In addition, ASV is limited to elements which are soluble in Hg and whose redox potentials lie between the oxidation potential of Hg and the potential at which hydrogen evolves in the solution (from the reduction of H^+). ACSV, despite having a greater elemental range than ASV, is still limited compared to the multi-element capabilities of ICP-MS. In addition, ACSV is affected by the presence of dissolved organic matter. This material can complex with the metals of interest, thereby preventing their adsorption to the electrode and consequently yielding incorrect results.

1.6 Current methods of sample introduction for ICP-MS.

1.6.1 Direct aspiration (continuous nebulisation).

Direct aspiration was the first method of sample introduction employed in ICP-MS and still remains the most frequently used. The sample is pumped directly into the nebuliser at a flow rate of 0.5 to 1.0 ml min^{-1} and analysed when the signal reaches a steady state. The short term stability, in terms of relative standard deviation over a 10 minute period, obtainable with this mode of sample introduction is typically less than 2% across the mass range. As described earlier, direct aspiration is limited to samples containing less than 0.2% m/v TDS. Above this level either the nebuliser, torch injector or sampling cones will eventually become blocked by salt deposition. Despite this limitation, the low detection limits offered by ICP-MS often allow the sample to be diluted, to reduce the TDS concentration, without severely degrading accuracy and precision.

1.6.2 Chromatographic sample introduction procedures.

The use of chromatographic techniques as a means of sample introduction for ICP-MS is widely cited. The major benefits conferred by this procedure are the potential for elemental speciation[44] and matrix separation.[45,46] Reversed phase high performance liquid chromatography has been successfully used for the speciation of arsenic[47,48], lead[49] and tin compounds.[50] In each case, aqueous mobile phases containing only small amounts of organic solvents and low buffer concentrations were used to avoid the complications associated with aspirating organic substances into the plasma. The chromatographic eluent could be passed directly into the nebuliser and spray chamber of the ICP-MS without any instrumental alteration.

Gas chromatography (GC) has been used in conjunction with ICP-MS for the separation and quantitation of organolead compounds.[51] One major advantage of linking GC to ICP-MS is an improvement in sensitivity, because the chromatographic eluent can be passed directly into the ICP torch, without first having to pass through the relatively inefficient nebuliser and spray chamber system. A second advantage of GC-ICP-MS is that samples entering the plasma are 'dry' and so solvent related interferences are dramatically reduced. This has the potential to markedly improve analysis of ^{56}Fe species in particular, because of the reduction of $^{40}Ar^{16}O^{+}$. In recent years, efforts have been made by Caruso *et al.*[52,53] to interface supercritical fluid chromatography to ICP-MS. In the work cited, organotin compounds of environmental interest were studied. As with GC the chromatographic eluent, in the form of a gas, could be passed directly into the torch, thereby giving improved detection limits over conventional nebulisation. In a later publication on the same subject,[54] the use of multi-element, time resolved data acquisition was described by these workers and the results generally found to be similar to those obtained by single ion monitoring.

1.6.3 Electrothermal vaporisation (ETV).

ETV is a technique in which a few microlitres of the sample are placed on an electrically heated platform, usually made from graphite. The platform, which may be tube, rod or trough shaped, is heated stepwise, while the carrier gas purges through it and on to the detector. First, the solvent is evaporated at low temperature. Next, the sample is ashed at a higher temperature, to volatilise any organic components in the matrix. Finally, the current is rapidly increased to ramp the temperature up to 2000 to 3000°C which atomises the sample analytes into the carrier gas above the platform.

One of the main benefits of ETV as a method of sample introduction for ICP-MS is the desolvation of the sample prior to its transport into the plasma. This leads to a significant reduction in the formation of solvent related polyatomic interferences, such as ArO^+, making the analysis of iron at low concentrations possible.[55] A second major advantage offered by ETV is improved analyte transport to the plasma (60 to 80%), compared to conventional nebulisation (1 to 4%), leading to lower detection limits. In addition, samples in organic solvents can be analysed using this technique and for each analysis only a small sample volume is required (typically 5 to 100 µl). The coupling of ETV with ICP-MS was first described by Gray and Date.[56] In this study, measurements of the principal isotope ratios of sulphur were described with a measurement precision (as % RSD) of < 1%, a result not possible with conventional nebulisation, because of interference from O_2^+ at m/z 32. Gregoire[57] used ETV-ICP-MS to study the platinum group elements in geological samples, after decomposition of the materials. Nickel nitrate was added to the samples as a matrix modifier to improve the response and isotope dilution was used to obtain quantitative results. Marshall and Franks[58] explored the potential of ETV-ICP-MS for performing accurate, multi-element analysis of the transient ETV peaks. In this study, simultaneous calibrations were obtained for 21 elements at ng ml^{-1} concentrations in aqueous samples. This work also indicated how the use of ETV could significantly improve ICP-MS analysis of ^{31}P in the presence of the $^{14}N^{16}O^1H$ interference, by using the ETV system to evaporate off water in the sample prior to volatilising ^{31}P. However, it was also demonstrated that interference free analysis of V in hydrochloric acid ($^{35}Cl^{16}O$ on ^{51}V) and Ti in sulphuric acid ($^{32}S^{16}O$ on ^{48}Ti, $^{32}S^{16}O^1H$ and $^{33}S^{16}O$ on ^{49}Ti) could not be completely achieved with the ETV system studied, although some improvements were made. This work also remarked on the known ETV disadvantage of memory effects, and their corresponding influence on detection limits. In a study by Park and Hall,[59] analysis of tungsten was affected by a

memory effect caused by carbide formation during the ashing stage. These workers also found it necessary to calibrate using an isotope dilution methodology to improve the precision in the results.

1.6.4 Vapour generation.

Vapour generation as a method for sample introduction in ICP-MS is restricted to analysis of those elements which form volatile vapours, such as hydrides, under mild reaction conditions in the laboratory (arsenic, germanium and lead for example), or those which can be readily reduced to an atomic vapour (mercury). This technique benefits from separation of the analyte species from the matrix prior to analysis, via a gas-liquid separator, and gives improved detection limits, because the hydrides or mercury vapour formed are transported directly into the ICP torch. Continuous hydride generation (HG) coupled to ICP-MS was applied for the detection of arsenic, selenium, antimony, bismuth and tellurium in aqueous samples, by Powell *et al.*[60]. The results illustrated improved detection limits compared to conventional nebulisation and a reduction in some matrix interferences. However, this study also revealed a memory effect on analysis of mercury. Wang *et al.*[61] studied the isotope ratios of lead using isotope dilution HG-ICP-MS and found that the presence of iron and copper in the samples significantly interfered with the hydride generation process. Methods were developed to successfully reduce these interferences. Branch *et al.*[62] used a tubular gas-liquid separator design to eliminate the interference of $^{40}Ar^{35}Cl^{+}$ on arsenic by HG-ICP-MS. This interference is a problem in conventional nebulisation and also arises in HG-ICP-MS when U-shaped gas-liquid separators are employed. This group obtained accurate results for arsenic in a reference water sample. A method has been reported by Stroh *et al.*[63] for the determination of arsenic, antimony and mercury in fresh and sea water using flow

injection (FI) coupled to ICP-MS, in conjunction with vapour generation. In this study, a commercial FI manifold, which incorporated a gas-liquid separator, was used. Reference materials were analysed and the results indicated that the method was suitable for trace determinations of these elements in environmental samples.

1.6.5 Slurry nebulisation.

The principal limitation for analysis of slurries by ICP-MS is its relatively low tolerance to particulate matter in the sample. The maximum particle size that can be transmitted through a standard 3 mm diameter torch injector, without blocking it, is < 5 μm. In a study by Ebdon and Collier[64], based on ICP-AES, use of a torch with an injector diameter of 4 mm was shown to allow successful transmission of particles up to 16 μm in diameter, but such particles cause instability in the plasma and correspondingly decrease the precision of the analysis. Once in the plasma, the slurry particles undergo atomisation and ionisation in the same way as liquid droplets, but in addition, these particles must also be completely fragmented. This requires a hotter plasma than is necessary for solution nebulisation, so for slurried samples the rf power for the plasma needs to be increased. In the study by Ebdon and Collier, a plasma rf power of 1.5 kW was used, compared to the standard 1.35 kW used for solution analysis. In addition, fractionation of the slurry, because of its particle size distribution, was shown to occur as the sample passed through the system. The main cause of this fractionation was found to be the spray chamber and the authors concluded that, of the spray chambers tested, the most severe fractionation was observed with the standard Scott double pass design. Fractionation can lead to inaccurate results if the different particle size granules have different elemental compositions. In addition to these problems, it is also necessary to add a dispersant to the sample, to prevent the suspended particles in the slurry

flocculating and precipitating out. It is clear from the above statements that sample preparation for slurry nebulisation is an involved process and can be regarded as a distinct disadvantage of the technique. With these difficulties in mind, Williams *et al.*[65] carried out a study on the feasibility of slurry nebulisation for ICP-MS. In this work, soil and catalyst samples were ground to a particle size of <10 μm, using a 'bottle and (zirconia) bead method', and prepared in a 0.05% w/v slurry, using tetrasodium pyrophosphate as the dispersant. In the 'bottle and bead' grinding method, the powdered sample was mixed with a small volume of the dispersant in a plastic bottle and about 10g of zirconia or agate beads were added. This mixture was then shaken on a laboratory shaker for 12 - 15 h. After this time, the finely ground sample was made up to the required volume and analysed immediately. The choice of dispersant was important as its presence in the sample lead to additional polyatomic interferences. Phosphate type dispersants are acceptable because the $^{31}P^{16}O^{+}$ and $^{31}P^{16}O^{1}H^{+}$ interferences that consequently appear in the ICP-MS spectra only affect analysis of one element (Ti). The analysis of a slurry concentration of 0.05% w/v allowed use of simple, aqueous standards to quantify the analytes in the slurried sample, because the viscosities of samples and standards were matched. In a later study, Ebdon *et al.*[66] adopted a similar approach to apply slurry nebulisation to ICP-MS analysis of coal. Jarvis and Williams[67] extended the methodology of slurry nebulisation to analysis of geological reference materials. In this work, the total solids concentration was 0.2% w/v, which pushed the ICP-MS instrumentation to its practical limits. To prevent nebuliser blockage at this solids concentration, a De Galan high solids nebuliser was used, and to prevent injector blockage, a 3 mm injector was used in place of the standard 1.5 mm version. With this approach, typical precisions of 10% relative standard deviation were obtained both within samples and between samples. Relatively good agreement was found for 14 out of 16 elements analysed in the reference materials. Only Cd and Bi were significantly

incorrect, probably because of the low concentrations of these elements in the samples. In addition to the studies described above, slurry nebulisation has also been successfully applied by Jarvis,[68] for the analysis of rare earth elements in whole coal samples. Grinding of the samples using agate beads in place of zirconia beads was found to be necessary because of contamination from the bead material. This publication also discussed the advantages and disadvantages of slurry nebulisation for ICP-MS. More recently, Jarvis' group have applied slurry nebulisation ICP-MS to analysis of the platinum group elements and gold in coal samples and reference materials.[69] Application of slurry nebulisation is still relatively limited in ICP-MS and seems to be of greatest use in the field of geological and geochemical analysis. The complex sample preparation and instrument optimisation required leads to low sample throughput and in practice, the technique may also require an experienced analyst if useful data are to be obtained on a routine basis.

1.6.6 Laser ablation (LA).

Laser ablation is used for the analysis of solid samples. The surface of the material is vaporised by an incident laser beam and the ablated products transported to the ICP-MS by an argon carrier flow. The technique generally has poorer limits of detection and decreased precision compared to solution nebulisation. The often non-homogeneous nature of the samples analysed can also lead to poor reproducibility and matrix matching standards to samples to obtain quantitative results is difficult. Use of an internal standard is required to compensate for factors such as variation in the laser power from sample to sample. Aside from these drawbacks, LA-ICP-MS shows reduced oxide interference levels, because the sample is transported into the plasma as a dry vapour. Analysis of iron and sulphur at low levels is therefore possible. Also, a wide range of sample types

ranging from rocks and minerals to ceramics and steels can be directly analysed, with little sample preparation. A preliminary study into LA-ICP-MS for analysis of geological materials was described by Gray in the mid 1980's.[70] This early study highlighted some of the practical difficulties encountered with the technique and also revealed that the detection limits obtained were not improved compared to solution nebulisation. Poor precisions were obtained for individual isotopes in reference materials, but better results were demonstrated for lead isotope ratio determinations. Arrowsmith[71] used the technique to obtain quantitative results for several elements including copper, arsenic and chromium in a reference steel sample. The use of ^{60}Ni as the internal standard yielded a precision for quantitative analysis of around 5% RSD and detection limits in the low $\mu g\ g^{-1}$ range were obtained. A study by Hager[72] described quantitative analysis of a number of trace components in copper, steel and aluminium standards and compared the results obtained by Q-switch and free-running ablation modes. An agreement of $\pm$ 50% was generally observed between the two modes of ablation. Marshall *et al.*[73] described the use of LA-ICP-MS for direct determination of trace elements in plastic materials. The presence of carbon in the samples permitted the use of ^{13}C as an internal standard to compensate for variations in ablation and transport of the ablated material to the plasma. Semi-quantitative values within a factor of two of the accepted values could be obtained in this way. The use of calibration materials of similar composition to the plastics analysed yielded more accurate results, although the inherent laser ablation problem of matrix matching samples and standards was still apparent. Further work by Franks *et al.*[74] extended the application of LA-ICP-MS to analysis of zeolites and glass fibres for major and trace elements, with good semi-quantitative results being obtained for fused samples. A group at NERC, lead by Kym Jarvis, have also recently described the application of LA-ICP-MS to quantitative analysis of several elements in silicate materials[75] and platinum group elements and gold in rock and ore samples.[76] The LA-

ICP-MS studies described up to this point have all been based on the use of infra-red (IR) lasers, but more recently, pioneering studies have been carried out using ultra-violet (UV) lasers, by Jeffries' group at University College, Wales. This group have compared the performance of the UV laser with an IR device, in terms of element fractionation arising during ablation of the samples.[77] This work illustrated that the UV laser caused significantly less fractionation than the IR laser. Jeffries *et al.*[78] have also used the UV laser to measure heavy metals in shellfish from the Welsh coast. In this article, the high spatial resolution capabilities of the UV laser (<10 μm spot size) compared to the IR device were highlighted.

These studies indicate that, despite its limitations, LA-ICP-MS is becoming a practical and accepted method for trace element analysis in solid samples.

1.6.7 Flow injection analysis (FIA).

1.6.7.1 Historical background of FIA.

Prior to the 1950's, the flow methods used for chemical analysis were based on merging the sample and reagent streams continuously on route to the detector. This approach was found to be plagued with contamination problems, due to sample carry over between analyses. To prevent sample carry over, some means of separating one sample from the next was necessary. The principle of air segmentation, to keep successive samples apart, was introduced in 1957, by Skeggs.[79] This effective approach significantly reduced sample carry over and remained the principle solution to the problem until the 1970's. Then, in a pioneering study performed at the Technical University of Denmark, Ruzicka and Hansen illustrated the potential of injecting samples directly into an unsegmented carrier stream.[80] They demonstrated that this approach gave faster sample throughput

than segmented flow analysis and also essentially eliminated sample carry over. This system was also simpler to operate, required lower reagent volumes and was potentially more versatile. Ruzicka and Hansen christened this new technique 'flow injection analysis'.[80]

1.6.7.2 Principles of FIA.

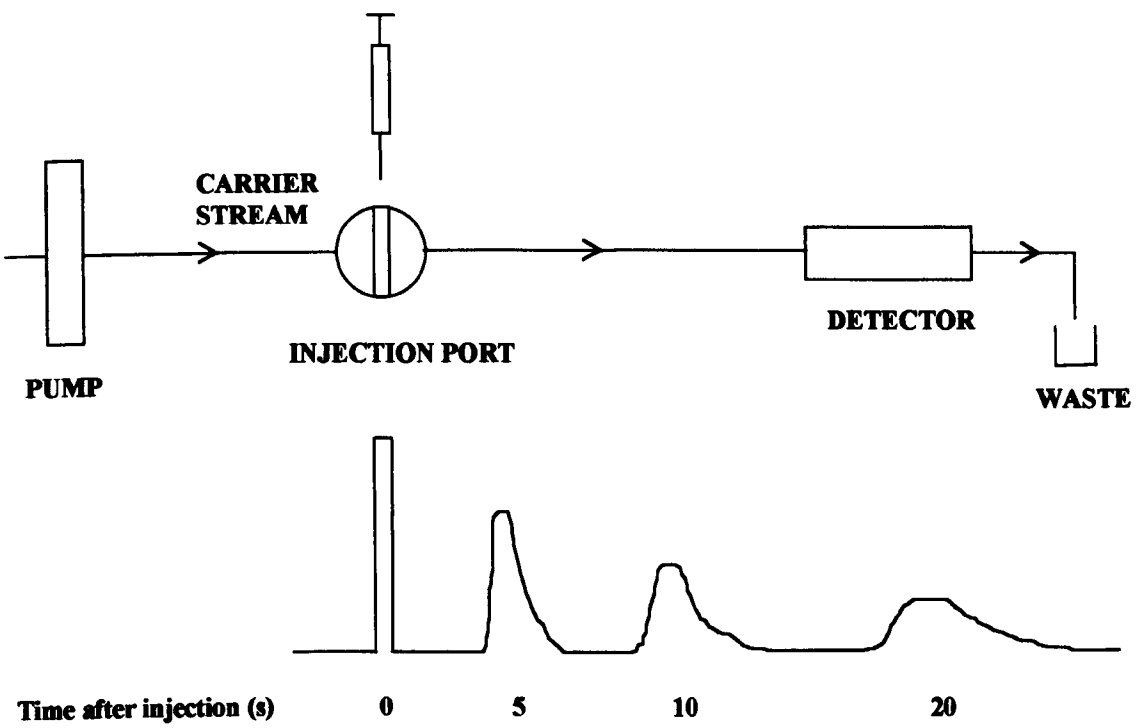
Flow injection analysis is an unsegmented continuous flow method, in which a small sample volume (typically 10 - 1000 μl) is injected as a discrete sample slug into a liquid stream, usually flowing at rates of 1 to 4 ml min^{-1}. Once in the stream, the sample may be manipulated in a variety of ways to facilitate detection by any one of a range of techniques including UV, electrochemical and atomic spectrometry. The output signal appears as a transient peak, the shape of which is usually skewed, rather than Gaussian, with the gradient being sharper on entry of the sample to the detector. The peak profile from injection to detection for a simple flow injection manifold, is presented in Figure 1.3. This type of peak profile is produced because of the kinetic process of dispersion. Dispersion arises from diffusion and convection of the sample slug as it passes along the apparatus tubing from injector to detector. One the one hand, dispersion is undesirable as it leads to sample dilution and a consequent drop in sensitivity, but on the other hand it can be desirable if the sample contains high matrix element concentrations, which could affect detection, or strong acid, which could damage the detector. Diffusion occurs both along the axis of the tube, giving a horizontal concentration gradient, and across the tubing, giving a concentration gradient perpendicular to the direction of flow. The latter process has the greater effect in terms of broadening the sample slug. Convective dispersion leads to a horizontal concentration gradient, because it generates a laminar liquid flow with the sample molecules in contact with the walls of the tubing having a

low velocity and the molecules in the centre of the stream having twice the average velocity. Dispersion is quantified in flow injection systems by the equation:

$$\textbf{Dispersion, } D = C_o/C_m \qquad \text{(equation 1.2)}$$

where C_o is the initial sample concentration before injection and C_m is the maximum concentration of sample that passes through the detector. So, if the signal intensity of a directly aspirated sample is 10 units and the maximum signal intensity of an injected slug of the same sample is 5 units, the dispersion i.e. extent of dilution is 2.

Figure 1.3. Simple flow injection manifold with peak profile from injection to detection.



1.6.7.3 FIA coupled to ICP-MS.

The ease of introducing a liquid sample into a continuous stream facilitates the direct coupling of FIA to any detector system which is capable of dealing with a low liquid flow. ICP-MS fulfils this requirement and the use of flow injection as a method of sample introduction for the instrument has been characterised in a study by Dean *et al.*[81] This work highlighted the benefits of the method for analysis of samples of high salt concentration, high acidity and high viscosity. Hutton and Eaton[82] employed a flow injection strategy for ICP-MS analysis of aluminium and diluted brine solutions, containing up to 2% m/v dissolved solids. The authors illustrated how continuous nebulisation of these samples lead to blockage of the interface cones and that the rate of blockage was faster for the more refractory aluminium solutions. Flow injection sample introduction dramatically reduced the blockage problem, but could not completely eliminate it. A study by Richner described a procedure for analysing the trace components in high purity nickel.[83] In this case the solids loading was 3% m/v which would have rapidly blocked the torch injector or sampler cone if continuous aspiration had been employed. A study into the signal characteristics and precision, both short and long term, for samples injected in a matrix of 3% sodium chloride was carried out by Stroh *et al.*[84] These workers described improved limits of detection by flow injection compared to continuous aspiration, in addition to acceptable accuracy and precision (generally < 5% RSD). The main difficulty with using flow injection in conjunction with ICP-MS lies with the data acquisition. Monitoring a transient peak with a scanning mass spectrometer can lead to mass bias effects on the spectrum obtained, especially for peaks of very short duration, which decrease accuracy and precision, even at high scan rates. Also, the larger the mass range or the more peaks selected for analysis the shorter the dwell time at each mass and so the poorer the detection limit. For this reason, up until

recently two approaches have been adopted, namely single ion monitoring and mass scanning over a selected time window across the centre of the peak. However, the first choice does not utilise the multi-element capabilities of the instrument and the second choice is only suitable for peaks of long duration, typically longer than 30 seconds. The development of time resolved acquisition (TRA) has enabled multi-element determinations to be performed on transient peaks and the preliminary use of this facility with flow injection has been described by Ebdon *et al.*[85] These workers described the multi-element benefits of this type of acquisition, but also highlighted the drawback of the lengthy data processing which is currently associated with it.

In addition to the flow injection studies described above, the technique has also been applied to on-line preconcentration and matrix separation for ICP-MS. This thesis is principally concerned with developments in this area and the subject is covered in detail in later chapters. For this reason, it will not be considered further here.

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CHAPTER 2

REAGENTS FOR PRECONCENTRATION AND MATRIX SEPARATION

2.1 Introduction.

Preconcentration is the process whereby an analyte species is enriched prior to analysis. This is necessary when the target analyte is present in the sample at concentrations below or very close to the detection limit of the analysis. The analyte may be enriched using procedures based on solvent extraction (liquid - liquid), co-precipitation, or sorbent extraction (liquid - solid). The use of reagents which selectively bind specific elements while excluding others also facilitates quantitative separation of trace analyte species from matrix elements present at much larger concentrations. In this way, many matrix linked interferences can be reduced or removed entirely from a particular analysis. Increased concern with the levels of environmental contamination has led to the development of a wide variety of reagents and procedures for preconcentration and matrix separation. Recent years have seen a general shift from batch to on-line procedures leading to more rapid, less easily contaminated analyses. Although the use of on-line solvent extraction,[1,2,3,4,5] on-line precipitation[6,7] and on-line co-precipitation[8,9] for trace element enrichment have been reported in the literature, these methods are rarely used. In the case of solvent extraction, this is mainly due to the limited range of phase ratios (i.e. partition ratios between the organic and aqueous phases) that can be achieved, which results in these systems offering relatively poor analyte enrichment. In practical terms, on-line solvent extraction also requires a high degree of operator skill. For on-line

precipitation, the main requirement is that the precipitate formed must have low solubility in both the carrier stream and sample matrices. Since there are few precipitating reagents which fulfil these criteria, applications of on-line precipitation are inherently limited. Co-precipitation, on the other hand, depends on trapping the selected analytes in the matrix of a reagent which precipitates on mixing with the sample. The precipitate is collected on an in-line filter, then backflushed and analysed. This technique is therefore not subject to the limitations of direct precipitation, and has a much wider range of application. However, this approach has been rarely exploited principally because of the difficulties encountered with transporting the precipitate to the detector and with increasing back-pressure as the in-line filter becomes progressively blocked. The problem of filter blockage has largely been overcome by the use of knotted reactors. With this type of reactor, liquid flow through the tight coils generates a centrifugal force which effectively pushes the precipitate onto the walls of the reactor tubing, where it becomes adsorbed. The central channel of the reactor tubing doesn't become blocked, so the flow restriction encountered with in-line filters does not arise. This technique is quite effective, but often a supplementary in-line filter is also required to improve analyte recovery. Furthermore, it has been shown that relatively poor recoveries are obtained with on-line co-precipitation because in the flowing stream the process only has a short time in which to occur.[9]

By far the most widely applied technique for on-line preconcentration is sorbent extraction, in which the analyte species are retained on a suitable material packed into a column incorporated in the flow manifold. Packing materials which have been used include ion-exchange resins, immobilised chelating agents, and chromatographic materials (for retaining analyte chelates prepared on- or off-line).

2.2 Ion-exchange resins.

Ion-exchange can be defined as "the reversible interchange of ions between a solid and a solution phase".[10] Ions bound to the solid surface can generally be readily displaced by altering the pH or by mixing the solid with a concentrated solution of a counter ion. The ion-exchange process can be represented by the equations:

$$M^-A^+ + B^+ \longrightarrow M^-B^+ + A^+ \qquad \textit{Cation exchange resin}$$

$$M^+A^- + B^- \longrightarrow M^+B^- + A^- \qquad \textit{Anion exchange resin}$$

where M^-/M^+ represents, the charged support and A^+/A^- and B^+/B^- represent cations and anions on the resin surface and in solution. Cation exchange resins can either be strong or weak acid exchangers, depending on the type of functional group grafted to the support surface. Carboxylic acid groups on the support confer weak acidity to the resin, and consequently weaker binding of the analytes to the resin. Sulphonic acid groups on the other hand, confer strong acidity to the resin and offer stronger analyte binding. For anion exchange resins, two main types are available; the Type 1 resins, based on trialkylamine groups, and the Type 2 resins based on dimethylethanolamine. The latter types of resin are less basic and more unstable than the former.[11]

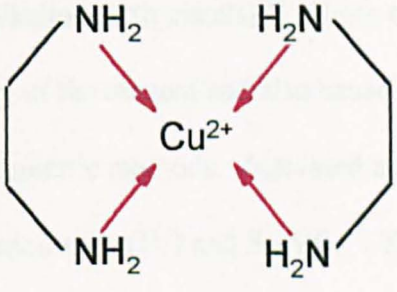
Ion-exchange reagents have found widespread application for trace analysis, especially of metals in environmental samples. These reagents are routinely used for water purification and effluent stream clean-up. The relatively high capacities of the exchange materials have also lead to their frequent use for preconcentration and separation from interfering species of opposite charge. However, ion-exchange resins are limited in the fact that they have relatively poor selectivity. A cation exchange resin, for example, will

simultaneously retain a broad spectrum of cations from the alkali and alkaline earth elements through to the transition group elements. This can be a problem if the target analytes are in an interfering matrix, such as sea water, which has an equal affinity for the ion-exchange resin.

2.3 Chelating resins.

Chelating resins differ from ion-exchange resins in that the support material is coated with a functional group which can abstract ions from solution by forming stable chelates on the resin surface. Depending on the pH, chelating resins may also illustrate some ion-exchange characteristics. Chelating resins have much greater selectivity compared to ion-exchange resins. This is because the chelation process results from the interaction of lone pairs on the atoms of the chelating group with unoccupied electronic 'd' orbitals of the metal ion, as illustrated in the example below (Figure 2.1). In this example, the chelate formed between Cu^{2+} and ethylenediamine is sketched, with the chelate bonds illustrated as red arrows.

Figure 2.1. A schematic illustration of chelate formation - Cu^{2+} with ethylenediamine.



Metal ions which possess vacant 'd' orbitals (e.g. Ni^{2+}, Cu^{2+}) are capable of forming strong chelates whereas those ions that do not will only form very weak complexes (e.g. Ca^{2+}, Na^{+}). It is this selectivity against matrix species which has fuelled the popularity of

immobilised chelating reagents for use in trace element preconcentration and matrix separation systems.

2.4 Acidic and basic alumina ion-exchange reagents.

In contrast to the ion exchangers described previously, Yamada and co-workers utilised a different type of ion-exchange preconcentration reagent.[12] In this study, an acidic alumina microcolumn was used to enrich sulphur, in the form of sulphate, from high purity iron certified reference materials, using a flow injection system. In this way it was possible to separate sulphur from the iron matrix since the latter was present as a cationic species. Several different types of alumina were tested as were the effects of acids, iron and other elements on the process. On-line standard additions gave a ng ml^{-1} limit of detection. The use of alumina as a preconcentration and matrix separation reagent for analysis of Pb in potable waters,[13] Cr(III) in human urine[14] and uranium[15] and transition metal cations[16] in natural waters has also been reported. It should be noted here that activated alumina behaves somewhat like a cation exchange resin, in as much as it is not particularly selective against matrix cations. To this end, the material has been reported to show only minimal retention of Na^+ and K^+ (alkali metals), but substantial retention retention of Ca^{2+} and Mg^{2+} (alkaline earth metals).[16] These retained matrix species may reduce the retention efficiency of the reagent and also cause interference problems during detection using atomic spectrometric methods. Activated alumina has also been successfully applied to speciation of Se(IV) and Se(VI).[17] This was achieved by first determining Se(IV) in the samples, by preconcentrating this species on basic alumina, then reducing Se(VI) to Se(IV) on-line to make a total Se determination. In this way, Se(VI) was determined by difference, rather than an absolute determination being made. A study of the speciation of Cr(III) and Cr(VI) in natural waters by McLeod *et al.*[18].

utilised the amphoteric nature of alumina to first selectively preconcentrate cationic Cr(III), under basic conditions, and then to selectively retain anionic Cr(VI) under acidic conditions. Using this approach an absolute determination of Cr(VI) could be made.

2.5 Support materials for immobilised chelating reagents and ion exchange resins.

Solid support materials used for immobilisation purposes include polymeric resins, such as formaldehyde / phenol resins, cross linked polymers, such as polystyrene / divinylbenzene, cellulose and porous glass structures, such as silica and controlled pore glass (CPG). Polymeric supports are generally prone to changes in volume particularly when subjected to changes in pH and solution composition. In contrast, porous glass materials do not change volume under these conditions. CPG is physically robust and its reactive surface is easily modified. These characteristics are desirable if the material is intended to be incorporated into a flow system. The support material must also be inert and chemically stable under the conditions in which it is to be used.

2.6 Polymer based chelating resins.

One functional group which has been widely exploited for preconcentration, because of its excellent chelating properties, is the iminodiacetate group. The most frequently encountered preconcentration media which contain this group are polymeric resins in which the iminodiacetate group is chemically immobilised onto a polymer backbone. A commercially produced form of this resin, Chelex-100, in which the iminodiacetate units are chemically insolubilised on 1% cross-linked polystyrene, was used by Olsen *et al.*[19] in the first published on-line preconcentration study. These workers utilised the resin to preconcentrate several trace elements from a sea water matrix with subsequent detection

by AAS. The results indicated that on-line preconcentration offered reproducible and accurate elemental determinations at low concentrations (sub-ng ml^{-1}) with a much reduced chance of accidental sample contamination. Hartenstein *et al.*[20] described a method in which a small ion exchange column of Chelex-100 was used to preconcentrate several metal ions from aqueous solution, prior to simultaneous detection using ICP-AES. Good precision (< 5%), high sampling rate (30 h^{-1}) and detection limits around 20 times better than those of continuous nebulisation were obtained. Chelex-100 was also used for preconcentrating lead from sea water, in a study of lead isotope ratios.[21] The preconcentration step yielded a detection limit for lead of 0.1 pg ml^{-1} and precisions better than 3% RSD were obtained for the isotope ratios measurement. One drawback with Chelex-100 is its tendency to increase in volume by up to 100% when converted from its acidic form to a salt form.[22] This property complicates use of the resin in packed columns for on-line preconcentration studies. In the light of this disadvantage of Chelex-100, other polymer based iminodiacetate resins have been developed which possess a more highly cross-linked polymer backbone and are therefore less subject to dimensional changes. Hirata *et al.*[23,24] have described the use of one such resin, Muromac-A1, in an on-line system for the study of trace elements in simple water matrices. The method was validated by the analysis of several standard reference materials, prepared by an off-line digestion procedure. The results were in agreement with the certified values and the method showed good precision (< 2% RSD). The use of an iminodiacetic acid / ethyl cellulose (IDAEC) resin, as another alternative to Chelex-100 has also been assessed for use in preconcentration.[25] A column containing IDAEC was incorporated into a computer assisted on-line flow system with ICP-AES detection. A range of metals of clinical and environmental interest were analysed in sea water, urine and water. The IDAEC resin performance was compared to that of carboxymethylated polyethyleneimine-polymethylenepolyphenylene (CPPI) and

Chelex-100 in terms of sample throughput, recovery and preconcentration factor. IDAEC was the most efficient and gave the best long term performance. In this study a sampling rate of 12 h^{-1} and detection limits enhanced by up to 100 times were achieved.

2.7 Silica and controlled pore glass reagents.

2.7.1 Preparation, nature and properties of silica and CPG.

Silica is generally available commercially in the form of silica gel. This material is usually prepared by coagulating and drying colloidal silica particles, to form a rigid three dimensional matrix. CPG is a particular form of silica gel, prepared by phase separation of a homogeneous borosilicate glass followed by dissolution of the boron-rich glass phase by strong acid.[26] The product of this process is a silica-rich, highly porous glass. Through careful control of this process, silica can be commercially produced with a variety of particle sizes and surface areas. Silica has been widely exploited for many years as a base support in liquid chromatography, particularly for reversed phase applications, and a wealth of information regarding its properties has been amassed. The other principle features and advantages of this material as an immobilisation support are tabulated below (Table 2.1)

Table 2.1 Features and advantages of silica as an immobilisation support.

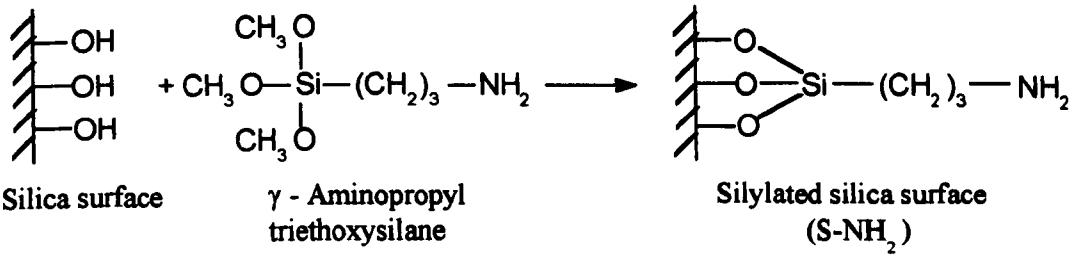
Feature	Advantage
Highly reactive surface chemistry	Readily modifiable with organic groups
Good mechanical robustness	Stable even under high pressure conditions
Stable across wide pH range (2-9)	Tolerates changes in solution pH
Dimensionally stable	Does not swell or contract

It is clear from Table 2.1 that silica has impressive credentials as a support material for chelate immobilisation, particularly for on-line applications, but inevitably there are some disadvantages. These are principally degradation of silica above pH 9 due to alkaline hydrolysis and, in the case of immobilised chelating reagents, low analyte capacity caused by the rigid nature of the silica matrix. This prevents expansion of the material with increasing analyte uptake, leading to steric obstruction of the reactive sites.[25] Steric obstruction on the silica surface also reduces the number of chelating groups that can be attached during preparation of the material. Silica based reagents therefore have inherently lower capacities compared to their polymeric counterparts.

2.7.2 The chemistry behind chelate immobilisation on silica and CPG.

As indicated above, silica and CPG possess a highly reactive surface which can be readily modified with organic functional groups to yield a variety of immobilised species. The process of surface modification usually begins with a silylation reaction which covalently grafts a particular group onto the material. The most well documented silylation method, first described by Weetall[27] and later by Hill,[28] involves reaction of the silica substrate with γ-aminopropyltriethoxysilane to cover the surface with covalently bound amino groups. These groups can be further reacted with di-aldehydes to yield aldehyde groups, which can then be reacted with amino groups on the species to be immobilised, thereby finally yielding the desired immobilised reagent. A typical reaction pathway for the surface modification of silica is illustrated below (Figure 2.2).[29]

Figure 2.2. A typical reaction pathway for the surface modification of silica.

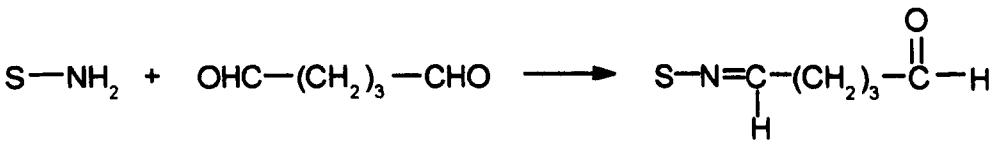


Silica surface

γ - Aminopropyl triethoxysilane

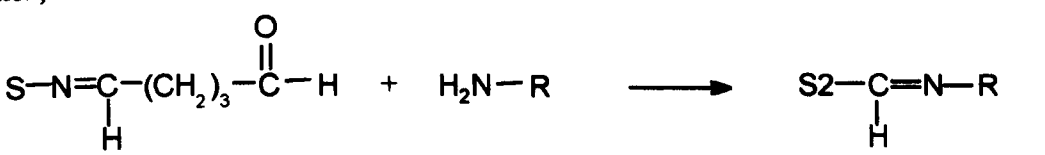
Silylated silica surface ($S\text{-}NH_2$)

then,



Glutaraldehyde

then,



Any molecule containing a reactive primary NH_2

Immobilised species

This silica modification technique was first exploited for immobilising enzymes[30] and was extended to chelating reagents as a direct result of this original work. Since these early studies, a steady stream of silica immobilised chelate papers have appeared in the literature. The principal chelate of interest has been 8-hydroxyquinoline (8-HQ), for which a large amount of data has now been amassed. In contrast, silica based iminodiacetate chelates have been given relatively little attention. This is probably because of the intense interest shown in polymer based iminodiacetate resins, notably Chelex-100, which is reflected by the enormous number of papers pertaining to this type

of resin (> 1000 since 1970). The same is true for CPG immobilised amines and dithiocarbamates, for which there are few papers. At its most esoteric, the study of CPG based chelates includes immobilised algae, bacteria and yeast for which a steady stream of articles are continuing to appear. The CPG based chelating reagents which have been reported are reviewed in detail in section 2.7.3 and 2.7.4.

2.7.3 CPG immobilised 8-hydroxyquinoline (CPG-8-HQ).

Of the CPG and silica immobilised chelates which have been developed for the purpose of trace element preconcentration, CPG-8-HQ is by far the most popular. The first reported use of CPG-8-HQ for the preconcentration of trace metals was by Hill,[28] in 1973. In this article, which presented a benchmark procedure for preparing CPG-8-HQ (see section 2.3.2 above), the potential of the material for retaining a range of transition metal elements was suggested. The ability of CPG-8-HQ to quantitatively separate transition metals from strong solutions of Na, Ca, K and Mg salts was illustrated. Sugawara *et al.*[31] swiftly followed Hill's work with a more comprehensive evaluation of the performance of CPG-8-HQ. In this work, the effect of pH, equilibration time, other complexing agents and increasing concentrations of NaCl on analyte retention by CPG-8-HQ were investigated. It was found that pH was an important factor on analyte retention whereas the presence of increasing amounts of NaCl had little effect on the retention efficiency. However, these workers also identified the potential problem of competing complexing species from their observation that retention of Fe by CPG-8-HQ was strongly reduced in the presence of citric acid. In this work, a retention capacity for Fe of around 0.06 mmol g^{-1} was measured, which, although low, was considered by the authors to be sufficient for specific, low concentration work. The selectivity of CPG-8-HQ for transition metal retention was the feature of greatest importance for Moorhead

and Davis[32] in their brief article describing a clean-up procedure for solutions of NaSCN. These workers required ultra clean solutions of concentrated NaSCN for anodic stripping voltammetry analysis of Ga. They found that after passing these solutions (at pH 9) twice through a column of commercial CPG-8-HQ (manufactured by the Pierce Chemical Co. at this time), considerable reductions in the concentrations of Cu, Zn, Cd and Pb were obtained. The promising results of these early studies opened the door for the subsequent exploitation of the material for separation and preconcentration of trace elements from natural waters and notoriously difficult, complex matrix samples such as sea waters. Among the first to assess the use of CPG-8-HQ for preconcentration from natural waters were Guedas da Mota *et al.*[33] This group designed an automated system to process 2 litre samples of water (adjusted with an acetate buffer to pH 4.6) through a column containing around 0.5 g of CPG-8-HQ. This study focused on Cu only, but the authors concluded that the procedure could easily be extended to the analysis of several other heavy elements. Furthermore, attention was drawn to the shorter analysis times possible using CPG-8-HQ instead of polymer based materials, because of the lack of swelling and better flow characteristics of the former. Following this study of natural waters, Sturgeon *et al.*[34] took the next step with their first study into the application of silica immobilised 8-HQ for analysis of trace metals in sea water samples. These workers used an immobilised 8-HQ reagent prepared in-house (using the procedure described by Hill), to preconcentrate and separate the elements Cd, Pb, Zn, Cu, Fe, Mn, Ni and Co from sea water. An off-line approach was taken, and the final samples were analysed using graphite furnace AAS. Using preconcentration factors of 50 (for a near shore sea water) and 90 (for an open ocean sea water) accurate results, compared to accepted values, were obtained for all the selected elements. To underline the success of their procedure, the authors also presented part of this work in a second publication, specifically directed at marine science[35]. The affinity of silica immobilised 8-HQ for

chelating a range of trace metals was put to good effect by Eskew *et al.*[36] for purifying plant nutrient solutions. These authors illustrated that a column containing the 8-HQ reagent retained >99% of radioactively labelled Ni and Zn added to various nutrient salt solutions. This retention efficiency was calculated by measuring the change in radioactivity of the spiked nutrient solutions before and after their passage through the column. It was around this time, that Olsen *et al.*[19] presented their pioneering study into the possibility of on-line preconcentration and matrix separation, using the technique of flow injection. The encouraging results of this study, which was the first of its kind, promptly triggered intense interest in this area, and unsurprisingly, silica immobilised 8-HQ with its excellent flow characteristics featured heavily in the work that followed. It was Malamas *et al.*,[37] in Sweden, who presented the first article describing the direct, on-line coupling of a silica immobilised 8-HQ preconcentration and matrix separation flow system to a flame AAS instrument. This study discussed in detail the factors involved in directly coupling the flow system to the detector and explored the effects of pH, eluent composition and interfering ions on the retention of several transition metals, Cd, Pb and Ca. These authors confirmed the expected significant effect of solution pH and also illustrated that the retention efficiency of the 8-HQ column (using Cd as the test element) was not reduced by the presence of high concentrations of the matrix element Ca, but suppressed by >50% in the presence of 15 ppm of Cu. This suppressive effect, caused by the greater affinity of Cu than Cd for the chelation sites on the surface of the material, still remains a limitation in this technique. Later the same year, Fang *et al.*[38] included silica immobilised 8-HQ in an on-line preconcentration study, using a two column system to increase sample throughput, with flame AAS detection. These workers achieved a sampling rate of 60 per hour, with preconcentration factors ranging from 50 to 105 for Cu, Zn, Pb and Cd. The performance of the preconcentration reagents Chelex-100, 122 resin and silica immobilised 8-HQ were compared in the study.

In general, the 8-HQ reagent was considered to be the most convenient for use in the flow system, but suffered a partial drawback in that its relatively low exchange capacity (expressed in mmol g^{-1}) slightly compromised the results obtained for heavy elements in sea water. The problem of low exchange capacity was subsequently addressed by Marshall and Mottola,[39] in a paper discussing the on-line preconcentration performance of a silica immobilised 8-HQ material, prepared using a more efficient synthetic route. In this work, the breakthrough capacity of the material for Cu, at a flow rate of 4.5 ml min^{-1}, was found to be only 20% of the batch capacity. This result illustrated the limiting effects of kinetic factors on the chelation process. Despite this observation, the authors obtained results for Cu in EPA waters which agreed well with the accepted values. Nakashima *et al.*[40] used silica 8-HQ to preconcentrate several transition group elements from sea water, off-line from the detection system. Relatively large sample volumes (> 50 ml) were processed to facilitate detection of open ocean concentrations of the selected elements. In contrast, Fang and Welz[41] constructed a flow injection manifold incorporating CPG-8-HQ which consumed less than 2 ml of sample and could be operated with a sampling frequency of 120 samples per hour. The atomic absorption detector (AAS) for this manifold was connected on-line. These workers improved the precision of the method by using conical preconcentration columns with sample elution from the wide to the narrow end of the column. Also in this study, preconcentration from a synthetic sea water matrix was described. It is known that 8-HQ (immobilised or free) is not entirely selective against alkaline earth metals, particularly magnesium, for which the 8-HQ complex is relatively stable.[42] This element particularly affects preconcentration of heavy elements, such as cadmium, and consequently this element gave variable and low recoveries in this study, until a 1:1 dilution of the sample with water was employed. This dilution procedure was effective because it reduced the

concentration of the matrix components and hence reduced the effect of the matrix on analyte retention.

The advent of the highly matrix sensitive technique of ICP-MS has heralded a new era for the science of preconcentration and matrix separation. Already, silica immobilised 8-HQ has been tentatively assessed for use in both off- and on-line matrix separation prior to analysis using ICP-MS, with some promising results. In Chapter 3 of this thesis, on-line matrix separation and preconcentration for ICP-MS using silica immobilised 8-HQ is explored, and the few articles which have appeared to date on the subject are discussed in detail therein.

2.7.4 Unusual CPG and silica immobilised chelating reagents.

Since the original work of Weetall *et al.* described earlier, the chemistry behind the immobilisation of organic compounds on silica has been widely exploited. Shortly after Weetall, Hercules *et al.*[43] published a paper describing the reaction between glass fibre and a silylising reagent containing a free amino group. The species so formed was further reacted with carbon disulphide to produce a glass immobilised dithiocarbamate chelating material. This reagent was used to preconcentrate metals such as Pb and Hg from water, for subsequent analysis by electron spectroscopy. Two years later, Leyden *et al.*[44] published their first paper on the subject, describing a procedure for the preparation of an *N*-substituted diamine on silica for preconcentration of Cu, Hg and Pb from aqueous solution, prior to analysis by X-ray fluorescence (XRF). Further work by Leyden's group extended the use of amino silylated silica to preconcentration of trace metals, again using XRF detection. The first study examined the performance of immobilised ethylenediamine and its dithiocarbamate, several other primary and secondary diamines and their corresponding dithiocarbamates and also the silyl xanthate

(i.e. product of the reaction of silanol groups with carbon disulphide) of silica gel itself.[45] The effect of pH on the uptake of numerous elements including Hg, Pb, Ag and Cu was investigated. In this study, the capacity of each material for Cu and Zn was found to be in the range 0.5 - 1.0 mmol g^{-1} which is notably greater than that of CPG-8-HQ (typically 0.05 to 0.1 mmol g^{-1}). The authors concluded that the chelating reagents produced showed sufficient selectivity against matrix species such as Na and Ca, were sufficiently stable and had adequate capacity for use as trace element preconcentration media. In a third study using the same materials, the effects of ionic strength and NaCl concentration on the binding of transition metals to the silica surface were investigated.[46] Relatively little reduction in recovery was found for each of the elements analysed with up to 1 mol dm^{-3} NaCl, which reflected the high formation constants of the analyte complexes formed with the silica reagents. Linear calibrations across 2 to 3 orders of magnitude were obtained for the diamine and dithiocarbamate reagents respectively, with curvature developing above a level of 60% saturation of each material. This performance range, although relatively limited, is still more than enough for trace element analysis. Other papers by Leyden explored the use of these diamine functionalised silica reagents in their protonated form for the retention of phosphate,[47] and a range of oxyanions, including tungstate, molybdate and arsenate.[48] This work also led to an elegant method for separating anionic uranium carbonate complexes from molybdate species on the basis of the pH selectivity of these species on the protonated diamine reagent.[49]

In contrast to the functionalised silica approach described so far, Kosmulski *et al.* used unmodified CPG to adsorb Eu(III) and Ce(III) from basic and neutral solutions, with a view to adopting the technique to remove radiocerium from nuclear fission waste waters[50,51,52,53]. Optimum adsorption in the presence of 0.1 mol dm^{-3} NaCl was found to occur above pH 8 and, interestingly, in the presence of lower concentrations of NaCl, this optimum pH was found to shift to around pH 7. Although effective for this

application, unmodified CPG is considerably less selective than its chelate functionalised analogues and at pH values above 8, substantial retention of Ca and Na will occur through ion exchange of these elements with the silanol groups on the CPG surface.

As an aside from the extensive work carried out on CPG immobilised 8-HQ described in section 2.7.3, Devi *et al.*[54] assessed the performance of CPG immobilised 8-quinolinol-5-sulphonic acid for preconcentrating several elements, including Cu, Cd, Hg and Pb. This chelate has an electrophilic $-SO_3H$ group located opposite the chelating hydroxyl group on the quinoline ring. This should, by resonance in the aromatic ring, reduce the electron density around the hydroxyl group, and change the chelation properties. Despite the presence of the $-SO_3H$ group, the reagent was found to exhibit similar pH behaviour to its 8-HQ relative. Capacities measured for the material were stated to be as high as 8 mmol g^{-1}, which is in stark contrast to previously reported values for 8-HQ of around 0.1 mmol g^{-1}. The inability of the CPG support to expand leads to lower capacities and decreased coverage of the surface by the chelating group, so the high capacity reported is unexpected. The consideration by Devi *et al.*[54] that electrophilic groups attached to the quinolinol system might influence the chelating properties of this species was further explored by Elmahadi and Greenway[55] in their recent study of CPG immobilised chloroxine. This analogue of 8-HQ has a electrophilic chlorine atom attached in the 5 position on the quinoline ring. In this study immobilised chloroxine was found to give maximum preconcentration efficiencies at slightly higher pH values compared to immobilised 8-HQ, for the elements Cu, Zn, Cd, Co and Pb. These workers concluded that the presence of the chlorine atom had caused the change in optimum preconcentration pH, because of the increased acidity it conferred to the hydroxyl proton through its electron withdrawing effect. Habib and Townshend[56] developed the subject further in a paper which described the behaviour of a CPG immobilised dialkyldithiocarbamate reagent. The elements Co, Cu, Hg and Rh could be retained by

this reagent. For quantitative elution of Hg, thiourea was required in the eluent acid, to displace this element from its strong complex with the sulphur atoms of the dithiocarbamate. This material was also reported to have a high capacity (up to 4 mmol g^{-1}).

Kobayashi and Miyazaki[57] reported the preparation and properties of a highly selective CPG reagent, based on immobilised salicylideneamino-2-thiophenol, for the preconcentration of ionic Al at trace levels. The procedure was affected by Fe^{3+} and Cr^{3+} interferences but these could be removed by adding hydroxylamine hydrochloride as a reducing agent. An operating pH of at least 6 was required for quantitative recovery of Al. By preconcentrating by a factor of 100, accurate analysis of Al in drinking water samples was achieved.

A novel approach to metal preconcentration was described by Elmahadi and Greenway, in two articles published in the early 1990's. These articles described the use of the amino acid cysteine[58] and the algae *Selenestrum capricormutum*,[59] immobilised on CPG, for preconcentration in an on-line system. In both these papers, Hg, Pb and a range of transition group elements were analysed. The effects of pH were studied and the variations in the optimum pH for retention of each element observed. Both articles also addressed the influence of concomitant elements in the solution on the retention of the target analytes. In general, Co was observed to be the most seriously affected with up to an 18% reduction in retention when Cu, Pb or Cd were present at 20 μg ml^{-1}. For Zn and Cd, the presence of Pb caused some retention suppression. These observations clearly show that there is a problem if the sample to be analysed contains high levels of elements which can chelate with the column material. These elements will swamp the available sites and suppress retention of the analytes of interest. In some cases, careful selection of the pH can bias against retention of the major competing elements. It may also be possible to selectively precipitate or adsorb the problem elements before

preconcentration. Either way, prior knowledge of the sample composition is required which complicates the analysis.

The analytical use of CPG immobilised algae was taken a step further by Elmahadi and Greenway[60] in a paper which described not only trace element preconcentration of Ag and Cu but also Cr speciation. This work utilised a dual column system to show how two immobilised algae, *Selenestrum capricornutum* and *Chlamydomonus reinhartii* could be used to retain cationic Cr^{3+} and anionic Cr(VI) respectively and how, under flow injection conditions, the two analytes could be separately eluted from the columns into a flame AAS. This interesting study illustrated the potential use of CPG immobilised reagents for wider applications of trace element analysis.

The novel approach taken by Elmahadi and Greenway was subsequently adopted by Maquiera *et al.*[61], to evaluate the behaviour of a CPG immobilised yeast, *Saccharomyces cerevisiae* in an on-line preconcentration system. The reasoning behind the choice of a biological yeast material was that the cell wall of this organism is covered with a variety of chelating functional groups, notably -SH, which should facilitate quantitative complexation of a wide range of elements. The material was found to quantitatively retain Zn, Cd and Cu from pH 7.5 up to pH 9.5, in a phosphate buffer. For Fe, retention decreased sharply from an optimum at pH 6 and for Pb, an optimum centred around pH 7.5 was observed. High concentrations (5 $\mu g\ ml^{-1}$) of a range of elements was observed to cause some suppression in retention of the target analytes, especially in the case of Cu. Capacities of the material were similar to those reported for CPG-8-HQ. A second paper by Maquieira *et al.*[62] detailed a similar method, this time using an immobilised cyanobacteria. Although bacterias, yeasts and other micro-organisms have the capability to chelate many different metals, they have the disadvantage in that they are more prone to degradation than simple chelating agents and are also more susceptible to algal growth.

2.7.5 CPG immobilised ethylenediaminetetra-acetate (EDTA) and iminodiacetate (IDA).

Of the many literature references to chelating reagents covalently immobilised onto controlled pore glass, only a scant few describe the use of EDTA and IDA. The first paper on the subject appeared in 1978, courtesy of Guedas da Mota *et al.*[63]. In this preliminary paper, the chelating properties of a commercial glass immobilised EDTA reagent (ED3A Pierce 23520), now no longer available, were investigated for Cd(II), Zn(II), Cu(II) and Pb(II). The stability of the ED3A complexes of the selected elements and the capacity of the reagent for these elements was studied. Increasing amounts of ED3A were added to acetate buffered solutions of each element and the free ion concentration after equilibrating the mixture. Using a logarithmic plot of metal ion concentration versus ED3A added, element capacities of 0.05 to 0.08 meq/g were calculated for the elements studied. These workers chose to study Zn and Cd at pH 6.7 and Cu and Pb at pH 5.7, but also briefly described attempts to evaluate stability constants at other pH values. This initial study also found that Cu and Cd could be separated from Pb and Zn, by simply adjusting the pH, because of the different complex formation constants of these two pairs of elements. A second paper by Guedas da Mota *et al.*[64] developed the study of ED3A further by applying the reagent to the separation of Cu(II), Pb(II) and Zn(II) from the non-chelating matrix components of sea water. In this work, the ED3A reagent was packed in a column and the sea water samples (5 l) pumped through it, after adjustment to pH 5.6 with acetate buffer. After loading, the column was eluted with a small volume of hydrochloric acid, yielding a preconcentration factor of nearly 400 in addition to matrix separation. Finally, the eluate was analysed using flame AAS. The paper states that the total labour time required to perform the analysis was only 15 min but this time did not include the 17 h taken for the sea water samples to pass through the column. Thorough investigation of the effects of eluent acid

strength, sample loading flow rate, eluent acid volume and sodium chloride on the recoveries of Cu, Zn and Pb revealed that flow rate and eluent acid strength were of greatest importance. Several coastal North Sea waters were also analysed and the results found to compare well with an independent analysis. Allen *et al.*[65] investigated the application of ED3A to preconcentration of aluminium from natural waters. These workers highlighted the lack of literature studies of this material by indicating that "Studies of [ED3A] have been restricted to Cu and Pb extraction...." and then proceeding to cite only the work of Guedas da Mota *et al.*[63,64] described above. The study by Allen *et al.*[65] compared the performance characteristics of packed columns of ED3A and CPG-8-HQ. The effect of pH, in acetate buffered solutions, on Al retention was evaluated and the distribution coefficients of Al between each resin and the solution calculated. This study illustrated that ED3A yielded the more stable Al complex and was far superior at retaining Al than was CPG-8-HQ. It was also found that a pH of at least 4 was required to achieve quantitative retention of Al. Below pH 4, retention decreased and this was suggested to be a result of competition between H and Al ions on the surface of each chelating material. At this point, the trail of ED3A seemed to go cold. The material was no longer manufactured, presumably because of competition from cheaper, high capacity polymer based resins, and no further work appeared in the literature.

Recently, a new CPG based iminodiacetate reagent has become available for trace element preconcentration and matrix separation. The performance of this material has been compared with two commercially available polymeric resins in a preliminary matrix separation and preconcentration study of the first row transition group elements, using AAS detection (see list of publications in the Appendix). In this study, the new CPG-IDA resin was found to perform more effectively under flow conditions than the

polymeric resins mainly because unlike these resins, it was not susceptible to volume changes with changes in the flow composition and pH.

Later in this thesis, in Chapters 4 and 5, the application of this new CPG-IDA reagent to on-line matrix separation, using ICP-MS detection, will be discussed in detail.

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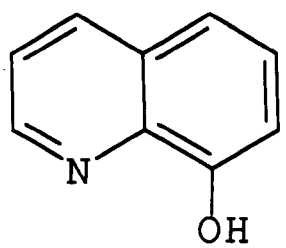
CHAPTER 3

ON-LINE MATRIX SEPARATION AND PRECONCENTRATION USING 8-HYDROXYQUINOLINE IMMOBILISED ONTO CONTROLLED PORE GLASS (CPG)

3.1 Introduction.

Chelating reagents play an important role throughout the whole field of chemistry. These compounds have been exploited for a variety of applications, including the production of catalysts, dyestuffs and liquid chromatography substrates. However, by far the widest area of application of chelating reagents has been for selective extraction and preconcentration of trace elements. Of the chelating compounds which have been used, 8-hydroxyquinoline (8-HQ) is certainly one of the most popular. This is principally because it can readily form stable, uncharged chelates with a large number of elements, while having a very low affinity for matrix species such as Na^+, K^+ and Ca^{2+}. The structure of 8-HQ is illustrated below (Figure 3.1).

Figure 3.1. Structure of 8-HQ.



Chelation occurs via the N atom and the hydroxyl group in the 8 position of the aromatic quinoline system. The presence of both a basic N atom and an acidic OH group in the molecule makes its complexing ability strongly dependent on pH. In acidic solutions, the OH group of 8-HQ is protonated. The dissociation reactions of the protonated molecule, together with the corresponding dissociation constants (pK) are as follows:

$$H_2Ox^+ \rightleftharpoons HOx + H^+ \qquad pK_1\ 5.02$$
$$HOx \rightleftharpoons Ox^- + H^+ \qquad pK_2\ 9.81$$

In the scheme presented above H_2Ox^+ represents protonated 8-HQ, HOx represents 8-HQ itself and Ox^- represents 8-HQ, minus its hydroxyl proton. It is clear from these reactions that at high pH values, the Ox- species will predominate. Under these conditions, the molecule contains the lone pair of the N atom and two lone pairs on the O atom, which allows the molecule to chelate strongly. Unfortunately, the negative charge also leads to some ionic binding, albeit weak, of species such as Ca^{2+} and Mg^{2+}, which show little or no chelating ability. Therefore, under high pH conditions, the selectivity of 8-HQ against matrix elements (i.e. Ca^{2+}, Mg^{2+}, Na^+ etc) is slightly compromised. Under low pH conditions, the protonated H_2Ox^+ species predominates. This species still contains the lone pair on the N atom, but the lone pair on the OH group has been used to form a dative bond with the proton. The chelating ability of 8-HQ under these conditions is therefore markedly reduced. Ideally, the region of pH 5 to 7 is the most favourable for elemental chelation using 8-HQ. The many literature references to the use of immobilised 8-HQ for trace element preconcentration in conjunction with atomic spectrometric detection were summarised in Chapter 2 of this thesis. This extensive literature list indicates that, despite the limitations of 8-HQ outlined above, this

reagent has been and still remains one of the most popular reagents for preconcentration and matrix separation.

3.2 Use of immobilised 8-HQ for preconcentration and matrix separation in ICP-MS.

Many samples have analyte concentrations which are below the ng ml^{-1} level. These concentrations cannot be determined by flame AAS unless the sample is greatly enriched. As indicated earlier, large preconcentration factors have been used in off-line studies, but are not generally practical for use in on-line systems. However, the arrival of ICP-MS, with its very low detection limit capabilities, has facilitated on-line analysis of samples with sub ng ml^{-1} analyte concentrations, using only small preconcentration factors. For ICP-MS, the issue is not so much one of preconcentration, but rather more one of matrix separation. As described in detail in Chapter 1 of this Thesis, direct analysis of saline samples by ICP-MS is plagued with problems. In summary, these problems are:

(1) Injector and interface cones blockage by sample deposition.
(2) Matrix related polyatomic interferences overlapping with the isotopes of interest.[1,2]
(3) Ionisation suppression within the plasma.

The tolerance of ICP-MS to dissolved solids is lower than that of ICP-AES and much lower than that of flame AAS. Furthermore, the effect of matrix atomisation and ionisation has also been reported to be greater for ICP-MS than ICP-AES.[3] The operation of ICP-MS instrumentation is also more costly than flame AAS, because of the use of argon gas, or ICP-AES, because of the vacuum pumping systems required, so lengthy sample pre-treatment while the instrument is running is undesirable. On the

other hand the multi-element and low detection limit capabilities of ICP-MS are highly desirable features, particularly for analysis of sea waters and biological samples. For these reasons, attention has been directed in recent years to developing both off- and on-line matrix separation systems for ICP-MS that are as efficient and as rapid as possible. In the relatively few studies which have been reported in this area to date, immobilised 8-HQ has featured strongly. Indeed, it was this material that was used in the first study of this kind, performed by McLaren *et al.*[4] at the National Research Council of Canada. In this study, 8 elements in a coastal sea water reference material (CASS-1) were analysed by ICP-MS after off-line preconcentration on a silica immobilised 8-HQ reagent, prepared in-house. To achieve quantitative retention of the selected elements the preconcentration process was carried out at pH 8. The extremely low detection limit capabilities of the ICP-MS instrumentation used allowed accurate results to be obtained using a preconcentration factor of just 50, compared to a factor of 225 that had been necessary in a similar, previous study, in which graphite furnace AAS detection had been used.[5] The authors also highlighted the added advantage of the isotopic measurement capabilities of ICP-MS, by presenting accurate isotope dilution results for 6 of the selected elements. Following this initial investigation, the Canadian group went on to publish three further papers in this area. In the first of these three articles,[6] the same preconcentration procedure described above was used to measure 7 elements, including U, in an open ocean water reference material (NASS-2). The analyte concentrations in this sample were generally somewhat lower than in the CASS-1 sample analysed previously, but nonetheless, accurate results were obtained for 6 of the 7 elements using an isotope dilution approach. Only the data for Mo were inaccurate, a result which the authors concluded to be due to insufficient equilibration time after spiking the samples with the enriched ^{100}Mo isotope, prior to preconcentration and the final isotope dilution

measurement. In the second article, which reported results for several different ICP-MS applications, McLaren *et al.*[7] included data for the coastal sea water reference material CASS-2, obtained using isotope dilution and off-line preconcentration on silica immobilised 8-HQ. This article served to further underline the analytical value of ICP-MS and the success of the silica immobilised 8-HQ off-line preconcentration / matrix separation procedure. In the last of the these three "follow-up" papers,[8] the Canadians used a 90 fold off-line preconcentration procedure, with isotope dilution quantitation, to measure a total of 11 elements in an open ocean water reference material (NASS-3). This detailed article described the use of two separate pH conditions to separately preconcentrate Fe and Mo (pH 3) and the other 9 elements (pH 8). This approach overcame the earlier inaccuracies experienced with Mo, when only pH 8 was used. In order to obtain accurate isotope dilution results for Fe in this study, an argon-nitrogen mixed gas plasma was used to essentially eliminate the ArO^+ interferences on Fe at m/z 56 and 57.

Up until this time, preconcentration using immobilised 8-HQ had only been applied to analysis of the transition group metals and some heavy elements. This range of elemental application was extended by Orians and Boyle[9] in their study of Ti, Ga and In in sea water. These elements were known to be present at picomolar concentrations, which combined with the sea water matrix problems, made direct analysis of these samples practically impossible. Orians and Boyle employed an off-line preconcentration procedure, using 8-HQ immobilised on a polymeric vinyl support (TSK-8HQ) as the preconcentration medium. The method developed was based on passing 4 litre volumes of each sea water sample, buffered to pH 4 prior to analysis, through the column followed by a wash step and finally elution with 10 ml of HNO_3. In this way a 400 fold preconcentration could be achieved. This was sufficient for the analysis of Ti but, even

for ICP-MS, a further evaporative concentration step was necessary to facilitate analysis of Ga and In. The procedure was very time consuming because a maximum flow rate of only 5 ml min^{-1} could be operated through the column. Another, later study in which the elemental application of immobilised 8-HQ for preconcentration was extended further was reported by Esser *et al.*[10] These workers used silica immobilised 8-HQ to preconcentrate the rare earth elements from natural water samples, as part of a sample preparation procedure, prior to analysis by ICP-MS. The procedure was carried out at an optimum pH of 8, at which value significant retention of Ca and, more importantly, Ba occurred. The presence of Ba in samples which are to be analysed for rare earth elements introduces problems in ICP-MS because of isobaric interference of BaO species on these elements. Esser *et al.*[10] circumvented the problem of Ba in the samples by adding a second column downstream of the first, which contained an organophosphorous extractant (RE-Spec) capable of selectively retaining the rare earth and transuranic elements in acidic samples. Using this elegant approach, rare earth analytes retained on the silica immobilised 8-HQ column could be eluted, complete with Ba, with approximately 1 N acid onto the RE-Spec column, which retained only the rare earth elements while allowing Ba to flow to waste. Finally, the retained rare earth species could be eluted with 0.015 N acid, and then analysed by ICP-MS. In this way, almost complete separation of the rare earth elements from Ba was achieved and accurate results were obtained, using isotope dilution, for several sea and ground water samples. On-line preconcentration using immobilised 8-HQ with ICP-MS detection is still a relatively new science, for which few papers have appeared to date. Beauchemin and Berman[11] were the first to report the use of silica immobilised 8-HQ in an on-line matrix separation system for ICP-MS. In this preliminary investigation, a riverine reference material of low salinity, SLRS-1, was analysed for six elements and the results found to

agree well with the expected values. The use of a low salinity sample allowed the matrix separation efficiency to be monitored throughout as only a low salt concentration passed into the ICP-MS. However, this approach did not test the matrix separation system to its full extent, so a further analysis of the more saline open ocean reference material, NASS-2 was carried out. The results for Mn, Mo, Cd and U were reported to compare well with the expected values, but data were not presented for Co, Ni, Cu or Pb. The authors reported a spectral interference of CaO on Ni, even with the low salinity sample. This interference was due to the presence of residual matrix in the sample eluted from the column into the plasma. The design of the manifold used in this study was such that the sample and eluent acid passed along the same stream, albeit at different times. It seems likely, therefore, that contamination could have arisen from the residual presence of some of the original, untreated sample in the system. This hypothesis is further supported by the lack of data for Cu, Ni and Co for the more saline NASS-2 sample, since each of these elements are affected by saline matrix related interferences, and would therefore have been difficult to analyse accurately using the manifold design reported.

The original on-line preconcentration for ICP-MS work of Beauchemin and Berman was later extended by McLaren *et al.*,[12] in a study based on the incorporation of a column of silica immobilised 8-HQ into a commercially available automated flow injection manifold. This automated manifold, supplied by Dionex, was used to analyse the first row transition elements, Cd and Pb at a sampling rate of around 8 minutes per sample. The manifold was operated with sequence of steps that included a column wash with buffer after sample loading. In this way, the weakly bound residual matrix species (Ca and Mg) could be removed, prior to elution of the analytes of interest into the ICP-MS. The matrix removal achieved in this work lead to an improvement in the results obtained for Cu and Ni compared to the original study by Beauchemin and Berman, the results for

which were compromised by matrix related interferences. The results for Co, however, were higher than the certified value which suggested that $^{42}Ca^{16}O^{1}H$ arising from residual Ca may still have been a problem. The on-line method developed in this work was adopted again by McLaren's group for the evaluation of lead isotope ratios in fresh and saline waters, using ICP-MS.[13] In this study, the precision of the isotope ratio measurements was shown to be close to the mathematically predicted precision values. The rapidity of the analysis was considered to be advantageous, but this lead to the disadvantage that the transient analytical signals were too short in duration for accurate and precise data to be obtained for the low abundance ^{204}Pb isotope. The method was applied for quantitative analysis of the reference water materials NASS-3 and NASS-4 and for isotope ratio measurements for NASS-3 and SRM-981. In both cases accurate results were obtained compared to the certified values, illustrating the effectiveness of CPG-8-HQ for this application.

In this chapter, a rapid on-line matrix separation system for ICP-MS, using a flow injection manifold incorporating a mini-column of CPG-8-HQ, for the determination of several trace elements in saline samples is described. The immobilised chelate is located in the loop of a manual flow injection valve. The retained analytes are eluted counter current to the sample flow to yield improved, less dispersed peaks. The system described is based on fixed volume injection, rather than time based sampling, and gives a 5-fold preconcentration in addition to the required matrix separation. The manifold design also offers very low sample consumption. Data from the transient eluted peaks has been collected using the multi-element time resolved analysis facility supplied with the instrument. Results illustrating the effectiveness of the matrix separation process with respect to the $^{40}Ar^{23}Na$ interference on ^{63}Cu are presented together with validation of the procedure using coastal and estuarine certified reference materials.

3.3 Performance characteristics of on-line preconcentration and matrix separation systems.

3.3.1 Advantages over batch systems.

The general advantages of on-line preconcentration and matrix separation compared to batch analysis systems are well known and can be summarised as follows:

(1) High sample throughput.

(2) Low sample and reagent consumption.

(3) Excellent reproducibility.

(4) Low risk of contamination since the sample manipulation occurs within a closed system.

(5) Relatively inexpensive and easy to automate.

The principle disadvantage of this approach is that the procedure takes place under non-equilibrium conditions. This could introduce a discrepancy in the results obtained if, for some reason, the analysis conditions differ between standards and samples.

3.3.2 Analytical features.

The performance of on-line matrix separation and preconcentration techniques can be assessed in several ways, including the preconcentration factors obtainable, the efficiency of the process and the analytical speed. In a comprehensive monograph on the subject, Fang[14] has classified the various important parameters in terms of five basic equations.

(1) Enrichment factor (EF). This term is the value most regularly used for characterising on-line preconcentration systems. It is a measurement of the ratio between the analyte concentrations in the eluted concentrate (C_e) and the original sample (C_s), and is calculated from the equation:

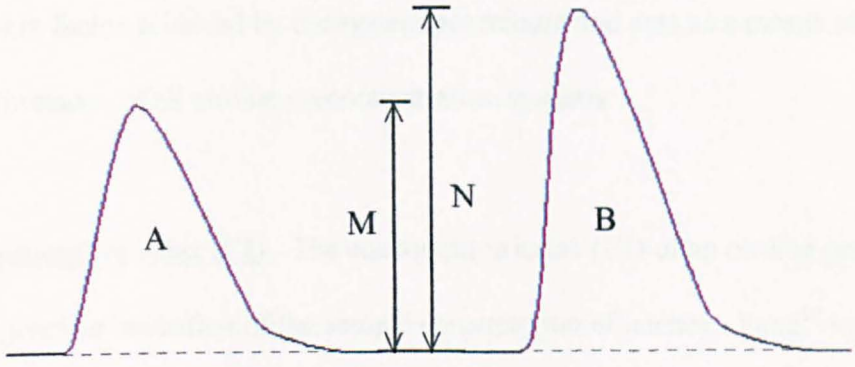
$$EF = C_e/C_s$$

In practice this value may be difficult to measure directly since the analyte concentration in the sample is not accurately known. For this reason, an acceptable approximation of the EF can be calculated by measuring the ratio of the slopes of the analyte calibrations obtained before and after preconcentration. For analysis of highly saline samples, however, this approach may not be possible since direct analysis of such samples may not be practical. In this case, the EF value must be estimated from the ratio of the sample and eluent volumes (if these are known accurately) and the retention efficiency for each analyte.

(2) Enhancement factor (N). This feature is of importance when the signal response changes between preconcentration and direct analysis for reasons other than analyte enrichment. For example, if the preconcentrated sample is eluted in a solvent matrix which differs from that of the standards, the analyte response may be enhanced or suppressed. This situation may also arise in matrix separation systems based on counter current elution (see section 3.4.6), when measurements are based on peak height rather than peak area. In this case, the calibration solutions must be injected via the valve used for the column eluent, because identical volumes must be used in order to equate the standard response to the analyte response. Since the analytes are loaded in a narrow

band at one end of the column, during elution by acid flowing in the opposite direction, peaks are produced which are sharper than the more dispersed calibration solution peaks. Hence, the peak height of the eluted analytes is greater than the equivalent calibration solution peak height, and so analyte concentrations are measured which are too high. This situation is presented graphically below (Figure 3.2).

Figure 3.2. Effect of counter current elution on peak height enhancement.



Calibration solution, 10 ng ml^{-1} Eluted analyte, X ng ml^{-1}

Area A = Area B, therefore X = 10 = calibration solution concentration

Height N > M, therefore X > 10 > calibration solution concentration i.e. too high

It has been shown by Fang *et al.*[15] that when independent enhancement factors are present their effects will influence the EF according to the equation:

$$N_t = N_1 \times N_2 \times \ldots\ldots \times N_n \times EF$$

where N_t, N_1, N_2, N_n equal the total, first, second and nth enhancement factor respectively and N can be less than unity.

(3) Concentration efficiency (CE). This value is supplementary to EF in the sense that the operating efficiency of any on-line preconcentration system depends not only on the analyte enrichment factor (EF) but also on the rate of sample throughput. The concentration efficiency of the system can be defined by the equation:

$$CE = EF \times (f/60)$$

where f is the sampling frequency per hour. The CE value therefore represents the enrichment factor achieved by the system per minute and acts as a means of normalising the performance of all on-line preconcentration systems.

(4) Consumptive index (CI). The consumptive index (CI) of an on-line preconcentration system gives an indication of the sample consumption efficiency. Fang[19] has defined CI as a measurement of the volume of sample required to achieve unit EF, using the equation:

$$CI = V_s/EF$$

where V_s is the sample volume (in ml) used during the analysis. An assessment of the CI value is of greatest importance when only a limited quantity of sample is available.

(5) Phase transfer factor (P). In on-line preconcentration systems, the transfer of analyte from the sample through to the eluted concentrate is often incomplete as a result of low analyte capacity of the preconcentration medium or insufficient equilibrium time between the flowing sample and the preconcentration sorbent. Evaluation of the phase transfer

factor is important if it is suspected that different transfer efficiencies are being achieved for the samples and the standards, because of matrix interference effects. The value of P can be calculated from the equation:

$$P = m_e/m_s$$

where m_e and m_s are the mass of analyte in the original sample and the eluted concentrate respectively. For on-line column preconcentration systems, the phase transfer factor is more commonly referred to in terms of the percentage retention efficiency, which is a measurement of the analyte recovery after preconcentration.

3.4 Experimental.

3.4.1 Reagents.

High purity deionised water (18MΩ cm resistivity, Elgastat UHQ PS, Elga Ltd, UK) was used throughout this study. Elemental stock solutions (100 μg ml^{-1}, SpectrosoL, BDH, Poole, Dorset, UK) were used in the preparation of calibration solutions. The reagents 8-hydroxyquinoline, 3-aminopropyltriethoxysilane and *p*-nitrobenzoylchloride (Sigma, Poole, Dorset, UK), absolute ethanol (Hayman, Witham, Essex, UK), sodium dithionite (BDH, Poole, Dorset, UK), and concentrated hydrochloric acid (Fisons, Loughborough, Leicestershire, UK) were used in the immobilisation procedure. Controlled pore glass (CPG, 100-125 μm particle size, Fluka, Gillingham, Dorset, UK) was used as the immobilisation support. Ammonium acetate buffer (Sigma) was prepared from the solid and purified prior to use by passing through a column of Chelex-100 (Sigma) under

gravity. Adjustments in pH were made using glacial acetic acid or aqueous ammonia, as appropriate. The ammonia solution was prepared by isothermal distillation of concentrated aqueous ammonia (Beecroft and Partners, Rotherham, UK). Synthetic sea water was prepared by dissolving sodium chloride, sodium sulphate, sodium hydrogencarbonate, magnesium chloride, calcium chloride and potassium chloride in water, using the procedure described by Van Berkel *et al.*[16] Samples of the coastal (CASS-2) and estuarine (SLEW-1) certified reference materials (CRM's; National Research Council of Canada, Ottawa, Canada) were introduced to the manifold without pre-treatment. All analytical work was performed without the use of clean room facilities.

3.4.2 Instrumentation.

Throughout the course of this work, the ICP-MS measurements were made using a VG PlasmaQuad 2 Plus (VG Elemental, Winsford, Cheshire, UK). The instrument was routinely calibrated and optimised prior to operation using a tune solution containing the elements Be, Mg, Co, Y, La, Eu and Bi at 10 ng ml^{-1} in a matrix of 5% nitric acid. The transient analyte peaks produced by the matrix separation system were monitored using the TRVision® time resolved analysis software supplied with the instrument (see Section 3.2.2). Data acquisition and instrument operating parameters are given in Table 3.1. The ICP-AES measurements required for batch and dynamic capacity evaluations were made using a Perkin-Elmer Plasma 40 (Perkin-Elmer, Beaconsfield, Bucks., UK). The instrument operating parameters are given in Table 3.1. Data output from the ICP was recorded using a chart recorder (BBC, Servoscript Ltd., Surrey, UK).

Table 3.1. Instrument operating parameters for the ICP-MS and ICP-AES.

ICP-AES instrument.

Aerosol gas flow rate (l min^{-1}): 0.75 Intermediate gas flow rate (l min^{-1}): 0.6

Outer gas flow (l min^{-1}): 12.0 Nebuliser: Cross flow type

Spray chamber: Ryton

ICP-AES data acquisition parameters.

Emission wavelength for Mn (nm): 257.610

Chart recorder settings: 2 V fsd, 1 cm min^{-1}.

ICP-MS instrument.

Forward power (W): 1350 Reflected power (W): < 5

Aerosol gas flow rate (l min^{-1}): 0.94 Intermediate gas flow rate (l min^{-1}): 1.0

Outer gas flow (l min^{-1}): 13.0 Nebuliser: De Galan type

Spray chamber: Scott double pass, water cooled to 10°C

ICP-MS time resolved analysis data acquisition parameters.

Time per slice (s): 1.00 Points per peak: 3, peak jumping

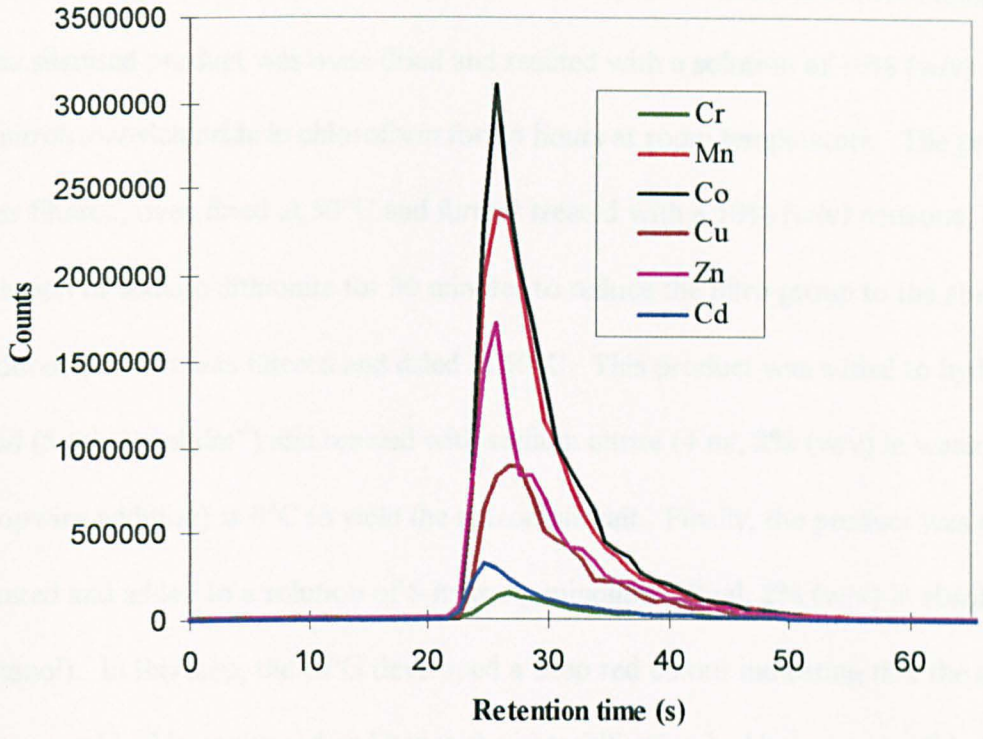
Detector mode: Pulse counting

Selected isotopes: ^{48}Ti, ^{51}V, ^{55}Mn, ^{59}Co, ^{60}Ni, ^{63}Cu, ^{64}Zn, ^{65}Cu, ^{107}Ag, ^{114}Cd, ^{115}In, ^{140}Ce, ^{208}Pb

3.4.3 Time resolved data acquisition.

Accurate analysis of transient peaks using quadrupole ICP-MS is inherently difficult owing to the fact that the technique operates sequentially and hence cannot measure all the selected isotopes simultaneously. However, this is a relatively minor problem for today's instruments which can achieve scan rates fast enough to cover the complete Periodic Table mass range in less than a second. The difficulty encountered now is one of how to cope with the large quantity of data produced from each scan of the mass spectrometer. For analysis of non transient signals, such as those arising from continuous nebulisation of a sample, data for each successive scan can be rapidly accumulated in a series of electronic channels on board the instrument, where each channel corresponds to a particular isotope mass. At the end of the acquisition, all the data accumulated in the channels can be transferred to the computer in one step. This hardware facility, referred to as the multi-channel analyser (MCA), eliminates the time-consuming and inefficient need to download data at the end of every scan. For analysis of transient signals, however, the situation is more complex. The MCA approach cannot be adopted because accumulation of data for each scan simply gives a final summated spectrum, with no visual description of the peak profile. In order to for it to be possible to generate a peak profile, data must be downloaded at the end of each scan. Until recently, this has been a problem for transient peak analysis by ICP-MS, but new developments in computer software and hardware have now made it possible to rapidly download data for each scan. This technique is referred to as time resolved acquisition (TRA) and a typical peak profile obtained using it is presented below (Figure 3.3).

Figure 3.3. Typical peak profile obtained using TRA data acquisition.



By using TRA differences between individual element peaks can be identified. For example, in the above figure, which depicts an elution profile from a matrix separation column, Cu is seen to elute later than either Zn or Cd. This example serves to illustrate that TRA is an effective method of data acquisition for ICP-MS sample introduction systems which produce transient peaks, such as laser ablation, electrothermal vaporisation, flow injection, capillary electrophoresis and gas, liquid or supercritical fluid chromatography.

3.4.4 Immobilisation procedure for 8-hydroxyquinoline.

A similar procedure to that described by Habib[17] for the preparation of CPG immobilised dialkyldithiocarbamates was used. CPG (1.0g) was first activated by boiling it in 20 ml of 10% (v/v) nitric acid for 30 minutes. The product was filtered and dried in an oven at

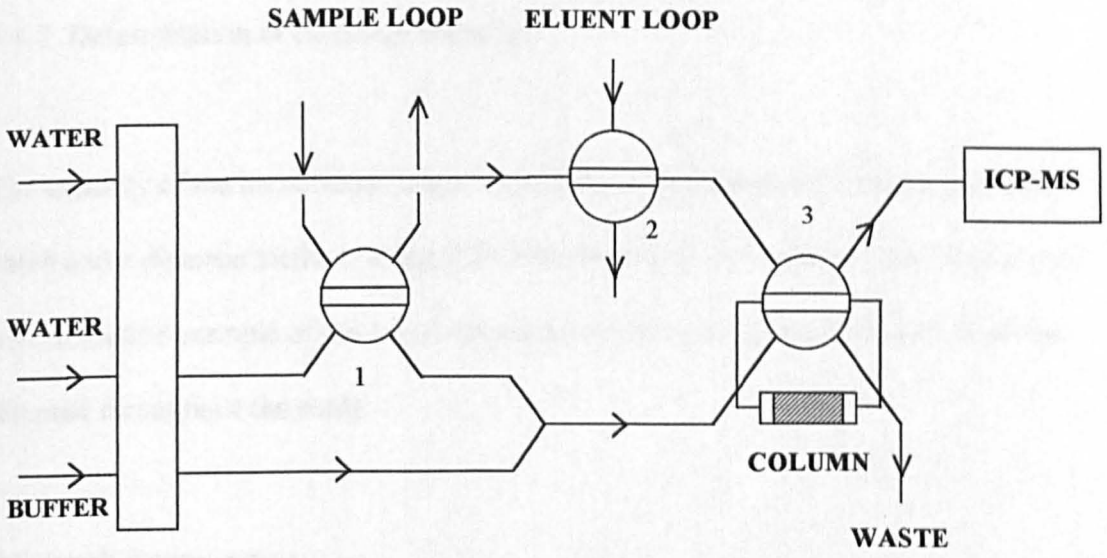
80°C for three hours and subsequently silanised by reaction with 10ml of 10% (v/v) 3-aminopropyltriethoxysilane in anhydrous toluene, for 15 minutes at room temperature. The silanised product was oven dried and reacted with a solution of 10% (w/v) *p*-nitrobenzoylchloride in chloroform for 24 hours at room temperature. The product was filtered, oven dried at 50°C and further treated with a 10% (w/v) aqueous, boiling solution of sodium dithionite for 30 minutes to reduce the nitro group to the amine. The reduced product was filtered and dried at 50°C. This product was added to hydrochloric acid (5 ml, 2 mol dm^{-3}) and reacted with sodium nitrite (4 ml, 2% (w/v) in water, dropwise addition) at 0°C to yield the diazonium salt. Finally, the product was rapidly filtered and added to a solution of 8-hydroxyquinoline (20 ml, 2% (w/v) in absolute ethanol). In this step, the CPG developed a deep red colour indicating that the diazo compound had been formed and hence the immobilisation had been successful. The final product was filtered, washed with hydrochloric acid (2 mol dm^{-3}) and water and stored in a vacuum dessicator.

3.4.5 The matrix separation manifold

The matrix separation manifold is illustrated in Figure 3.4. It was designed to facilitate rapid processing to increase the sampling frequency above that achieved in earlier studies.[16,17] The immobilised 8-hydroxyquinoline was contained within a glass mini-column (2 cm x 3 mm i.d., Omnifit, Cambridge, UK) incorporated in the loop of a manual 4-way injection valve (Rheodyne 5020, Supelco Ltd, Poole, Dorset, UK). All the manifold connections were made using 0.8 mm i.d. PTFE tubing. The reagents were pumped at 1.0 ml min^{-1} using a peristaltic pump (Gilson Minipuls 3, Anachem, Luton,

UK), resulting in a matrix separation flow rate of 2.0 ml min^{-1} and an elution flow rate of 1.0 ml min^{-1}.

Figure 3.4. The manual flow injection manifold.



1 - Sample loading valve

2 - Eluent loading valve

3 - Switching valve (to switch column between loading and elution streams)

3.4.6 Matrix separation procedure.

For matrix separation, the first valve (1) (Figure 3.4) served as the sample injection port and the second (2) as the acid eluent port. The third valve (3) allowed matrix species unretained by the column to run to waste during the separation process, while aspirating water into the ICP-MS. On elution, valve 3 served to direct the eluted analytes into the ICP-MS. Sample volumes of 0.5 ml were loaded on to the column via valve 1 and analytes retained on the column were eluted via valve 3, using 0.1 ml of 2.0 mol dm^{-3} nitric acid. In this way, a preconcentration factor of 5 was achieved, in addition to matrix separation. With this manifold design, the sample and eluent streams flowed in opposite directions through the column. This design yielded sharper eluted peaks and

prevented compression of the packing material at one end of the column, thereby avoiding the build up of back pressure. Cross contamination was minimised by constructing the eluent and sampling streams separately from each other.

3.4.7 Determination of exchange capacity.

The capacity of the immobilised 8-hydroxyquinoline was determined for Mn, by both a batch and a dynamic method, using ICP-AES detection. This element was chosen as a representative example of the transition group elements, to which most attention was directed throughout the study.

(a) Batch determination.

A solution of Mn (20 $\mu g\ ml^{-1}$) was prepared in ammonium acetate buffer (0.1 mol dm^{-3}, pH 6.0) and added to 0.05 g of dry immobilised 8-hydroxyquinoline. The mixture was equilibrated, with stirring, overnight. The solution was filtered and the concentration of Mn in the supernatant liquid measured versus the original concentration. The drop in concentration of Mn was used to evaluate the capacity, using equation 3.1.

$$C_b = (c_i - c_f)v / [m*1000*M_r] \qquad \text{(equation 3.1)}$$

where C_b is the batch capacity (in mmol g^{-1}), c_i and c_f are the concentrations of the metal before and after equilibrium (in $\mu g\ ml^{-1}$), M_r is the relative atomic mass of the metal and v is the volume of solution (in ml) equilibrated with a mass m (in g) of immobilised chelate.

(b) Dynamic determination.

In order to determination the capacity of the CPG-8-HQ material under dynamic conditions, the flow injection manifold was coupled to an ICP-AES instrument equipped with a chart recorder. It was necessary to couple the manifold to an ICP-AES instead of the ICP-MS in this capacity evaluation because the Mn concentrations eluted from the column after loading were too high to be passed into the ICP-MS. Aqueous solutions of Mn were prepared across the range 0 to 700 $\mu g\ ml^{-1}$. Dry immobilised 8-HQ (0.04 g) was packed into a glass mini-column. It was necessary to operate the manifold with a stronger ammonium acetate buffer concentration of 2 mol dm^{-3} because of the high acidity of the Mn solutions, arising from the 5% (v/v) HNO_3 acid matrix of the original 1000 $\mu g\ ml^{-1}$ Mn stock solution. Nitric acid eluent (2.0 mol dm^{-3}) was used. Three repeat analyses were made at each concentration and the results evaluated in terms of the eluted peak height. The concentration beyond which no further increase in peak height was obtained was deemed to represent the dynamic capacity limit. The dynamic capacity was evaluated using equation 3.2.

$$C_d = cv_s / [m*1000*M_r] \qquad \text{(equation 3.2)}$$

where C_d is the dynamic capacity (in mmol g^{-1}), c is the concentration of the metal at the dynamic capacity limit (in $\mu g\ ml^{-1}$), v_s is the volume of the injected sample (in ml), m is the mass of immobilised chelate in the column (in g) and M_r is the relative atomic mass of the metal under evaluation.

3.5 Results and Discussion.

3.5.1 Manifold design.

The manifold developed for the project incorporated several design features to simplify and improve the matrix separation procedure. To make the method more rapid and more economical in terms of sample consumption, a small sample volume was utilised. To reduce the analysis time further, CPG-8-HQ was selected as the chelating reagent because column washing and conditioning could be achieved very rapidly between samples, compared with iminodiacetate resins. As polymeric based chelating resins are subject to volume changes between matrix separation and elution, the dimensionally stable material CPG was selected as the support. This material can also be readily functionalised because of reactive silanol groups on its surface, to yield useful, insoluble, ion-exchange / chelating reagents. The application of counter current matrix separation and elution yielded sharper transient peaks and avoided compacting the column material at one end of the column, thereby reducing the risk of back pressure still further and ensuring good flow stability.

3.5.2 Analytical features of the manifold.

Earlier in this chapter (section 3.3.2) a description of some of the fundamental features of on-line matrix separation systems, according to Fang,[19] was presented. The values of these fundamental features, where appropriate, for the manifold used in this study are given below (Table 3.2).

Table 3.2. Analytical features according to Fang[19] for the manifold used in this study.

Feature	Equation	Measured value
Enrichment factor, EF	$EF = C_e/C_s$	≈5
Concentration efficiency, CE	CE = EF x (f/60)	1.25
Consumptive index, CI	$CI = V_e/EF$	0.1

The low EF value indicates that the system has a relatively small preconcentration ability, but since the system was developed for ICP-MS analysis, a high degree of analyte enrichment was not considered necessary. The CE value reflects the rate of sample analysis, which for this system, was around 15 per hour (see Table 3.3). This sampling rate was greater than that reported in the original on-line CPG-8-HQ study by Beauchemin and Berman[11] or in the later studies by McLaren's group.[12,13] The low CI value shows that the matrix separation system developed in this study was highly efficient in terms of sample consumption.

3.5.3 Optimisation of the matrix separation procedure.

The procedure was optimised with respect to the parameters of buffer concentration, buffer pH and eluent acid volume and concentration, using a univariate approach. Samples were prepared by spiking a synthetic sea water solution with the selected analytes, followed by acidification with one drop of concentrated nitric acid. Acidification was performed in accordance with the accepted procedure of maintaining the sample integrity with respect to the trace metal content.[18] It was found that eluent volumes below 0.1 ml yielded badly dispersed peaks giving poor reproducibility and

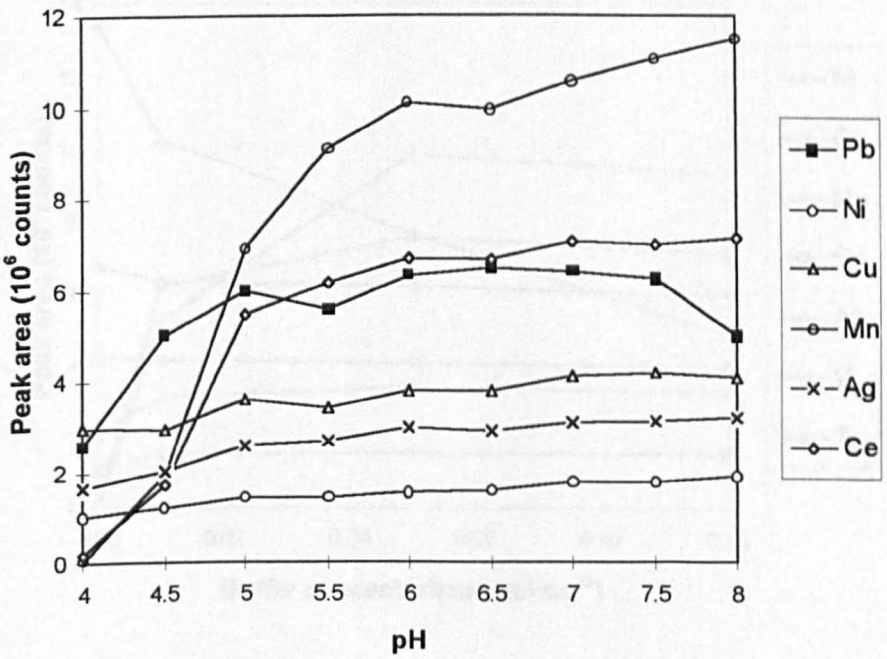
volumes greater than 0.1 ml gave a lower preconcentration factor. For these reasons an eluent volume of 0.1 ml was selected, without further optimisation. The complete optimum conditions are given in Table 3.3.

Table 3.3. Optimum matrix separation and preconcentration conditions for the 8-HQ study.

Buffer conditions: Ammonium acetate, 0.05 mol dm^{-3}, pH 6.0
Matrix separation flow rate (ml min^{-1}): 2.0
Elution flow rate (ml min^{-1}): 1.0
Nitric acid eluent concentration (mol dm^{-3}): 2.0
Total analysis time (min $sample^{-1}$): 4

The response of the column to some of the selected analytes with changing buffer pH is shown in Figure 3.5.

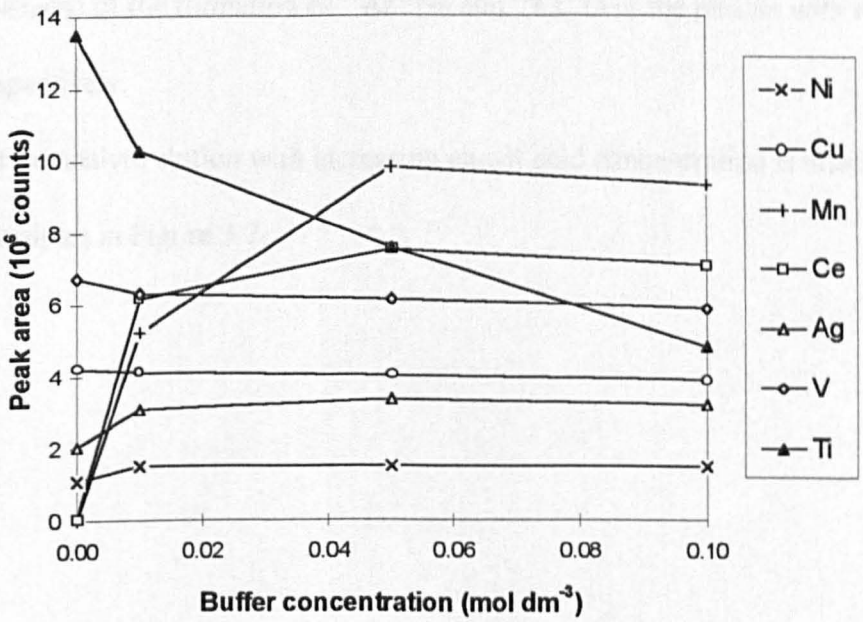
Figure 3.5. Effect of buffer pH on analyte retention for the 8-HQ column.



In Figure 3.5, the elements shown were in the following oxidation states; Pb^{2+}, Ni^{2+}, Cu^{2+}, Mn^{2+}, Ag^{+} and Ce^{3+}. Data were also obtained for V (as VO^{2+}), Co^{2+}, Zn^{2+}, Cd^{2+} and Ti^{4+}, but these are not shown in Figure 3.5 for clarity reasons. The pH curves of Zn and Cd were analogous to that of Pb, whereas the pH behaviour of V and Co was similar to that of Cu. The results for Ti appeared to show an increase in retention efficiency with increasing pH, but this apparent trend was determined to be due to increasing retention of Ca (interference of ^{48}Ca on ^{48}Ti). Since the optimum buffer pH for matrix separation varies between elements a compromise pH must be selected for multi-element analysis. On the basis of the element responses illustrated in Figure 3.5, pH 6.0 was chosen, as values below this gave decreased retention of some analytes. Values above this level gave lower buffer capacity (up to pH 8) and beyond pH 8 gradual hydrolysis of the CPG based chelating material would occur.

The effect of the ammonium acetate buffer concentration on the retention of selected elements is illustrated in Figure 3.6.

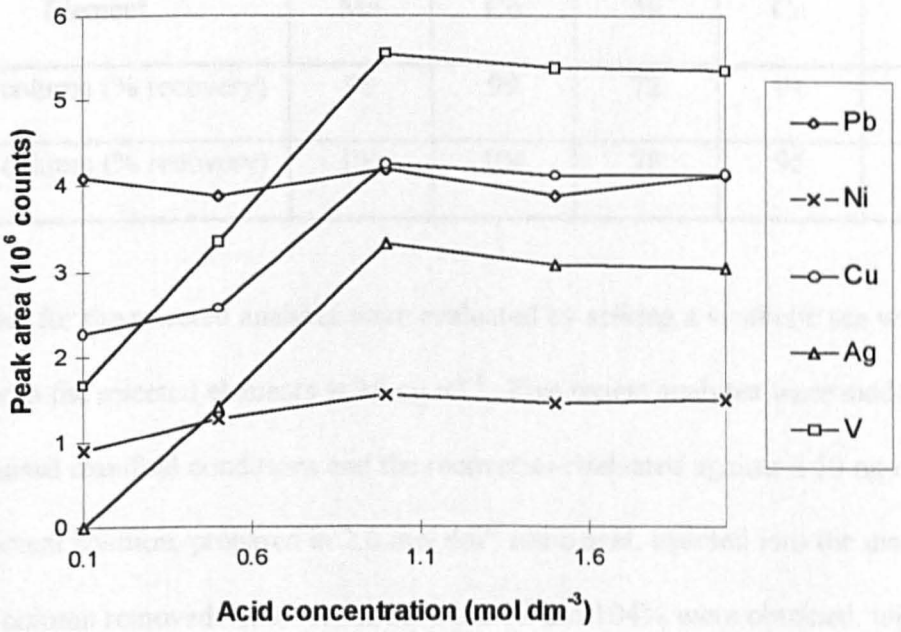
Figure 3.6. Effect of the buffer concentration on analyte retention.



In Figure 3.6, the elements shown were in the following oxidation states; Ni^{2+}, Cu^{2+}, Mn^{2+}, Ce^{3+}, Ag^{+}, V (as VO^{2+}) and Ti^{4+}. Data were also obtained for Co^{2+}, Zn^{2+}, Pb^{2+} and Cd^{2+}, but these are not shown in Figure 3.6 for clarity reasons. The effect of buffer concentration on all these elements was analogous to that shown for Ce. The results in Figure 3.6 show that the buffering process is effective at a minimum concentration of 0.05 mol dm^{-3}. Since the optimum matrix separation pH lies outside of ±1 pH unit of the buffer pK_a,[19] the buffer capacity is significantly lower than the maximum at pH 4.8. This observation is of interest since it might be expected that optimum pH for analyte retention should correspond more closely with the buffer pK_a. This effect is considered to be due to kinetic effects in complex formation close to the surface of the chelating material, the magnitude of the individual element complex formation constants and may also be influenced by ionic strength effects. The fact that the optimum pH lies outside of the $pK_a \pm 1$ region means that with decreasing buffer concentration, a decrease in both the analyte percentage retention and the measurement precision will occur. The anomalous result observed for ^{48}Ti is due to ionogenic retention of ^{48}Ca at low buffer concentrations. This effect is also slightly evident at zero buffer concentration for ^{63}Cu and ^{51}V because of the formation of $^{40}Ar^{23}Na$ and $^{35}Cl^{16}O$ in the plasma with residual Na and Cl respectively.

The effect on analyte elution with increasing eluent acid concentration is illustrated for selected analytes in Figure 3.7.

Figure 3.7. Effect of the acid concentration on analyte elution.



In Figure 3.7, the elements shown were in the following oxidation states; Pb^{2+}, Ni^{2+}, Cu^{2+}, Ag^{+} and V (as VO^{2+}). Data were also obtained for Co^{2+}, Zn^{2+}, Ti^{4+}, Mn^{2+}, $Ce^{3+}$and Cd^{2+}, but these are not shown in Figure 3.7 for clarity reasons. In this study, quantitative elution was observed for all the elements investigated at an acid concentration of 2.0 mol dm^{-3}. The acid concentration required for each individual element increased with the formation constant of the element / 8-hydroxyquinoline complex. Acid concentrations greater than 2.0 mol dm^{-3} were not investigated as these would risk degrading the column and shortening its working life. To determine the effect on the column of repeated exposure to the acid eluent, a second column was prepared from the same batch of immobilised 8-hydroxyquinoline and the two columns compared in terms of element recoveries (Table 3.4).

Table 3.4 Effect of repeated use on the performance of the 8-HQ column.

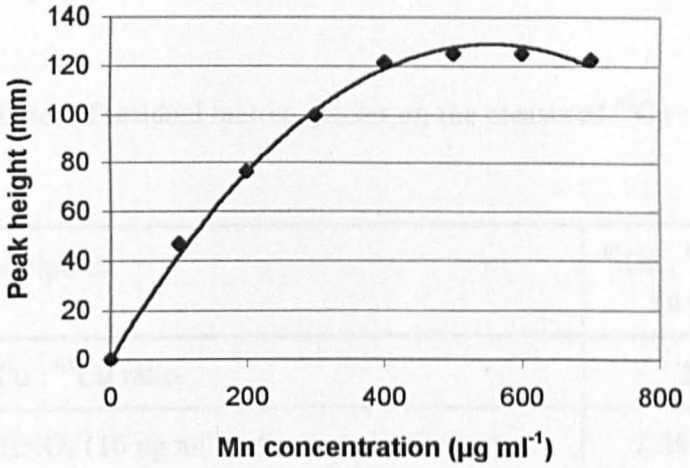
Element	Mn	Co	Ni	Cu	Ce
Aged column (% recovery)	98	99	72	91	89
Fresh column (% recovery)	101	104	78	95	95

Recoveries for the selected analytes were evaluated by spiking a synthetic sea water sample with the selected elements at 10 ng ml^{-1}. Five repeat analyses were made using the optimised manifold conditions and the recoveries evaluated against a 50 ng ml^{-1} multi-element solution, prepared in 2.0 mol dm^{-3} nitric acid, injected into the manifold with the column removed. Recoveries between 89 and 104% were obtained, with the exception of Ni which gave reduced recoveries of between 70 and 80%. The results showed that the original column performance was not significantly reduced, for the analytes measured, after use for approximately 300 hours.

3.5.4 Dynamic and batch column capacity evaluations.

Evaluation of the capacity of the immobilised 8-hydroxyquinoline material was performed using Mn. A batch capacity of 0.086 ± 0.009 mmol g^{-1} was measured. The dynamic capacity was calculated by loading increasing quantities of Mn (up to 700 μg ml^{-1}) on to the column and monitoring the eluted peak by ICP-AES. The concentration at which no further increase in Mn peak height on elution was observed was deemed to represent the dynamic capacity limit of the material. The results of this experiment are illustrated in Figure 3.8 below.

Figure 3.8. Dynamic capacity results (quantity of Mn loaded versus eluted peak height).



From Figure 3.8, it is evident that the dynamic capacity maximum occurs at a Mn concentration of 500 µg ml^{-1}. This gave a dynamic capacity measurement of 0.118 ± 0.003 mmol g^{-1}, which is comparable to the batch capacity measurement. This result is in contrast to previous observations of decreased capacity with dynamic operation.[10] This illustrates that under the conditions of dynamic operation used in this study, the column retains this analyte rapidly. These capacity values are consistent with earlier reported values for this material.[5,10,20] The capacities with respect to other elements have not currently been investigated but on the basis of recovery results it is evident that the capacity of the material is sufficient for a wide range of elements.

3.5.5 Evaluation of the effect of residual matrix species.

To evaluate the influence of residual matrix on the column following matrix separation and column washing, the isotope ratio of ^{63}Cu to ^{65}Cu was evaluated for a spiked pure water sample, a spiked sea water sample and for both the CRM's. If residual Na was present at an unacceptably high level, this ratio would be greater than the natural ratio because of the formation of the $^{40}Ar^{23}Na^{+}$ interference. Table 3.5 illustrates that residual

matrix does not significantly effect the isotope ratio measurement and therefore does not degrade the accuracy of the measurement.

Table 3.5 Effect of residual matrix species on the measured ^{63}Cu : ^{65}Cu ratio.

Sample description	^{63}Cu : ^{65}Cu ratio (n = 5)#
Natural ^{63}Cu : ^{65}Cu ratio	2.24
Cu in 5% HNO_3 (10 ng ml^{-1}), direct aspiration	2.29 ± 0.06
Cu in sea water (10 ng ml^{-1}), direct aspiration	9.77 ± 2.18
Cu injected in 2 mol dm^{-3} HNO_3 (50 ng ml^{-1})	2.19 ± 0.09
Cu matrix separated from spiked pure water (5 ng ml^{-1})	2.21 ± 0.05
Cu matrix separated from spiked sea water (5 ng ml^{-1})	2.18 ± 0.08
CASS-2 Cu ratio (1.5% salinity)	2.19 ± 0.06
SLEW-1 Cu ratio (3.5% salinity)	2.16 ± 0.08

Values quoted with 2 standard deviation range (95% confidence limits).

The natural ^{63}Cu : ^{65}Cu ratio is greater than the ratios measured in this study. This is primarily due to mass discrimination effects arising from the detector dead time (leading to lower count detection at higher count rates) and sequential mass scanning of the transient analyte peak. Since a copper solution of known ^{63}Cu : ^{65}Cu isotopic ratio was not available in this study, an approximate value of the instrumental mass bias was calculated based on the accepted ^{63}Cu : ^{65}Cu ratio of 2.24 (measured using thermal ionisation mass spectrometry), using equation 3.3 below.[21]

$$(A/B)_m = (A/B)_t \times (1 + \alpha n) \qquad \text{(equation 3.3)}$$

where $(A/B)_m$ and $(A/B)_t$ are the measured and true values of the isotopic ratio of isotope A and isotope B, respectively, a is the mass bias per unit mass and n is the difference in mass between the two isotopes. Using the isotope ratio measured for the 50 ng ml^{-1} Cu solution (in 2% HNO_3) injected via the manifold (i.e. 2.19) and equation 3.3 above, a negative mass bias of -0.02 was measured for the ^{63}Cu : ^{65}Cu ratio. This means any copper isotope ratios measured with this ICP-MS system must be corrected for mass discrimination by multiplying the measured ratio by $[1/(1 + an)]$. For copper, this multiplication value is 1.02.

3.5.6 Calibration and analysis of certified reference materials.

Linear calibrations were obtained over the range 0 to 10 ng ml^{-1} for analytes in spiked pure water and spiked synthetic sea water matrices, as described in Table 3.6.

Table 3.6 Comparison of calibration data from pure and saline solutions.

Calibrations from spiked pure water					
Parameter	Mn	Co	Cu	Ag	Ce
Precision (% RSD), 5 ng ml^{-1}, n=5	1.4	1.8	3.2	2.2	1.6
Least squares regression coefficient	0.9999	0.9999	0.9992	0.9984	0.9992
Sensitivity (10^5 counts / ng ml^{-1})	9.8	9.0	1.9	3.0	7.9
Calibrations from spiked synthetic sea water					
Parameter	Mn	Co	Cu	Ag	Ce
Precision (% RSD), 5 ng ml^{-1}, n=5	1.8	1.6	1.7	3.6	2.0
Least squares regression coefficient	0.9971	0.9985	0.9985	0.9996	0.9983
Sensitivity (10^5 counts / ng ml^{-1})	11.8	10.8	2.3	3.8	10.3

The two calibration sets compared well therefore validating the use of calibration solutions prepared in high purity water for quantifying analytes in more complex matrices. This procedure utilised much smaller volumes than would be required using a standard additions procedure and was less susceptible to contamination.

The matrix separation procedure was validated by analysis of the two CRM's SLEW-1 and CASS-2, which had similar analyte concentration but different salinities. The analytes were quantified by external calibration against acidified multi-element (Mn, Cu, Zn, Ni, Co, Cd, Pb) pure water standards processed through the manifold. Five repeat analyses were made at each concentration and for the CRM's. The calibrations generally showed good linearity with the exception of Zn, which was affected by reagent contamination. For the remaining analytes, least squares regression coefficients of 0.997 to 0.999 were obtained across the concentration ranges 0 to 20 ng ml^{-1} (Mn), 0 to 5 ng ml^{-1} (Cu, Ni) and 0 to 0.1 ng ml^{-1} (Cd, Pb, Co). Precisions (measured as relative standard deviation, RSD) for the selected analytes were in the range 0.9 to 5.5 %. The results obtained for the reference material analyses are presented in Table 3.7.

Table 3.7. Results for the reference material analyses.

Element	SLEW-1		CASS-2	
	Found*	Certified*	Found*	Certified*
Mn	12.7 ± 0.4	13.1 ± 0.8	2.18 ± 0.19	1.99 ± 0.15
Co	0.051 ± 0.003	0.046 ± 0.007	0.032 ± 0.002	0.025 ±0.006
Ni	0.738 ± 0.049	0.743 ± 0.078	0.299 ± 0.015	0.298 ± 0.036
Cu	1.68 ± 0.08	1.76 ± 0.09	0.706 ± 0.034	0.675 ± 0.039
Zn	0.90 ± 0.12	0.86 ± 0.15	1.95 ± 0.16	1.97 ± 0.12
Cd	0.011 ± 0.001	0.018 ± 0.003	0.011 ± 0.001	0.019 ± 0.004
Pb	0.026 ± 0.002	0.028 ± 0.007	0.019 ± 0.005	0.019 ± 0.006

* Concentrations in ng ml^{-1}, with ± 2 standard deviations (95% confidence limit).

For both materials, good agreement was obtained between the measured and certified concentrations for all the elements monitored. Direct aspiration of the saline CRM's, for comparison with the matrix separation procedure, was not attempted because of the associated problems of cone and injector blockage and signal suppression in the plasma. Injection of saline samples without prior matrix separation was observed to reduce the blockage problems, but significant levels of polyatomic interferences (Table 3.5) and signal suppression remained. These problems, coupled with the low concentrations present in the CRM's, render direct sample injection inappropriate for this analysis.

3.6 Conclusions.

A rapid on-line matrix separation procedure using a mini-column of CPG-immobilised 8-hydroxyquinoline was developed. The transient peak signals were successfully monitored using time resolved data acquisition. A sampling frequency of 15 h^{-1} was obtained with typical measurement precisions of <3% RSD. Comparable linear calibrations were obtained from spiked pure and sea water illustrating that external calibration using pure water solutions was suitable for quantifying Mn, Co, Ni, Cu, Zn, Cd, Ce, Ag and Pb, in more complex matrices. The matrix separation procedure was shown to be efficient by the negligible effect of the $^{40}Ar^{23}Na$ polyatomic interference on ^{63}Cu, in terms of the $^{63}Cu/^{65}Cu$ ratio. Application of the matrix separation manifold to the estuarine and coastal water CRM's SLEW-1 and CASS-2 yielded results which were in good agreement with the certified values. These results indicated that ultra-trace element analysis of complex matrix samples is feasible in the absence of clean room facilities. The further application of this manifold to analysis of open ocean sea water samples would require a greater degree of preconcentration than is necessary for the coastal and estuarine samples analysed in this study.

3.7 References.

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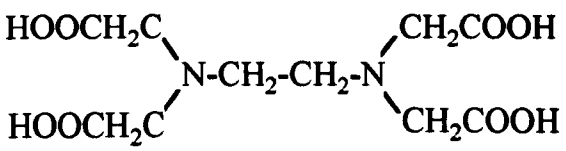
CHAPTER 4

ON-LINE MATRIX SEPARATION AND PRECONCENTRATION USING IMINODIACETIC ACID IMMOBILISED ONTO CONTROLLED PORE GLASS

4.1 Introduction.

The application of the iminodiacetate functional group for metal chelation has proved to be as popular as 8-hydroxyquinoline. Perhaps the most well known iminodiacetate based chelation reagent is ethylenediaminetetra-acetic acid (EDTA), the structure of which is presented below (Figure 4.1):

Figure 4.1. Structure of EDTA.



For EDTA, chelation can occur through either the N atoms of the tertiary amine groups or through the carbonyl and hydroxyl groups of the carboxylic acid units. As with 8-hydroxyquinoline, the presence of both the basic N atoms and the acidic OH groups in EDTA make its ability to complex metal elements strongly dependent on pH. In acidic solutions, EDTA exists in its free acid form. The dissociation reaction of the free acid groups, together with the corresponding dissociation constant (pK_a) are as follows:

$$RCOOH \rightleftharpoons RCOO^- + H^+ \qquad pK_a\ 4.76$$

In the scheme presented above RCOOH represents free EDTA and $RCOO^-$ represents the corresponding acid anion. It is clear from this dissociation reaction that at high pH values, the $RCOO^-$ species will predominate. Under these conditions, the molecule contains the lone pair of the N atom and two lone pairs on the O atom, a combination which facilitates strong chelation. The presence of the negative charge allows some ionic interactions to occur, which leads to weak binding of unwanted matrix species such as Na^+ and Ca^{2+}. Therefore, under high pH conditions, the selectivity of EDTA against these matrix elements is reduced. Under low pH conditions, the protonated RCOOH species predominates. This species contains lone electron pairs on the N and O atoms, so at low pH, EDTA still has significant chelation abilities. Furthermore, at low pH, the absence of a negative charge on the EDTA molecule confers a greater selectivity against matrix elements. However, at lower pH values, the chelation equilibrium favours displacement of the chelated metal back into solution which reduces the chelation efficiency. In practice, therefore, the region of pH 4 to 7 is generally the most favourable for elemental chelation using EDTA.

4.2 Use of immobilised EDTA for preconcentration and matrix separation.

Traditionally, polymer based resins of the iminodiacetate (IDA) type, have been extensively used[1,2] for preconcentration and matrix separation purposes. These have the disadvantage that they tend to swell and contract under changing solution conditions, such as pH and ionic composition. This can lead to back pressure problems in on-line systems and often necessitates lengthy conditioning of the column material between

samples, which reduces sample throughput. Polymeric resins have higher capacities than controlled pore glass materials, which can be beneficial if higher analyte concentrations are to be monitored or if a strongly retained analyte is suppressing retention of another, more weakly retained element. For this reason, several workers have assessed the use of controlled pore glass as the chelate support as described in Chapter 3. This material is dimensionally stable under changing flow conditions, has a reactive surface which can be easily functionalised and is chemically stable over a wide pH range. The active surface of this material also leads to rapid chelation of trace elements, which is a particularly desirable feature in on-line analysis, as analyte residence times are usually short (often less than 2 or 3 seconds). Early on-line matrix separation studies used columns of the polymer based Chelex-100 resin, incorporated into the flow manifold to separate several trace metals from sea water samples, with subsequent detection using AAS[3] and ICP-AES.[4] These studies highlighted that Chelex-100, which contains the IDA ligand covalently bonded onto a polystyrene - divinylbenzene support, is difficult to use in on-line systems because of the large volume changes it undergoes when converted from its acid form to a salt form. It also requires conditioning steps between each sample analysis which increases the analysis time. To address this difficulty, alternative more highly cross-linked IDA resins which are less susceptible to dimensional changes have recently been employed.[5,6] These resins are amenable for use in on-line systems but, like Chelex-100, require conditioning between samples. In contrast, the alternative, CPG support, which does not change in volume with changing solution composition, has been shown to be effective in on-line matrix studies.[7]

The location of the separation column in the flow manifold is an important factor in on-line matrix separation studies. In a system developed by Bloxham *et al.*[7], in which ICP-MS detection was used, the column was incorporated in the flow stream and a

separate valve was used to direct the matrix to waste downstream from it. Alternatively, it has been suggested that by incorporating the column within a loop across one valve, to allow direct switching between the sample and eluent streams, the need for a separate, additional valve can be circumvented.[8] Unfortunately, this arrangement allows some matrix to pass into the detector on switching the valve but if the connecting tubing is sufficiently short the residual matrix is kept to an acceptably low level. This design facilitates counter current elution of retained analytes which yields sharper, less dispersed peaks and gives a corresponding reduction in analysis time.

In this chapter, a rapid, automated on-line matrix separation system for ICP-MS, using a flow injection manifold is described. The manifold incorporates a mini-column of a new CPG based IDA resin, PROSEP® Chelating-1, for the determination of several trace elements in saline samples. This novel material combines the advantages of a CPG support with the efficient chelating performance of the IDA ligand. The immobilised chelate is contained within a glass mini-column, located in the loop of an automatic Teflon dual 6 port valve. The retained analytes are eluted counter current to the sample flow to minimise peak dispersion. The system described is based on fixed volume injection, rather than time based sampling, and gives a 10-fold preconcentration in addition to the required matrix separation, for a 3 ml sample volume. Data from the transient eluted peaks was collected using the peak jumping data acquisition mode of the instrument, with a suitable uptake delay to allow the eluted sample to travel from the column to the plasma. A full optimisation of the system is described, including recovery and capacity results. Validation of the procedure using coastal seawater (CASS-2) and estuarine water (SLEW-1) certified reference materials is also presented.

4.3 Preliminary investigation using a preliminary manifold design.

4.3.1 Experimental.

4.3.1.1 Reagents.

The CPG-IDA material, PROSEP Chelating-1 (Bioprocessing Ltd, Medomsley Road, Consett, Durham) was used as supplied. The preparation and structure of this material are commercially sensitive so cannot be described herein. Calibration solutions were prepared from elemental stock solutions (1000μg ml^{-1}, SpectrosoL, BDH, Poole, Dorset, UK). Ammonium acetate buffer (Sigma, Poole, Dorset, UK) was prepared from the solid and purified on-line during analysis. Adjustment to the buffer pH was made using glacial acetic acid or aqueous ammonia as appropriate. Measurement of pH was made using a portable pH probe (Piccolo 2, Hanna Instruments, Kings Langley, Herts, UK). Artificial sea water (Instant Ocean, Aquarium Systems, Mentor, Ohio, USA) was prepared by dissolving approximately 330 g of the powder in 10 litres of water. High purity de-ionized water (18MΩcm resistivity) (Elgastat UHQ PS, Elga, High Wycombe, Bucks, UK) was used throughout.

4.3.1.2 Instrumentation

As with the work described in Chapter 3, the ICP-MS measurements were made using a VG PlasmaQuad 2 Plus (VG Elemental, Winsford, Cheshire, UK). The instrument was calibrated before use, using a tune solution containing the elements Be, Co, Y, La, Eu and Bi at 10 ng ml^{-1} in 5% nitric acid. The transient analyte peaks were acquired in peak

jump mode, by first setting an uptake delay to allow for the injected sample to reach the plasma, then collecting data across the peak. The instrument operating parameters are given in Table 4.1. The matrix separation process was automated using a PrepLab liquid handling system (VG Elemental, Winsford, Cheshire, UK). This device was equipped with two peristaltic pumps, two switching valves (of the pinch valve type) and a Teflon dual 6-port injection valve.

Table 4.1 Operating parameters for the ICP-MS.

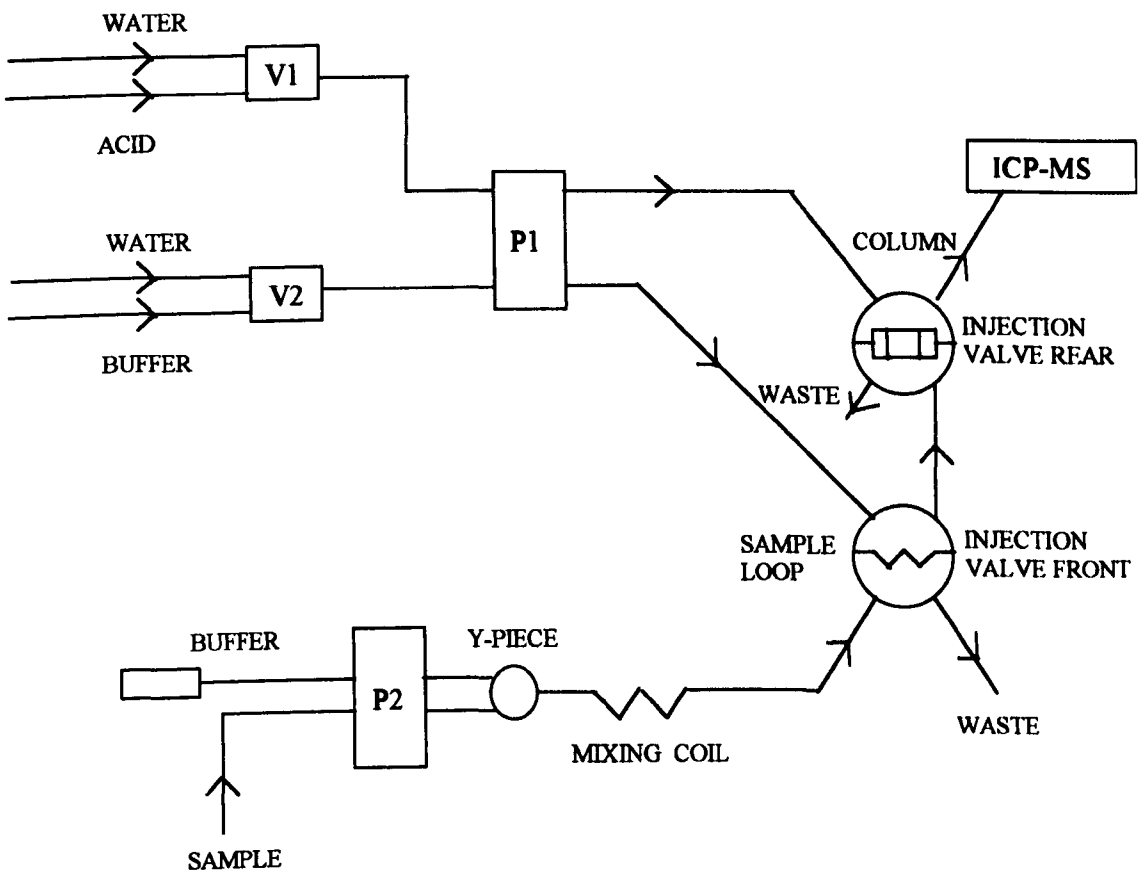
Instrument:	VG PlasmaQuad 2 Plus	
Forward power:	1350 W	Reflected power: < 5 W
Gas flow rates:	Outer gas:	13.0 l min^{-1}
	Intermediate gas:	1.0 l min^{-1}
	Aerosol gas:	0.94 l min^{-1}
Nebuliser:	De Galan type	
Spray chamber:	Glass, water cooled, 10°C	
Mode of data acquisition:	Peak jumping, 1 point per peak	
Dwell time:	10.24 ms	
Uptake delay:	18 s	
Acquisition time:	35 s	
Selected isotopes:	^{48}Ti, ^{51}V, ^{55}Mn, ^{59}Co, ^{60}Ni, ^{63}Cu, ^{64}Zn, ^{65}Cu, ^{107}Ag, ^{114}Cd, ^{140}Ce, ^{208}Pb	

4.3.1.3 Matrix separation procedure.

The manifold used in this study is illustrated in Figure 4.2. It incorporated a glass mini-column (2 cm x 3 mm i.d.) (Omnifit, Cambridge, UK), packed with the PROSEP IDA material located in a 3 ml volume loop across the Teflon dual 6 port valve (injection valve) on the PrepLab. The PrepLab was used to mix the sample on-line with buffer

before loading it into the sample loop, using a peristaltic pump (P2). The contents of this loop were then automatically injected onto the column, allowing the matrix and buffer to pass to waste. A post sample buffer wash was passed through the column via valve 2 (V2) to ensure that any residual matrix was removed, using a second peristaltic pump (P1). This was followed by a water wash via V2 for 1 min, to ensure that residual buffer was eluted from the system. Finally, the retained elements were eluted into the ICP-MS via valve 1 (V1) in the opposite direction to the matrix separation flow, thereby yielding sharp elution peaks.

Figure 4.2. The original automated matrix separation manifold.



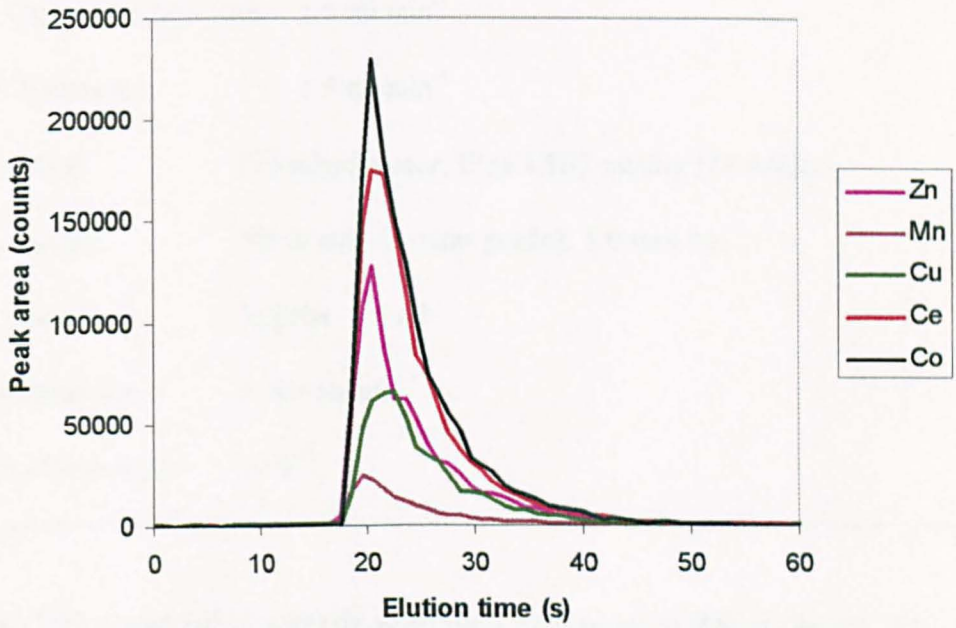
V1 - switch valve 1, V2 - switch valve 2

P1 - peristaltic pump 1, P2 - peristaltic pump 2

With this manifold design, a preconcentration factor of approximately 3 was obtained although the capacity of the material is sufficient to allow a much larger preconcentration. This would, however, lead to an increase in analysis time.

A typical example of the peak elution profiles obtained with this system is illustrated below (Figure 4.3).

Figure 4.3. Typical peak elution profile obtained with the system.



4.4 Results and Discussion.

4.4.1 Operating conditions for matrix separation.

The conditions used in this preliminary study are given in Table 4.2. At this stage of the study the system was not fully optimised. An ammonium acetate buffer at pH 5.5 was selected as this has been shown to be effective for the quantitative retention of a range of elements on IDA based resins.[9]

Table 4.2. Conditions selected for matrix separation using the PROSEP® Chelating-1 column.

Sample loop volume:	3 ml (i.e. 1.5 ml sample + 1.5 ml buffer)
Buffer conditions:	
On-line buffering:	Ammonium acetate, 2.0 mol dm^{-3}, pH 5.5, 1.5 ml min^{-1}
Post sample buffering:	Ammonium acetate, 1.0 mol dm^{-3}, pH 5.5, 0.5 ml
Matrix separation flow rate:	1.5 ml min^{-1}
Elution flow rate:	1.5 ml min^{-1}
Column wash:	Deionised water, Elga UHQ quality (18 MΩcm)
Column eluent:	Nitric acid (Aristar grade), 1.0 mol dm^{-3}
Eluent volume	Approx. 0.3 ml
Total analysis time:	6 min $sample^{-1}$
Sampling frequency:	10 h^{-1}

The manifold design led to a matrix separation flow equal to that of elution. For ICP-MS, typical sample uptake rates are between 0.7 and 1.5 ml min^{-1} and this dictated the choice of elution flow rate and hence that of matrix separation.

4.4.2 Analytical features of the manifold.

In Chapter 3, section 3.5.2, a selection of parameters assessing the fundamental features (according to Fang[10]) of the CPG-8-HQ manual on-line matrix separation system were presented. For the initial manifold design used in this study, the values of these parameters are tabulated below (Table 4.3).

Table 4.3 Analytical features of the automated manifold design used in this initial study.

Feature	Equation	Measured value
Enrichment factor, EF	$EF = C_e/C_s$	≈3
Concentration efficiency, CE	CE = EF x (f/60)	0.5
Consumptive index, CI	$CI = V_s/EF$	0.5

From the above table, it is clear that the initial design of the automated matrix separation manifold had a low EF value, reflecting the small preconcentration factor achieved with the system. Since the system was coupled to a highly sensitive ICP-MS detector, large analyte enrichment factors were not necessary, so the low EF value was not considered to be a limitation. The CE value measured for this system was lower than that obtained for the manual system described in chapter 3 which reflected the lower sample throughput of the latter system (10 samples h^{-1} compared to 15 samples h^{-1} for the manual system). Nonetheless, this sampling rate was still faster than that achieved in equivalent systems by other workers.[7,11] The relatively low CI value shows that this initial design of the automated matrix separation system was economical in sample usage.

4.4.3 Evaluation of the matrix separation efficiency.

The influence of residual matrix elements on the column following matrix separation was evaluated in terms of the ^{63}Cu : ^{65}Cu ratio as described previously (Chapter 3, section 3.5.5). Residual sodium in the eluted sample leads to the formation of $^{40}Ar^{23}Na^{+}$ in the plasma which overlaps with ^{63}Cu, giving an erroneously high result for this element. Gradual deposition of the residual salt on the sampler and skimmer cones also leads to a

continuous upward drift in the ^{63}Cu response, even if flow injection sample introduction is used. Table 4.4 illustrates that residual matrix problems did not arise in this study.

Table 4.4 Effect of residual matrix species on the measured ^{63}Cu : ^{65}Cu ratio.

Sample description	^{63}Cu : ^{65}Cu ratio#
Natural ^{63}Cu : ^{65}Cu ratio, accepted value	2.24
Cu in sea water (10 ng ml^{-1}), injected	5.31 ± 0.79
Cu in 2 mol dm^{-3} HNO_3 (50 ng ml^{-1}), injected	2.19 ± 0.09
Cu matrix separated from spiked pure water (5 ng ml^{-1})	2.09 ± 0.10
Cu matrix separated from spiked sea water (5 ng ml^{-1})	2.17 ± 0.12
Estuarine river water (~1.5% salinity), matrix separated	2.10 ± 0.04
Industrial process sample, matrix separated	2.11 ± 0.14

Values quoted with uncertainty (2 standard deviations, 95% confidence limit, n = 5), except natural ratio. For all real samples, n = 2.

The accepted ^{63}Cu:^{65}Cu ratio is slightly greater than those measured in this study because of mass discrimination effects and mass bias, due to sequential scanning of the transient peak. The ICP-MS instrument used in this work was shown to give a negative mass bias of -0.02 (see section 3.5.5).

4.4.4 Capacity and recovery evaluation for copper.

The capacity of PROSEP® Chelating-1 was evaluated for Cu, under batch conditions, using the compromise matrix separation pH and buffer conditions described in Table 4.2.

A result of 0.5 mmol g^{-1} for Cu was obtained, which is significantly greater than has been reported for the 8-hydroxyquinoline analogue of this material.[12] This capacity is sufficiently high to allow high preconcentration factors of over 100, even with the mini-column arrangement. For Cu, a recovery of 83% was obtained under the compromise pH conditions used. A more comprehensive recovery and capacity study was subsequently undertaken (see section 4.7.2).

4.4.5 Calibration and analysis of effluent samples.

Multi-element calibration solutions were prepared across the range 0 - 10 ng ml^{-1} in both pure water and artificial sea water matrices. Both sets of solutions were passed through the matrix separation manifold. Five repeat analyses were made for each solution. The results for both matrices compared well, as illustrated in Table 4.5, thereby facilitating the use of simple water external calibration for saline water analysis.

Table 4.5. Comparison of calibrations from pure and saline water samples.

Calibrations from spiked pure water					
Parameter	Mn	Co	Cu	Ce	Cd
RSD (%) at 5 ng ml^{-1} (n = 5)	2.5	2.6	3.9	1.8	4.5
Correlation coefficient, r	0.9990	0.9998	0.9982	0.9983	0.9965
Sensitivity (counts ng^{-1} $ml/10^5$)	0.02	0.18	0.09	0.16	0.03
Detection limit (3 s) (ng ml^{-1})	0.32	0.002	0.20	0.05	0.11

Table 4.5. continued.

Calibrations from spiked synthetic sea water					
Parameter	Mn	Co	Cu	Ce	Cd
RSD (%) at 5 ng ml^{-1} (n = 5)	1.6	1.1	1.6	2.2	2.7
Correlation coefficient, r	0.9970	0.9994	0.9889	0.9981	0.9981
Sensitivity (counts ng^{-1} $ml/10^5$)	0.02	0.17	0.11	0.16	0.03
Detection limit (3 s) (ng ml^{-1})	0.30	0.03	0.64	0.18	0.08

To validate this preliminary study, two independently analysed effluent samples were analysed and the results compared (Table 4.6).

Table 4.6 Analysis results for two saline industrial effluent samples.

	Sample 1		Sample 2	
Element	A	B	A	B
V	38	29	316	290
Co	117	110	624	621
Ni	20	<20	223	178
Cu	183	180	142	141
Mn	1399	1400	482	461

Results in ng ml^{-1}, based on 5 repeat measurements of each sample.

A = results obtained in this work, B = independent analysis results

The results in Table 4.6 show that reasonable agreement was obtained between the two data sets, particularly for Mn and Cu. The elements shown in Table 4.6 were the only analytes for which independent data were given, so data for other elements are not presented here. The independent analysis had been performed by simple dilution of the

samples prior to direct analysis by ICP-MS. The dilution methodology degraded the detection limits for some of the analytes of interest (notably Ni) and, because significant levels of Na were still present, analysis of ^{63}Cu was impractical. Furthermore, prolonged exposure of the sampling cones to even a diluted sample of this type would result in salting up, ionisation suppression and signal drift. Matrix separation was therefore considered to be the most effective approach to use for this analysis.

4.5 Conclusions.

The PROSEP IDA immobilised chelate was shown to be effective for the separation of V, Mn, Co, Ni, Cu, Zn, Ce, Cd and Pb from saline matrix samples. Comparable linear calibrations were obtained from both pure water and sea water matrices, illustrating that simple water matrix external calibration could be used for quantifying analytes in saline samples. A sampling rate of 10 per hour was achieved. Efficient matrix separation was demonstrated by the unaltered ^{63}Cu:^{65}Cu ratio measured for saline samples after matrix separation. Trace analytes measured in two effluent samples compared well with the results of an independent analysis, illustrating the practical application of the system.

4.6 Redesign of the flow manifold and detailed performance evaluation.

The initial manifold design employed in this study (Figure 4.2), although effective in its operation, was noted to have a few drawbacks. Firstly, with this manifold design, a 1:1 dilution of the sample with buffer occurred during loading, as the sample was mixed with buffer on-line prior to entering the sample loop. This dilution compromised the advantage of the preconcentration factor of the system. However, for samples containing relatively high concentrations of analytes which have a strong affinity for the

chelating reagent (e.g. Cu), suppression of the retention of other analytes present at much lower concentrations can occur. The dilution factor introduced during sample loop loading with this manifold design could therefore be beneficial in some analyses, since it would reduce the potential of suppressive interference problems during matrix separation (see Chapter 5 of this thesis). Secondly, the relatively large size of the main sample loop volume (3 ml) lead to considerable dispersion of the sample slug as it passed from this loop to the column, leading directly to an increase in the analysis time. For these reasons, the original manifold design was modified to (a) remove the dilution factor, (b) minimise dispersion to increase the sample throughput and (c) improve the limit of detection. The performance of the modified manifold was thoroughly evaluated.

4.6.1 Experimental.

4.6.1.1 Reagents.

The reagents used were the same as described previously (section 4.3.1.1).

4.6.1.2 Instrumentation.

The ICP-MS measurements were made using a VG PlasmaQuad 2 Plus instrument (VG Elemental, Winsford, Cheshire, UK). As with the initial manifold studies, the automated on-line matrix separation procedure was performed using the PrepLab liquid handling system (VG Elemental). Capacity evaluation measurements were made using a Perkin-Elmer Plasma 40 ICP-AES instrument, the operating parameters of which are given in Table 4.7(a) and 4.7(b).

Table 4.7(a). Operating parameters for the ICP-AES instrument.

ICP instrument.
Aerosol gas flow rate (l min^{-1}): approx. 0.75
Intermediate gas flow rate (l min^{-1}): 0.6
Outer gas flow (l min^{-1}): 12
Nebuliser: Cross flow
Spray chamber: Ryton

Table 4.7(b). ICP emission wavelengths.

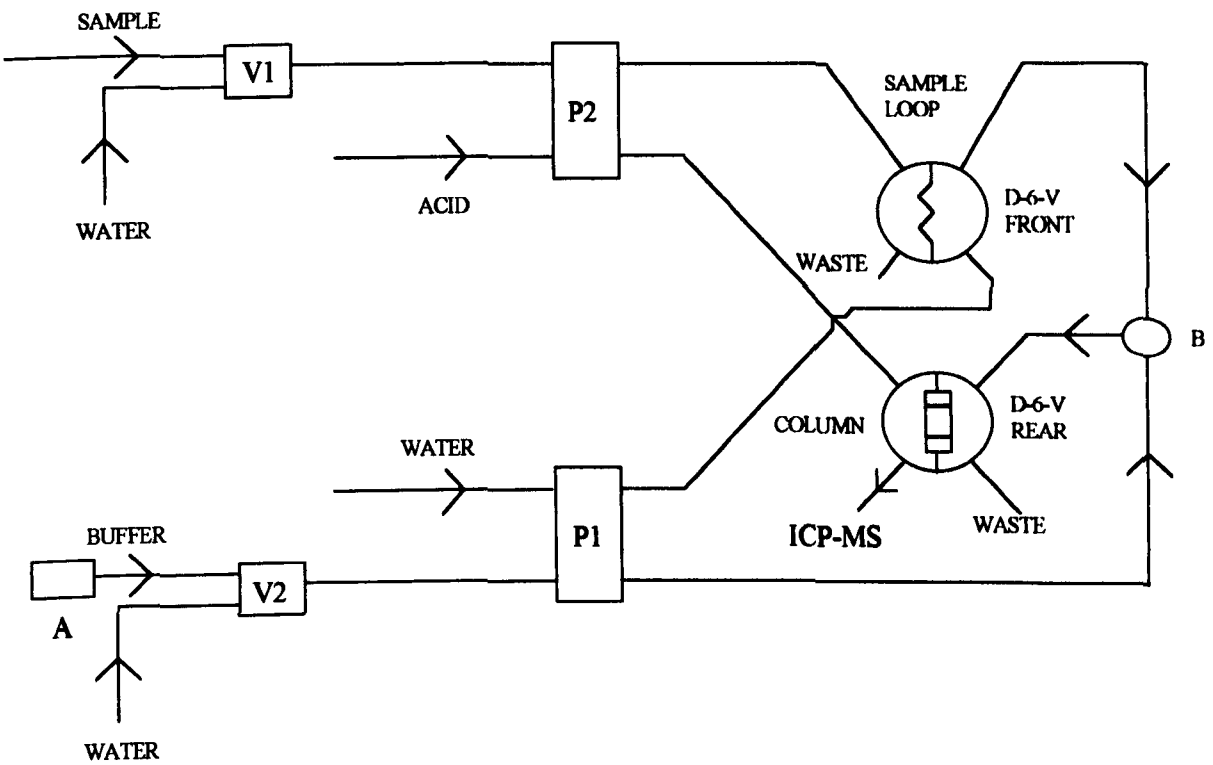
Element	Wavelength (nm)	Element	Wavelength (nm)
V	309.311	Cu	324.754
Cr	205.552	Zn	213.856
Mn	257.610	Cd	214.438
Fe	238.204	Ce	413.765
Co	238.892	Pb	220.353
Ni	221.647	U	385.958

4.6.1.3 The modified matrix separation manifold

The modified automated matrix separation manifold is illustrated in Figure 4.4. The sample loop (3 ml) was located across the front of the dual 6 port Teflon valve (injection valve) on the Preplab unit. The PROSEP® Chelating-1 material was contained within a glass mini-column (2.5 cm x 3 mm i.d., Omnifit, Cambridge, UK) incorporated in a loop

across the rear of the injection valve. All the manifold connections were made using 0.8 mm i.d. PTFE tubing. The reagents were pumped using the two peristaltic pumps on the Preplab. With the configuration used, the matrix separation flow rate could be varied but the eluent flow rate was fixed at 1.5 ml min^{-1}. Using the two valves supplied on the Preplab, both the sample uptake line and on-line buffer streams could be switched to water to rinse the sample loop between samples and the column after sample loading.

Figure 4.4. The modified automated matrix separation manifold.



V1 - switch valve 1, V2 - switch valve 2

P1 - peristaltic pump 1, P2 - peristaltic pump 2

4.6.1.4 Matrix separation procedure.

The automated matrix separation procedure began by opening the injection valve and valves 1 and 2 (V1 and V2), then starting peristaltic pump 2 (P2). This allowed sample

to pass into the sample loop and also eluent acid to pass as a continuous stream through the chelating column. After a period of 50 seconds, peristaltic pump 1 (P1) was started, to pump buffer through to the injection valve, via an on-line purification column containing Chelex-100 (A on Figure 4.4). After a further 30s, buffer had reached the injection valve and the sample loop was full. At this point, V1 closed followed immediately by the injection valve. Closing V1 stopped the sample uptake by switching to water and closing the injection valve allowed the sample loop contents to be eluted by water, then mixed with the buffer (at point B) and finally passed through the chelating column. In this way, trace elements in the buffered sample were retained on the column and the matrix and buffer species passed to waste. Once the sample had passed into the column, V2 switched from buffer to water to allow residual buffer and matrix species in the system to be rinsed to waste. After this rinse period, the injection valve and V1 were switched back to open. This allowed acid back through the column thereby eluting the retained analytes into the ICP-MS and simultaneously enabled a new sample to load during the ICP-MS analysis period. As the ICP-MS analysis time was similar to the initial 50s sample loading period, the latter could be skipped for subsequent repeats on the same sample by incorporation of a loop command in the Preplab program, thereby decreasing the total analysis time.

4.6.1.5 Analytical features of the modified automated manifold design.

In section 4.4.2, some of the fundamental parameters assessing the performance the initial design of the automated on-line matrix separation system were presented. For the modified manifold design, the values of these parameters were found to be as follows (Table 4.8).

Table 4.8 Analytical features of the automated manifold design used in this initial study.

Feature	Equation	Measured value
Enrichment factor, EF	$EF = C_e/C_s$	≈10
Concentration efficiency, CE	CE = EF x (f/60)	2
Consumptive index, CI	$CI = V_s/EF$	0.3

The results in the above table show that the modified automated matrix separation manifold design gave an increased EF value (EF = 10) compared to that of the initial design (EF = 3). The modified system therefore achieved an increased degree of preconcentration. The CE value of this system was increased relative to both the initial manifold design and the manual matrix separation system described in Chapter 3, for which CE values of 0.5 and 1.25 were obtained, respectively. This result showed that the redesigned automated manifold offered a significant improvement in the matrix separation efficiency compared to the previous two systems. Finally, the relatively low CI value (CI = 0.3) shows that the modified automated matrix separation system yielded a similar sample consumption efficiency to the initial manifold design (CI = 0.5).

4.6.1.6 Determination of exchange capacity and recovery.

The capacity of PROSEP® Chelating-1 was determined for a range of elements, using a batch method. Solutions of the selected analytes (200 μg ml^{-1}) were prepared in water (10 ml), then added to ammonium acetate buffer (1.5 mol dm^{-3}, 10 ml). Each solution was adjusted to the optimum pH for retention by the chelating reagent (generally pH 6.5, except for Cr and Mn, for which pH 8 was the optimum (see section 4.7.1)) and added

to 0.05 g of the dry chelating material. The mixtures were shaken and left to equilibrate overnight. The reduced concentration of each analyte in the supernatant solution was then measured versus the original concentration, using ICP-AES. The capacity of the material for each element was evaluated using equation 4.1.

$$C = (c_i - c_f)v / [m*1000*M_r] \qquad \text{(equation 4.1)}$$

where C is the capacity (in mmol g^{-1}), c_i and c_f are the concentrations of the element before and after equilibrium (in $\mu g\ ml^{-1}$), M_r is the relative atomic mass of the element (in $g\ mol^{-1}$) and v is the volume of solution (in ml) equilibrated with a mass m (in g) of PROSEP® Chelating-1. Recoveries for a range of elements were evaluated dynamically, for both a fresh and an aged column of PROSEP® Chelating-1, by passing an artificial sea water solution spiked with the selected analytes at 10 ng ml^{-1} through the automated flow injection manifold. The aged column had been in use for approximately 240 hours before the recovery study. A manual valve (Rheodyne 5020, Supelco, Poole, Dorset, UK), fitted with a 0.3 ml loop, was incorporated into the eluent acid stream of the manifold. Since the manifold was fitted with a 3 ml sample loop, a maximum preconcentration factor of 10 (assuming 100% analyte retention by the chelating material) could be achieved. Using this configuration the eluent acid, instead of being pumped continuously, was injected as a known, fixed volume via the manual valve into a water carrier stream. Recoveries were evaluated by comparing samples eluted from the column with a 100 ng ml^{-1} solution, prepared in the eluent matrix and injected via the manual valve.

4.7 Results and Discussion.

4.7.1 Optimisation of the matrix separation procedure.

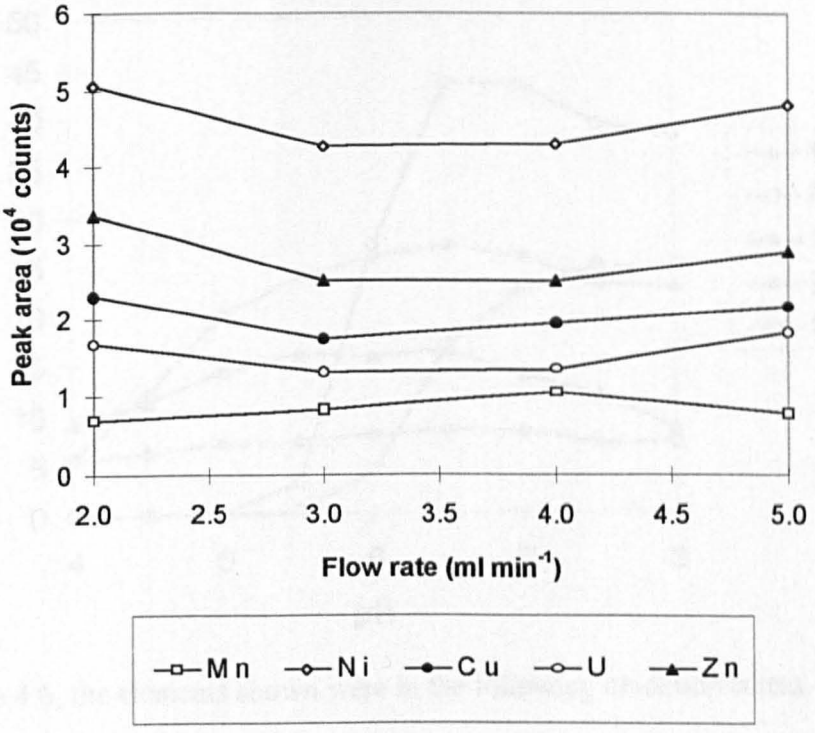
The procedure was optimised with respect to the matrix separation flow rate, buffer pH, buffer concentration and eluent acid concentration, using a univariate approach. For the optimisation procedure, samples were prepared by spiking a synthetic sea water solution with the selected analytes (10 ng ml^{-1}), followed by acidification with 0.1ml of concentrated nitric acid to 100 ml of sample. Acidification was performed to prevent trace element loss from solution by adsorption or precipitation. The complete set of optimum conditions are given in Table 4.9.

Table 4.9 Optimum conditions for matrix separation for the PROSEP® Chelating-1 column.

Sample loop volume:	3 ml
Buffer conditions:	Ammonium acetate, 1.5 mol dm^{-3}, pH 6.5
Matrix separation flow rate:	5.0 ml min^{-1}
Elution flow rate:	1.5 ml min^{-1}
Column wash:	Deionised water, Elga UHQ quality (18 MΩcm)
Column eluent:	Nitric acid (Aristar grade), 0.5 mol dm^{-3}:
Eluent volume:	Approx. 0.3 ml
Total analysis time:	5 min $sample^{-1}$

The effect of flow rate on the matrix separation procedure is illustrated for selected elements in Figure 4.5.

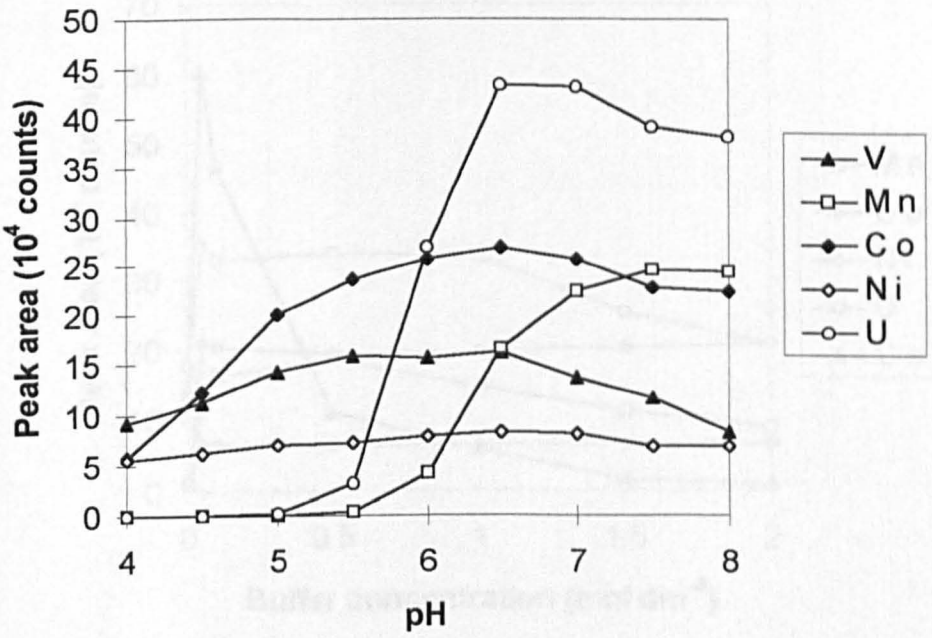
Figure 4.5 Effect of the matrix separation flow rate on analyte retention.



In Figure 4.5, the elements shown were in the following oxidation states; Mn^{2+}, Ni^{2+}, Cu^{2+}, U (as UO_2^{+}) and Zn^{2+}. Similar results were obtained for V (as VO^{2+}), Co^{2+}, Ce^{3+}, Cd^{2+}, Ag^{+} and Pb^{2+}, but these are not shown in Figure 4.5 for clarity reasons. Up to a flow rate of 5 ml min^{-1}, no significant change in response was observed for the elements determined. Faster flow rates than 5 ml min^{-1} introduced back pressure problems and slower rates lead to increased analysis time. Therefore 5 ml min^{-1} was selected for the rest of this study.

The response of the column to selected analytes with changing buffer pH is illustrated in Figure 4.6.

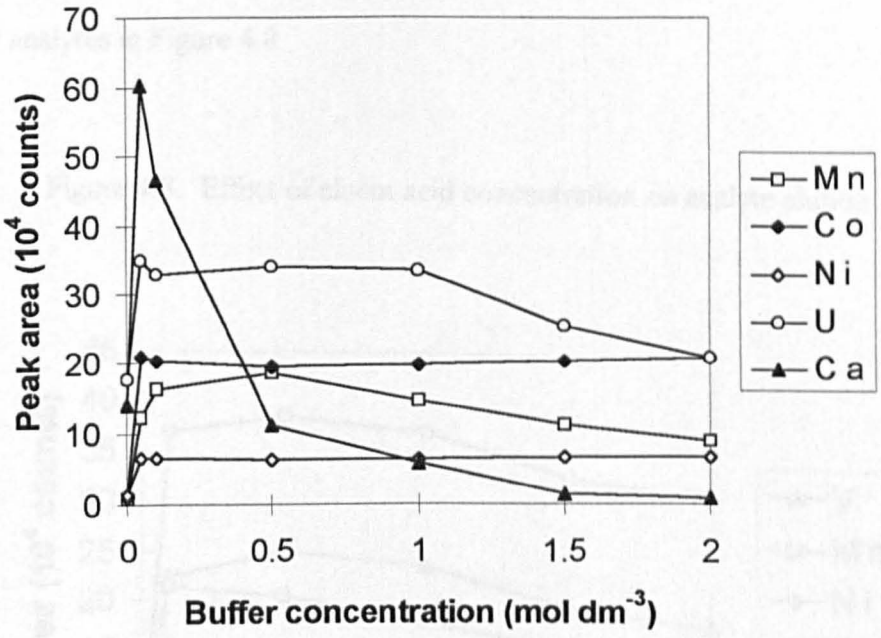
Figure 4.6 Effect of buffer pH on analyte retention.



In Figure 4.6, the elements shown were in the following oxidation states; V (as VO^{2+}), Mn^{2+}, Co^{2+}, Ni^{2+}, and U (as UO_2^{+}). Data were also obtained for Cu^{2+}, Zn^{2+}, Ag^{+}, Ce^{3+}, Cd^{2+} and Pb^{2+} but these are not shown in Figure 4.6 for clarity reasons. The pH behaviour of Zn, Ag, Ce and Cd was similar to that of Ni, whereas the pH response of Cu resembled that of V. Since the optimum buffer pH for matrix separation varies between elements a compromise pH must be selected for multi-element analysis. On the basis of the element responses shown in Figure 4.6, pH 6.5 was chosen, as values below this gave decreased retention of some analytes. Values above this level gave lower buffer capacity (up to pH 8) and also lead to increased retention of Ca, as the PROSEP® Chelating-1 has some affinity for this element.

The effect of the ammonium acetate buffer concentration on the retention of selected elements is illustrated in Figure 4.7.

Figure 4.7 Effect of buffer concentration on analyte retention.

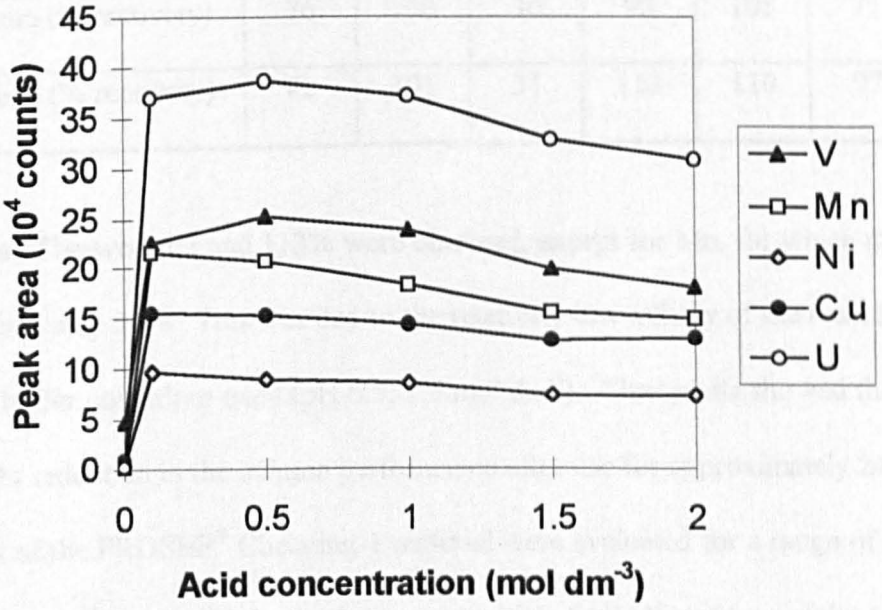


In Figure 4.7, the elements shown were in the following oxidation states; Mn^{2+}, Co^{2+}, Ni^{2+}, Pb^{2+}, U (as UO_2^{+}) and Ca^{2+}. Data were also obtained V (as VO^{2+}), Cu^{2+}, Zn^{2+}, Ag^{+} Cd^{2+} and Ce^{3+} but these are not shown in Figure 4.7 for clarity reasons. For all these elements the effect of buffer concentration was analogous to that of Co. The results show that the buffering process is effective for many elements at a concentration of 0.05 mol dm^{-3}. However, ionogenic retention of Ca at this level is high because the buffer cation concentration is insufficient to completely displace this element. Retention of ^{48}Ca interferes with ^{48}Ti and, at zero buffer concentration, ^{63}Cu and ^{51}V are also increased because of the formation of $^{40}Ar^{23}Na$ and $^{35}Cl^{16}O$ in the plasma with residual Na and Cl respectively. It was found that a buffer concentration of 1.5 mol dm^{-3} was

required to remove the Ca problem. This high concentration did not affect retention of the selected analytes, except Mn and U (as UO_2^+). These two elements show similar behaviour to Ca on PROSEP® Chelating-1 which is expected from the relative positions of these three metals in the Irving - Williams series.[13]

The effect on analyte elution with increasing eluent acid concentration is illustrated for selected analytes in Figure 4.8.

Figure 4.8. Effect of eluent acid concentration on analyte elution.



In Figure 4.8, the elements shown were in the following oxidation states; V (as VO^{2+}), Mn^{2+}, Ni^{2+}, Cu^{2+} and U (as UO_2^+). Data were also obtained for Co^{2+}, Zn^{2+}, Ag^+ Ce^{3+}, Cd^{2+} and Pb^{2+} but these are not shown in Figure 4.8 for clarity reasons. The effect of increasing the acid concentration on elution of these elements was similar to the response measured for Ni. In this study, quantitative elution was observed for all the elements investigated at an acid concentration of 0.5 mol dm^{-3}. Acid concentrations greater than 2.0 mol dm^{-3} were not investigated as these could degrade the column.

4.7.2 Recovery and capacity measurements for PROSEP® Chelating-1.

To determine the effect on the column of repeated use, a second column was prepared from the same batch of PROSEP® Chelating-1 and the two columns compared in terms of element recoveries (Table 4.10).

Table 4.10 Analyte recovery from a fresh and an aged column of PROSEP® Chelating-1.

Element	Ti	V	Mn	Co	Ni	Cu	U
Aged column (% recovery)	76	100	30	98	105	71	62
Fresh column (% recovery)	82	101	31	113	110	97	61

Recoveries of between 62 and 113% were obtained, except for Mn, for which the recovery was only 30%. This was due to the relatively low affinity of the resin for Mn under the buffer conditions used (pH 6.5, 1.5 mol dm^{-3}). The results showed that there was a slight reduction in the column performance after use for approximately 240 hours. Capacities of the PROSEP® Chelating-1 material were evaluated for a range of elements (Table 4.11) and found to be lower (by approximately 66%) than those of the polymeric based IDA resin, Chelex-100.[14] This is expected as the rigid CPG support cannot expand to allow greater access to the complexation sites. Nonetheless, the capacity of PROSEP® Chelating-1 is still more than sufficient for use in trace element studies.

Table 4.11. Measured capacity values for PROSEP® Chelating-1.

Element	Capacity (mmol g^{-1})	Element	Capacity (mmol g^{-1})
V	0.22	Cu	0.11
Cr	0.10	Zn	0.13
Mn	0.14	Cd	0.10
Fe	0.29	Ce	0.10
Co	0.13	Pb	0.13
Ni	0.11	U	0.04

4.7.3 Evaluation of the effect of residual matrix species.

In Chapter 3, section 3.5.5, a procedure for evaluating the influence of residual matrix on the ICP-MS results, using the ^{63}Cu : ^{65}Cu isotope ratio was described. This procedure was based on the observation that the ^{63}Cu : ^{65}Cu isotope ratio is anomalously high if Na is present in the plasma, due to polyatomic overlap of $^{40}Ar^{23}Na$ on ^{63}Cu. However, this method of identifying a residual matrix problem does not cover the possible interference of $^{33}S^{16}O_2^+$ on ^{65}Cu, which could yield a low ^{63}Cu : ^{65}Cu ratio. In this work, this potential inaccuracy was addressed by calculating data for the ^{48}Ti : ^{49}Ti ratio, in addition to the ^{63}Cu : ^{65}Cu ratio, to clarify the efficiency of the matrix separation procedure. The ^{48}Ti : ^{49}Ti ratio clearly identifies the effect of residual Ca on the process by virtue of the ^{48}Ca / ^{48}Ti isobaric overlap. Like ^{65}Cu, ^{49}Ti can be affected by a sulphur interference, $^{33}S^{16}O$, but since ^{33}S has a natural abundance of only 0.75%, all ^{33}S based interferences will be low. Furthermore, since the sulphate present in the sea water samples is not retained by the column, this source of interference should be negligible. Table 4.12

illustrates that residual matrix does not affect the isotope ratio measurement for ^{63}Cu : ^{65}Cu or ^{48}Ti : ^{49}Ti thereby illustrating that the matrix separation procedure is effective, with respect to both Na and Ca removal. The results presented for an injected artificial sea water sample (Table 4.12) clearly show a severe increase for both the ^{63}Cu : ^{65}Cu and ^{48}Ti : ^{49}Ti ratios, illustrating that use of flow injection, although effective in reducing blockage problems with high salt samples, cannot remove the associated polyatomic interferences. Matrix separation is therefore essential if accurate results are to be obtained.

Table 4.12. Effect of residual matrix species on the measured ^{63}Cu : ^{65}Cu and ^{48}Ti : ^{49}Ti isotope ratios.

Sample description	^{63}Cu : ^{65}Cu ratio*	^{48}Ti : ^{49}Ti ratio*
Natural ratio, accepted value	2.24	13.42
Sea water (10 ng ml^{-1}), 300 µl injected	5.55 ± 3.07	64.04 ± 5.43
0.5 mol dm^{-3} HNO_3 (10 ng ml^{-1}), injected	2.22 ± 0.10	13.45 ± 0.57
Matrix separation from spiked pure water (5 ng ml^{-1})	2.21 ± 0.05	13.42 ± 0.88
Matrix separation from spiked sea water (5 ng ml^{-1})	2.17 ± 0.12	13.45 ± 0.47

* values given with range of ± 2 standard deviations (5 replicates), except natural ratios.

4.7.4 Calibration and analysis of certified reference materials.

Using the ICP-MS, linear calibrations were obtained over the range 0 to 10 ng ml^{-1} for analytes in both spiked pure water and spiked synthetic sea water matrices, as described

in Table 4.13. The two calibration sets compared well therefore validating the use of simple pure water calibration solutions for quantifying analytes in more complex matrices.

Table 4.13 Comparison of calibrations obtained from pure and saline water samples.

Calibrations from spiked pure water.					
Parameter	^{55}Mn	^{59}Co	^{63}Cu	^{64}Zn	^{114}Cd
RSD (%) at 5 ng ml^{-1} (n = 5)	4.6	4.0	2.4	3.3	2.2
Correlation coefficient, r	0.9997	0.9998	0.9990	0.9989	0.9998
Sensitivity (counts ng^{-1} ml/10^4)	1.28	4.57	2.31	1.27	1.75
Detection limit (5 s, n = 5) (ng ml^{-1})	0.10	0.02	0.05	0.20	0.09
Calibrations from synthetic sea water.					
Parameter	^{55}Mn	^{59}Co	^{63}Cu	^{64}Zn	^{114}Cd
RSD (%) at 5 ng ml^{-1} (n = 5)	3.2	2.4	2.3	1.1	2.0
Correlation coefficient, r	0.9987	0.9997	0.9999	0.9956	0.9996
Sensitivity (counts ng^{-1} ml/10^4)	1.12	5.14	2.58	1.35	1.77
Detection limit (5 s, n = 5) (ng ml^{-1})	0.56	0.01	0.09	0.44	0.07

The matrix separation procedure was validated by analysis of the two certified reference materials SLEW-1 and CASS-2. The analytes were quantified by calibration against acidified multi-element (Mn, Cu, Zn, Ni, Co, Cd) pure water standards processed through the manifold. Three repeat analyses were made at each concentration and for the reference materials. The calibrations generally showed good linearity with least squares regression coefficients of 0.997 to 0.999 being obtained across the concentration

ranges 0 to 20 ng ml^{-1} (Mn), 0 to 4 ng ml^{-1} (Cu, Ni, Zn) and 0 to 0.1 ng ml^{-1} (Cd, Co). Precisions (measured as relative standard deviation, RSD) for the selected analytes were in the range 0.5 to 5.5 %. Results for the certified reference materials analysis are given in Table 4.14.

Table 4.14 Results for the CRM analysis.

Element	SLEW-1		CASS-2	
	Found*	Certified*	Found*	Certified*
Mn	7.97 ± 0.72	13.1 ± 0.8	1.84 ± 0.07	1.99 ± 0.15
Co	0.040 ± 0.003	0.046 ± 0.007	0.028 ± 0.001	0.025 ± 0.006
Ni	0.751 ± 0.074	0.743 ± 0.078	0.264 ± 0.006	0.298 ± 0.036
Cu	1.72 ± 0.30	1.76 ± 0.09	0.704 ± 0.10	0.675 ± 0.039
Zn	0.74 ± 0.03	0.86 ± 0.15	1.95 ± 0.05	1.97 ± 0.12
Cd	0.015 ± 0.002	0.018 ± 0.003	0.017 ± 0.002	0.019 ± 0.004

* Concentrations in ng ml^{-1}. Uncertainties expressed as 2 standard deviations of the instrument response to each analyte (95% confidence limit, n = 3).

For both materials, good agreement between the found and certified values was obtained for all the elements measured, except for Mn. A consistently low result was obtained for this element in both SLEW-1 and CASS-2. This was interpreted to be a consequence of the low affinity of PROSEP® Chelating-1 for Mn under the matrix separation conditions used (ammonium acetate buffer, 1.5 mol dm^{-3}, pH 6.5) (see Figure 4.5). Direct analysis of the reference materials, for comparison with the matrix separation procedure, could not be performed because this lead to cone and injector blockage and signal suppression

in the plasma. Injection of saline samples without prior matrix separation circumvented blockage problems, but significant polyatomic interferences and signal suppression remained. These problems, coupled with the low analyte concentrations present, made direct injection impractical for the reference material analysis.

4.8 Conclusions.

The CPG immobilised IDA material, PROSEP® Chelating-1, was shown to be an effective reagent for on-line matrix separation for ICP-MS. An efficient automated flow injection procedure, which facilitated acceptable sample throughput (12 h^{-1}) combined with low sample consumption and a 10 fold preconcentration factor, was developed for the study. Linear calibrations of comparable sensitivity were obtained from both spiked pure water and spiked sea water matrices, illustrating that the procedure was unaffected by high salinity levels. This permitted accurate measurement of Co, Ni, Cu, Zn and Cd in the saline certified reference materials CASS-2 and SLEW-1, using a calibration procedure based on simple water matrix standards processed through the manifold. It was found that accurate measurements of Mn in SLEW-1 could not be made, since this element gave a poor recovery from saline solution. This was believed to be due to matrix interference effects on the formation of the weak Mn-IDA complex. The effect of residual matrix on the process was determined to be negligible by monitoring the effect of $^{40}Ar^{23}Na$ on the ^{63}Cu : ^{65}Cu ratio and ^{48}Ca on the ^{48}Ti : ^{49}Ti ratio. Neither ratio measurement was increased above the expected level, therefore confirming effective matrix removal. Continued use of the PROSEP® Chelating-1 reagent (approximately 240 hours) was shown to lead to only a nominal reduction in column recovery. Batch capacity measurements for a range of elements showed that the reagent was comparable,

and in some cases superior, to CPG - 8-hydroxyquinoline materials, but lower than polymeric based chelators, due to the inflexibility of the CPG support. However, this rigidity, combined with the good chemical stability, physical robustness, rapid surface reactivity and wide elemental application of PROSEP® Chelating-1, made the material a highly effective reagent for the matrix separation procedure undertaken in this study.

4.9 References.

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CHAPTER 5

INTERFERENCE EFFECTS OF DISSOLVED ORGANIC MATTER AND CONCOMITANT ELEMENTS ON MATRIX SEPARATION

5.1 Introduction.

On-line matrix separation and preconcentration is now recognised as a generally effective method for sample pre-treatment prior to analysis by ICP-MS. If the matrix involved only contains high concentrations of elements which either do not chelate or chelate poorly with the immobilised reagent, the technique is ideal. However, problems arise when either high levels of strongly chelating elements, such as Cu, Co and Ni, or natural chelating compounds, arising from dissolved organic matter, are present in the sample.[1,2] Strongly chelating elements swamp the available sites on the immobilised chelate surface and prevent quantitative retention of other elements in the sample, leading to low analyte recovery and hence incorrect analysis results. This difficulty can only be circumvented if the problem elements can be selectively removed prior to the analytical matrix separation. This requires both prior knowledge of the sample composition and a method for selective element removal. Dilution of the sample or use of a large separation column may reduce the problem, but at the expense of analysis time and an increased contamination risk. In the case of natural chelating compounds, competitive reactions will occur, which could lead to a percentage of the analytes of interest passing directly to waste. This would also lead to low analysis results. The influence of natural chelating compounds and strongly chelating elements on trace element preconcentration and matrix separation in on-line systems has, surprisingly, received very little attention. In

studies where ICP-MS has been used as the detector there are, at the time of writing, no literature references which discuss this topic. For these reasons, this chapter will focus on and discuss the effects of the natural chelating organic material, humic acid, and the elements U, Mn, Cu, Co, Fe and Pb on the analyte retention efficiency of the modified matrix separation system presented in Chapter 4, section 4.6.4. The elements Mn and Fe were selected because they are often found at $\mu g\ ml^{-1}$ concentrations in river waters, Cu and Co were chosen as these elements have high complex formation constants with the IDA ligand and by way of contrast, U and Pb were selected as these metals form weaker IDA complexes.

5.2 The nature and properties of dissolved organic matter.

In all natural waters, a broad spectrum of substances are present. These include major elements, trace elements and a variety of organic molecules, ranging from simple species to polyfunctional macromolecules. The organic components in natural waters are grouped together under the general heading of dissolved organic matter (DOM). DOM can be broadly divided into two sub-groups, namely humic and non-humic substances. The non-humic substance category, which comprises organic molecules such as phenols, carbohydrates, proteins and amino acids, is well defined and characterised.[3] Humic substances, on the other hand, are brown-coloured, acidic species with complex, macromolecular structures which are poorly understood and difficult to characterise.[4] Humic substances can be further sub-divided, on the grounds of water solubility and molecular weight, into humic acids and fulvic acids. Fulvic acids are soluble in both alkaline and acidic aqueous solutions, and range in molecular weight from 500 - 1500 g mol^{-1}. In contrast, humic acids are soluble in alkaline aqueous solutions but insoluble in

acidic solutions below pH 2, and range in molecular weight from 1500 - 300,000 g mol^{-1}. In general, fulvic acids make up around 50% of the DOM in natural waters, whereas humic acids contribute only 5 - 10% to the DOM concentration. This level of humic acid equates to a concentration of around 5 to 50 ppm in solution, but even at this low level, the brown coloration caused by these substances is clearly visible. In fact, the intensity of coloration can be used as a means of measuring the level of DOM in natural waters. Humic substances, with their constituent fulvic and humic acids, contain a variety of functional groups including hydroxyl, amino, carboxyl and sulphydryl units, all of which have the ability to form complexes with trace metal elements in aquatic systems. Several observations have been made regarding this complexation ability. Firstly, chemical weathering of relatively insoluble minerals by water generally increases when humic substances are present.[5] This occurs because complexes form between the humic substances and the metal ions of the mineral and these are more soluble than the mineral itself. Secondly, complex formation with humic substances can reduce or enhance the bio-availability of trace metals, which is of importance for plant nutrition.[6] Thirdly, depending on the prevailing conditions of pH, precipitation of some metal - humic acid complexes can occur, leading to effective removal of the trace element from the system.[7] Finally, and perhaps most significantly, it is now well known that complexation of trace metals with these natural chelating molecules can be beneficial, if the elements in question are toxic in their uncomplexed form.[8,9]

The last two points mentioned above highlight a potential problem for trace metal analysis of natural water samples. If accurate, quantitative analysis is required, any material which has precipitated must be mobilised back into solution. For samples where analyte / matrix separation is performed prior to analysis (as described earlier in this thesis), competition may arise between the chelating humic substances present and the

static chelate medium used to retain the analytes of interest. This could lead to low measured values for the target elements. This problem can be exacerbated if the chosen analytes are present in other chemical forms, but this is out of the scope of the current work, so will not be discussed further here.

5.3 Experimental.

5.3.1 Reagents.

The general reagents and matrix separation manifold construction were the same as those described in Chapter 4 (sections 3.1.1 to 3.1.4). Humic acid, as the solid powder (BDH, Poole, Dorset, UK), was used in the interferences study.

5.3.2 Instrumentation.

ICP-MS measurements were made using a VG Elemental PlasmaQuad 2 Plus. The instrument was tuned and calibrated prior to operation using a solution containing Be, Mg, Co, Y, La, Eu and Bi at 10 ng ml^{-1} in 2% nitric acid. The transient analyte peaks were monitored in peak jumping mode, with the resulting peak areas normalised against an internal standard (^{103}Rh at 10 ng ml^{-1}). This was required in order to compensate for instrumental drift and potential suppression effects, due to high concentrations of the selected interference element entering the plasma after elution from the column. The data acquisition and instrument operating parameters used are listed in Table 5.1. The matrix separation procedure was carried out using an automated manifold, controlled by a PrepLab unit supplied by VG Elemental (Winsford, Cheshire, UK).

Table 5.1. Operating parameters for the ICP-MS instrument.

ICP-MS instrument.

Forward power (W): 1350	Reflected power (W): < 5
Aerosol gas flow rate (l min^{-1}): 0.94	Intermediate gas flow rate (l min^{-1}): 1.0
Outer gas flow (l min^{-1}): 13.0	Nebuliser: De Galan type
Spray chamber: Glass, water cooled, 10°C	

Peak jumping acquisition parameters.

Points per peak: 3

Dwell time: 10.24 ms

Detector mode: Pulse counting

Selected isotopes (ICP-MS).

Element: ^{48}Ti, ^{49}Ti, ^{51}V, ^{55}Mn, ^{59}Co, ^{60}Ni, ^{63}Cu, ^{65}Cu, ^{103}Rh, ^{114}Cd, ^{208}Pb, ^{238}U

5.3.3 Matrix separation manifold design and operation.

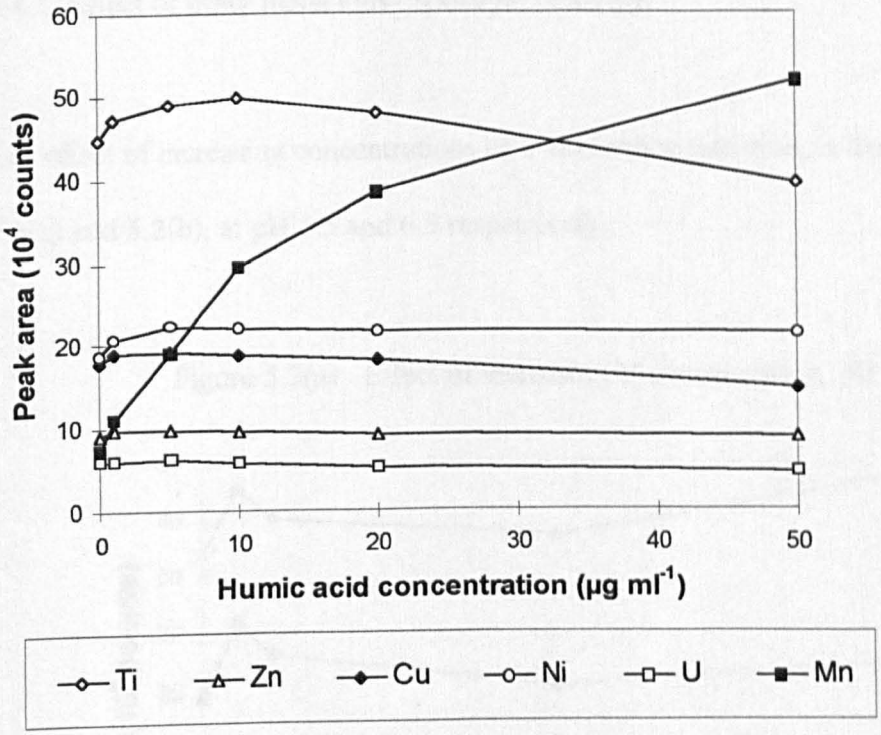
The design and operation of the manifold were the same as described previously (Chapter 4, sections 4.6.1.3 and 4.6.1.4).

5.4 Results and discussion.

5.4.1 Effect of humic acid on analyte recovery.

The effect of increasing concentrations of humic acid (0 - 50 μg ml^{-1}) on analyte retention is presented in Figure 5.1.

Figure 5.1 Effect of humic acid on analyte retention.



In Figure 5.1, the elements shown were in the following oxidation states; Ti^{4+}, Zn^{2+}, Cu^{2+}, Ni^{2+}, U (as UO_2^{+}) and Mn^{2+}. Similar results were obtained for V (as VO^{2+}), Co^{2+}, Ag^{+}, Ce^{3+}, Cd^{2+} and Pb^{2+} but these are not shown in Figure 5.1 for clarity reasons. Most of the selected analytes were found to be unaffected by the presence of up to 50 μg ml^{-1} of humic acid. Even copper, which is known to complex readily with humic acid,[10] showed only a small reduction in retention. This result is likely to be due to the much

stronger chelating affinity of the immobilised IDA ligands compared to the hydroxyl and amino groups of humic acid. Since humic acid is present in natural waters at levels of the order of 1 - 10 μg ml^{-1},[10] it is clear that in this study, real sample analysis is unaffected by the presence of this natural chelate. For Mn an apparent increase in retention with increasing humic acid concentration is observed. This anomaly is most probably caused by residual humic acid in the sample eluted to the plasma, giving rise to the $^{40}Ar^{14}N^{1}H$ polyatomic species which has an isobaric overlap with ^{55}Mn.

5.4.2 Effect of other metal ions on analyte recovery.

The effect of increasing concentrations of U on analyte retention, is illustrated in Figures 5.2(a) and 5.2(b), at pH 5.5 and 6.5 respectively.

Figure 5.2(a). Effect of increasing U concentration, pH 5.5.

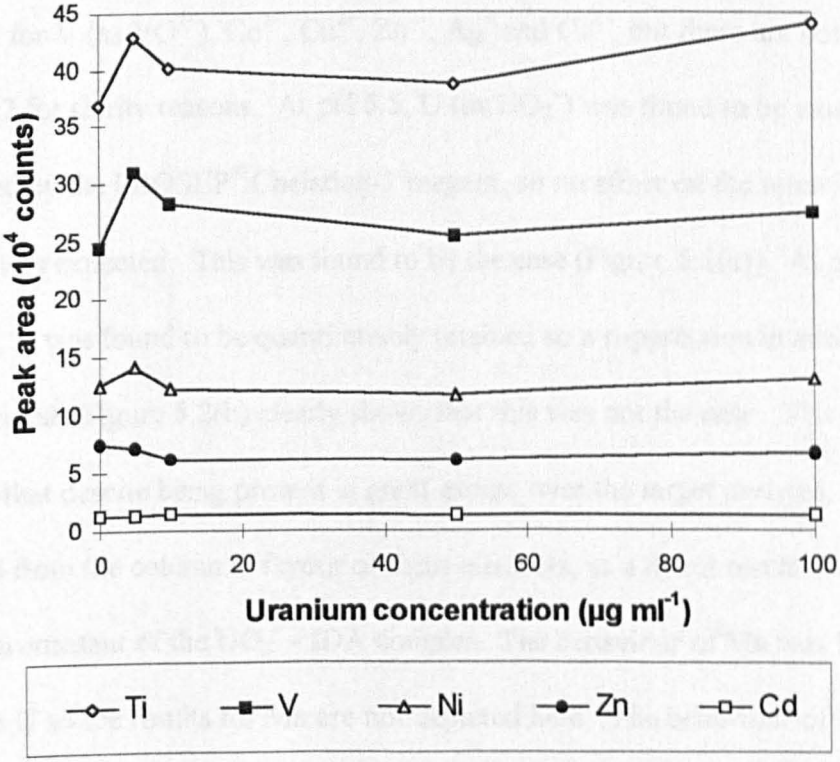
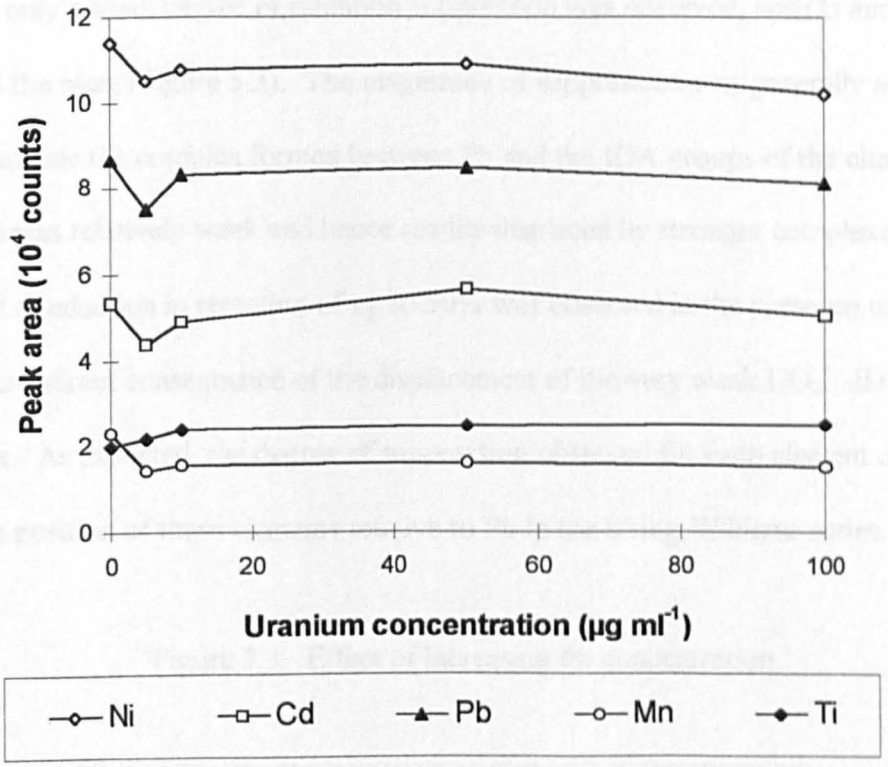


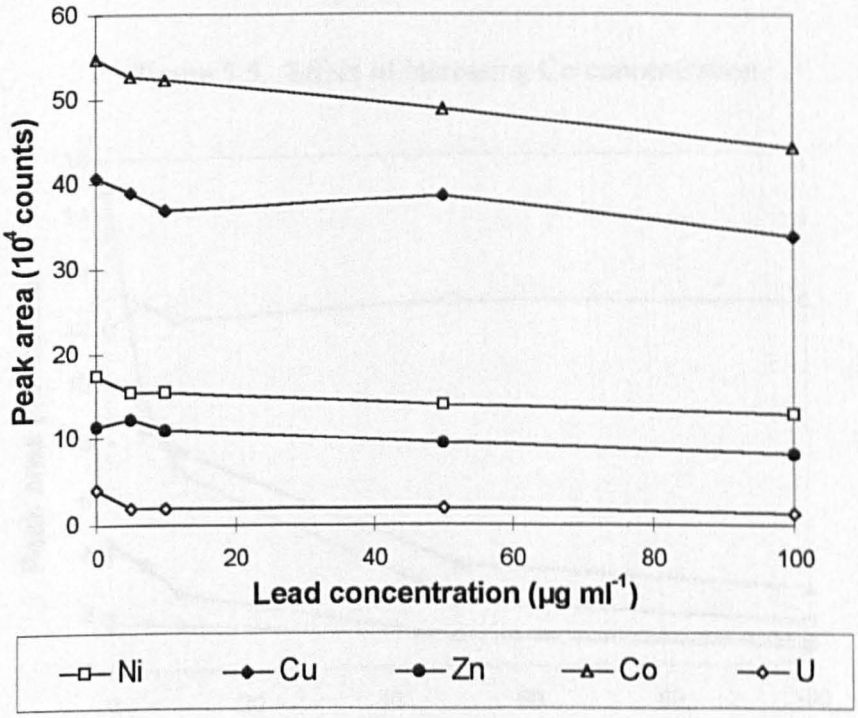
Figure 5.2(b). Effect of increasing U concentration, pH 6.5.



In Figure 5.2 (and subsequent Figures in this Chapter) the elements shown were in the following oxidation states; Ni^{2+}, Cd^{2+}, Pb^{2+}, Mn^{2+} and Ti^{4+}. Similar responses were observed for V (as VO^{2+}), Co^{2+}, Cu^{2+}, Zn^{2+}, Ag^{+} and Ce^{3+}, but these are not shown in Figure 5.2 for clarity reasons. At pH 5.5, U (as UO_2^{+}) was found to be essentially unretained by the PROSEP® Chelating-1 reagent, so no effect on the retention of other analytes was expected. This was found to be the case (Figure 5.2(a)). At pH 6.5, however, U was found to be quantitatively retained so a suppression in analyte retention was expected. Figure 5.2(b) clearly shows that this was not the case. This result suggests that despite being present in great excess over the target analytes, U was readily displaced from the column in favour of these elements, as a direct result of the low formation constant of the UO_2^{+} - IDA complex. The behaviour of Mn was found to be similar to U so the results for Mn are not depicted here. The behaviour of U and Mn is in accordance with the relative positions of these elements in the Irving - Williams

series.[11] The effect of increasing concentrations of Pb had a greater effect than U or Mn, but still only a small degree of retention suppression was observed, with U and Co being affected the most (Figure 5.3). The magnitude of suppression was generally small (up to 15%) because the complex formed between Pb and the IDA groups of the chelating material was relatively weak and hence readily displaced by stronger complexes. For uranium a reduction in retention of up to 50% was observed in the presence of 100 ppm of Pb, as a direct consequence of the displacement of the very weak UO_2^+ -IDA complex. As expected, the degree of suppression observed for each element correlates with the position of these elements relative to Pb in the Irving-Williams series.

Figure 5.3. Effect of increasing Pb concentration.



The results of the Co, Cu and Fe interference studies were a stark contrast to those of U, Mn, Zn and Pb. The IDA complexes of Co, Cu and Fe are highly stable and are relatively unaffected by pH.[11] As a result, significant reductions in analyte retention were observed for most of the analytes measured (Figures 5.4, 5.5 and 5.6).

Figure 5.4. Effect of increasing Cu concentration.

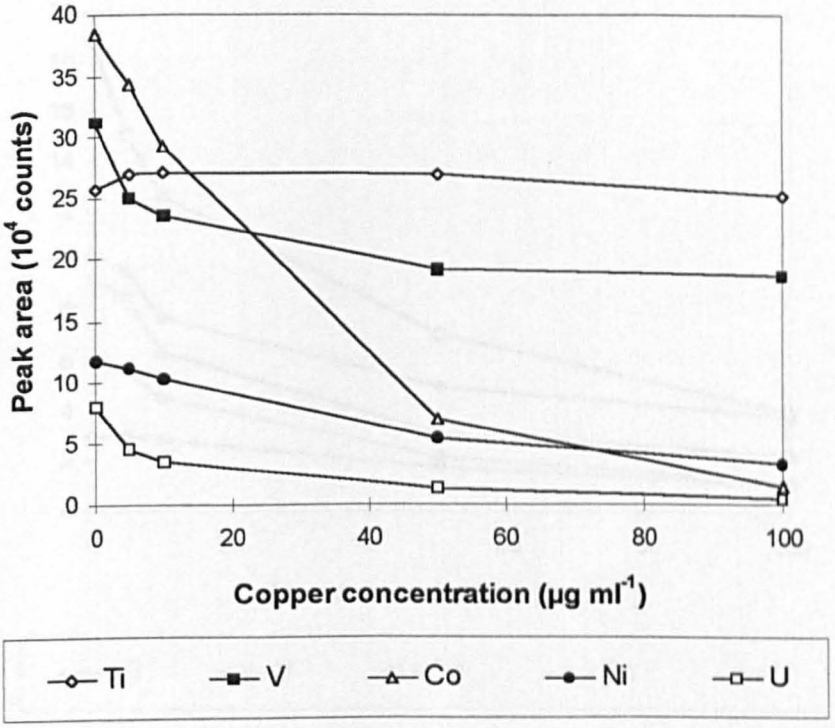


Figure 5.5. Effect of increasing Co concentration.

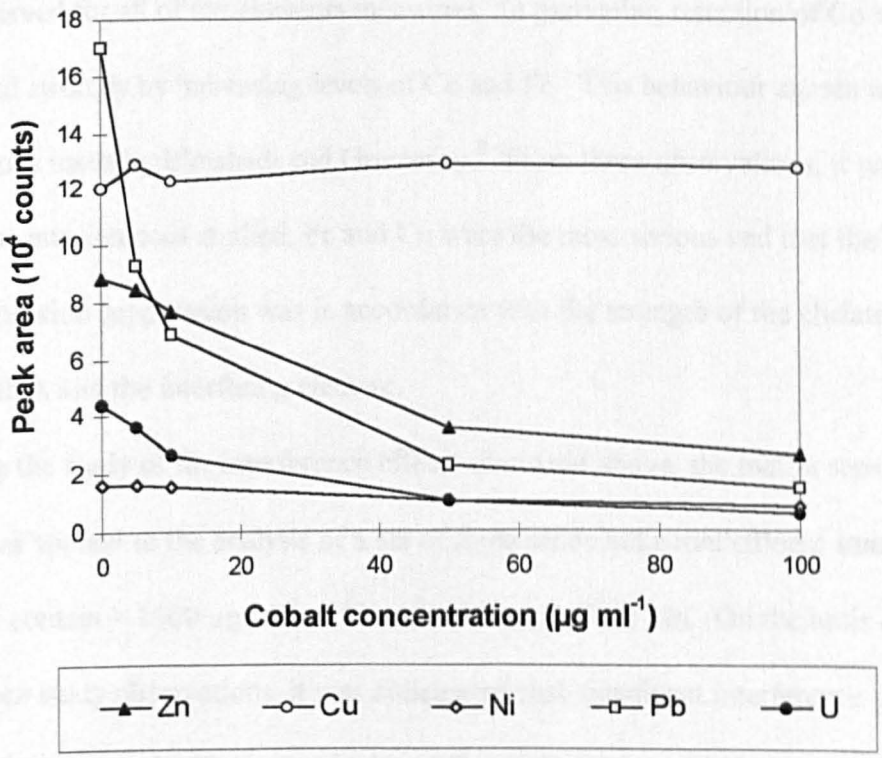
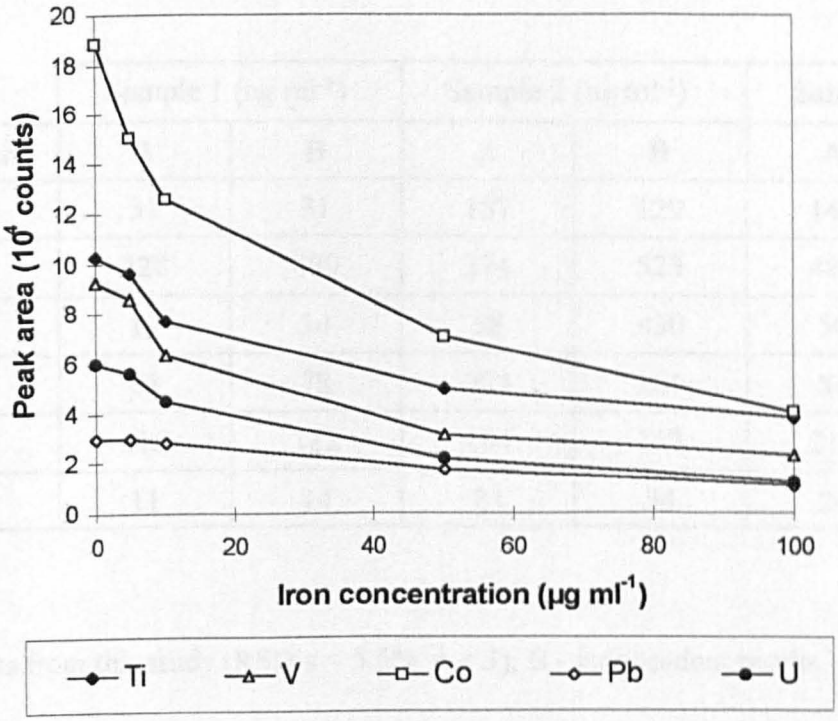


Figure 5.6. Effect of increasing Fe concentration



With increasing concentrations of Co, Cu and Fe varying degrees of analyte suppression were observed for all of the elements measured. In particular, retention of Co was suppressed strongly by increasing levels of Cu and Fe. This behaviour agrees with the observations made by Elmahadi and Greenway.[2] From these observations, it was clear that, of the interferences studied, Fe and Cu were the most serious and that the pattern of analyte retention suppression was in accordance with the strength of the chelate formed between IDA and the interfering element.

Following the study of the interference effects discussed above, the matrix separation system was applied to the analysis of a set of three saline industrial effluent samples, known to contain > 1500 µg ml^{-1} of Ti and > 500 µg ml^{-1} of Mn. On the basis of the interference study observations, it was anticipated that significant interference problems would be encountered with these samples. The results of the analysis are presented in Table 5.2.

Table 5.2. Analysis results for some saline industrial effluents.

	Sample 1 (ng ml^{-1})		Sample 2 (ng ml^{-1})		Sample 3 (ng ml^{-1})	
Element	A	B	A	B	A	B
V	31	31	157	129	140	126
Co	328	489	374	523	481	838
Ni	19	34	58	<30	50	38
Cu	68	78	284	261	54	58
Zn	115	112	164	153	212	213
Pb	11	14	24	34	24	40

A - results from this study (RSD's < 5.5%, n = 3), B - independent results.

It is clear from Table 5.2 that the results obtained for Co and Pb using the matrix separation system are lower than the expected values. In the case of Co, the measured results (A) are 60 - 70% of the expected values, further illustrating that this element is vulnerable to severe retention suppression when high concentrations of competing elements are present. In contrast, the results obtained for Cu, Zn and V are in reasonable agreement with the expected values. These elements are relatively unaffected by the high Ti and Mn concentrations in the samples, as a direct result of the strong complexes they form with the IDA ligand on the surface of PROSEP® Chelating-1.

5.5 Conclusions.

The effects of high concentrations of humic acid and several elements on a matrix separation system for ICP-MS were evaluated. The matrix separation system comprised

a commercial CPG immobilised iminodiacetate reagent, PROSEP® Chelating-1, incorporated into an automated manifold. Humic acid (0 - 50 μg ml^{-1}) was found to have no significant effect on the retention of a range of analytes. A small decrease in retention of Cu was observed, as expected from the known complexation affinity of humic acid for this element. The anomalous increase in response for Mn was considered to be due to the formation of $^{40}Ar^{14}N^{1}H$ in the plasma, resulting from residual humic acid in the eluent. Both Mn and U were observed to have little effect on analyte retention, even at pH 6.5 (optimum pH for U retention), mainly because of the low complexation constants of these two elements with the IDA groups of PROSEP® Chelating-1. In contrast, Cu and Fe were observed to have a significant effect on most of the elements analysed, as a direct result of the formation of their strong IDA complexes. The effect of high concentrations of Mn and Ti on analyte retention were clearly illustrated for three saline industrial effluent samples, by a significant drop in retention of Co and Pb.

5.6 References.

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CHAPTER 6

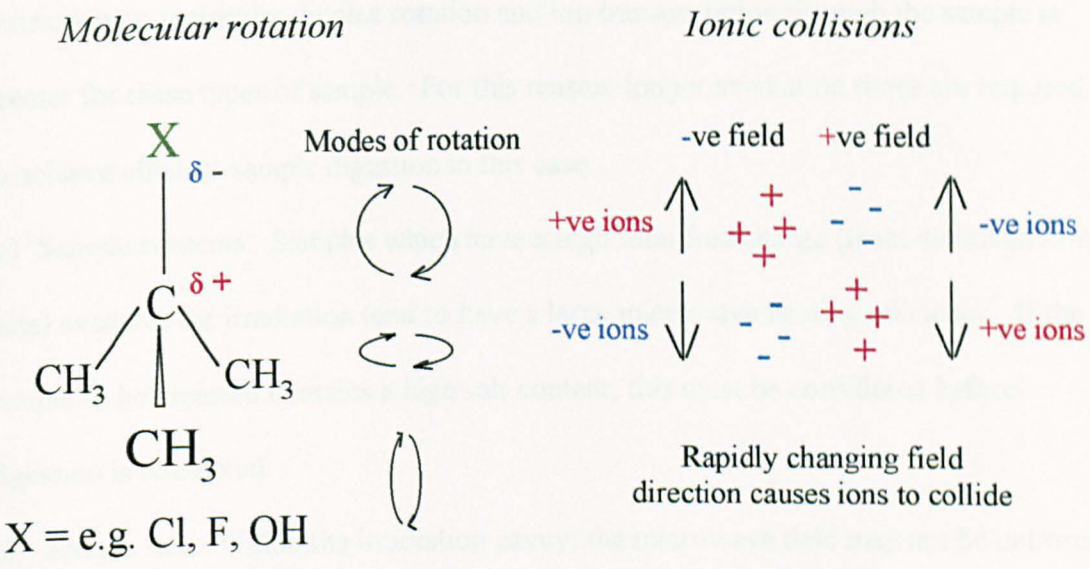
APPLICATIONS OF MICROWAVE DIGESTION IN COMBINATION WITH ICP-MS

6.1 Introduction.

Microwave radiation is a form of electromagnetic radiation with wavelengths in the range of 1 cm to 1 m, between radio waves and infra-red. This corresponds to a frequency range of 300 Mhz to 30 Ghz. Since microwave radiation is part of the electromagnetic spectrum, it is composed of an electric component and a magnetic component, which travel through space perpendicular to each other in the form of a transverse, oscillating sine wave, moving at the speed of light (3×10^8 m s^{-1} *in vacuo*). Microwaves are a versatile form of radiation finding uses in military radar systems, communication networks, spectrochemical analysis and more recently for domestic purposes in the form of the microwave oven. This latter application has also proved to be beneficial in the scientific arena for sample digestion, derivatisation and extraction, as described later in this chapter. The use of microwaves for both cooking and sample treatment purposes is made possible because of the way in which microwave radiation interacts with matter. In order for this interaction to be possible, the constituent molecules of the material must possess either a permanent dipole or a charged functional group. Absorption of microwave radiation by a substance leads to both an increase in molecular rotation (in contrast, absorption of infra-red radiation leads to an increase in molecular bond vibration) and an increase in ionic movement.[1] Both processes lead to an increase in temperature because of frictional heating, caused by (a) molecules spinning in

an effort to align themselves with the rapidly oscillating electric and magnetic fields of the incident microwave radiation and (b) ions colliding whilst trying to move with these fields. These processes are illustrated below (Figure 6.1). Since microwaves operate at GHz frequencies, the speed at which the molecular and ionic oscillations proceed is extremely fast. Consequently, the substance gains energy faster than it can dissipate it and heating occurs very rapidly.

Figure 6.1. Molecular spin and ion collisions induced by microwave irradiation.



6.2 Physical and chemical properties of microwave heating.

The increased efficiency and speed of microwave digestion compared to traditional hotplate digestions has lead to the rapid acceptance of this technique as a means of sample dissolution.[1] The improvements observed using microwave digestion result from the more severe digestion conditions of high pressure and temperature compared to open hotplate procedures. Since the digestion is generally performed in a closed system, there is also a reduced risk of airborne contamination entering the sample prior to analysis.

Although microwave digestion is inherently more effective than the traditional hot plate digestion approach, there are some important factors which affect the technique. These are summarised below.

(a) Temperature effects. As the temperature increases within the digestion vessel, the heating efficiency decreases because the hot sample is closer to its thermal capacity limit than it was when cold. When hot, the absorption capacity of the sample is therefore lower.

(b) Viscosity. Viscous solutions possess large intermolecular forces. Consequently, the restriction on molecular dipoles rotation and ion transportation through the sample is greater for these types of sample. For this reason, longer irradiation times are required to achieve efficient sample digestion in this case.

(c) Sample contents. Samples which have a high total free charge (from dissolved ionic salts) available for irradiation tend to have a large microwave heating efficiency. If the sample to be digested contains a high salt content, this must be considered before digestion is attempted.

(d) Sample size. Within the irradiation cavity, the microwave field may not be uniform and may instead be present as "cool" and "hot" spots of varying microwave strength. Consequently, a small sample located in a "cool" spot may not be efficiently irradiated, and therefore not heat up. For large or multiple samples it may only be necessary to increase the irradiation time or increase the microwave power.

At present, the majority of microwave digestions are carried out under batch conditions. In this case, the sample (typically 1g) is placed in a Teflon vessel, appropriately referred to as a digestion bomb, and a small quantity of the digestion acid (usually 10 ml) is added. Larger acid volumes are not recommended because this increases the risk of the

vessel exploding. A variety of digestion acid mixtures have been reported in the literature, depending on the type of sample to be digested, but the majority of methods are based on nitric acid. Once the sample and acid have been added, the vessel is capped with a lid which can either be designed to vent above a certain pressure or which contains a membrane which will rupture and safely vent the vessel in the event of the pressure rising to a dangerous level. During microwave irradiation, the sample undergoes a process of localised heating which leads to the formation of hot spots within the digestion vessel, whilst the vessel itself is not heated by the radiation These hot spots grow as the digestion proceeds until a point is reached where the temperature becomes uniform throughout the vessel. As the sample begins to digest, gases are evolved and the pressure increases rapidly. The increasing pressure raises the boiling temperature above that expected for the acid under normal atmospheric pressure thereby considerably increasing the severity of the digestion compared to the standard hotplate approach. Since the digestion occurs at elevated pressure, production of gas within the sample is avoided and this prevents the digestion mixture from boiling. Furthermore, the cooler, smooth walls of the digestion vessel do not facilitate nucleation of gas bubbles on the inside if the vessel. In contrast, with open hotplate digestions the vessel wall is in direct contact with the heat source and hence is hotter than the liquid. Since hotplate digestion operates at atmospheric pressure, nucleation of gas at the hot vessel walls occurs and boiling occurs. This contributes to the lower digestion efficiency achieved using hotplate digestion systems. The elevated sample temperature (referred to as 'super heating') experienced with microwave digestion is a notable feature of this technique. Microwave digestion also produces increased oxidising conditions through the formation of the NO_2^+ species by microwave induced dissociation of HNO_3. If HCl has also been added to the digestion mixture, this dissociation results in the production of the strongly

oxidising species, NO_2Cl. The sample is therefore considerably more viciously attacked under these conditions, leading to the rapid, efficient digestions observed.

6.3 Analytical applications of microwave digestion.

6.3.1 Microwave digestion studies using off-line detection.

The use of microwaves for chemical analysis purposes began with a report by Hesek and Wilson[2] in 1974, which described the application of microwaves for drying process control samples. This was soon followed by a paper by Abu-Samra et al.[3] in which a microwave system was used to reduce samples to ash, prior to analysis. Since that time, batch microwave digestion has become recognised as an efficient, rapid technique for sample preparation for a variety of applications. To this end, a number of papers have appeared in the literature which describe microwave digestion and dissolution methods for aqueous and marine samples,[4] biological samples,[5] soils, sediments and geological samples,[6] paints[7], food contact polymers[8] and even explosives.[9] The use of stopped flow microwave digestion, with off-line detection has also been reported. This approach has the principle advantages that a greater pressure can be achieved in the system, degassing is easier and the digestion is more efficient and reproducible. The main disadvantage is that the analysis time is increased over that of continuous flow systems. Gluodenis and Tyson[10] used a stopped flow approach to digest cocoa powder, horse kidney and coal samples prior to analysis using flame AAS. In this study, the samples were collected in volumetric flasks which were then diluted to volume. This procedure was time consuming and involved quite large dilution factors, which degraded the detection limit of the analysis. Nonetheless, acceptable agreement was found between the certified and

measured values for Fe, Zn, Cd and Mg in the horse kidney and cocoa samples. The coal samples gave lower than expected values for some elements because of incomplete digestion of the silicate material present. In this publication, the on-line digestion results were compared with batch microwave digestion results and it is noteworthy that the batch results consistently showed better agreement with the certified data than the on-line results. In contrast to the closed digestion vessel studies discussed up to this point, the use of open vessel microwave digestion has been reported for the leaching of organotin species from sediments and biomaterials.[11,12] In each of these studies, a commercially available open vessel microwave digestion system was used. The samples were placed in extraction tubes, open to atmosphere, which were then inserted into the microwave cavity on the device. In both studies, a low power, focussed microwave field was applied, which efficiently leached the organotin species from the samples whilst keeping the species intact. Off-line analysis by capillary GC was used in both studies, with flame photometric detection employed in the first study[11] and atomic emission detection, using a microwave induced plasma, in the second.[12]

Recently, a system for automated, continuous flow on-line microwave digestion has become commercially available. This system, which is the focus of the work described in this chapter, has been used for the determination of 9 elements, including As, Cr, and Pb, in environmental samples (using a combination of ETAAS, ICP-AES and ICP-MS)[13] and for the analysis of Pb in a range of reference materials (using ICP-MS detection).[14]

In the first study,[14] samples were prepared as slurries (0.5 - 1% (m/v)) in an acid medium containing HF, HNO_3 and HCl, and pumped through the microwave system under pressure. The digests were collected automatically using a sample collection arm on the microwave device, and analysed off-line. A sample throughput of between 6 and 8 samples per hour was achieved with unattended operation. With this system, good

agreement between the measured and certified concentrations in various marine sediment and biological reference materials was reported, although the results indicated problems with the accuracy for ^{52}Cr, because of an interference in the ICP-MS measurements from ArC^+ at m/z 52. In the second study,[15] carried out at NIST, the samples (10 ml) were introduced to the system as slurries in a dilute mixture of $HClO_4$, HNO_3 and HF. The digested samples were automatically collected, then analysed off-line by ICP-MS, using isotope dilution as the quantitation method. Using this approach the authors were successful in obtaining accurate data for the Pb concentration in peach leaves, air filters, urine, domestic sludge, household dust and paint reference materials. The authors suggested that although the samples had been collected off-line in this study, it was, in principle, feasible to directly couple the on-line microwave digestion unit to the ICP-MS instrument. This approach is discussed in detail later in this chapter.

6.3.2 Microwave digestion studies using on-line detection.

The use of microwaves for digesting and dissoluting samples under static or on-line flow conditions is a new concept in analytical chemistry. Directly coupling a flow through microwave digestion system to a detector is inherently complex, which largely explains the rather late development of this science and the relatively few papers currently published in the literature. Some of the difficulties which can be encountered have been described by Tsalev et al.[15] and be summarised as follows:

(1) Gas production from the digestion process and the associated pressure build-up.

(2) Incomplete sample digestion, due to short reaction times.

(3) Large percentage of non-absorbed power, leading to high reflected power.

(4) Flow disturbance and irreproducibility arising from sample matrix effects.

Furthermore, blockage of the flow system either before entry to the microwave cavity or during digestion can be a serious drawback to the technique. In spite of these difficulties, the potential advantages of increased sample throughput, reduced contamination and offered by on-line microwave digestion have established it as a topical area of scientific development.

The first documented study in which on-line microwave digestion was used in conjunction with atomic spectrometric detection appeared in 1986. This pioneering study, by Burguera and Burguera[17] used a simple flow injection manifold to mix blood samples (100µl) with a dilute HNO_3 / HCl acid mixture prior to passage through a 50cm long coil in the microwave cavity. The outlet of the system was connected directly to the nebuliser of a flame AAS. The sample and reagent streams were operated at a flow rate of around 2 ml min^{-1} each, leading to a residence time of 20 s for the sample to react in the microwave cavity. Quantitative recoveries were achieved for Cu, Zn and Fe but only by using matrix matched standards, because the system did not achieve complete sample digestion. Attempts to improve the digestion by increasing the microwave residence time lead to increased gas production, which caused pressure build-up and affected the flow stability. This initial study was later developed further by J.L. Burguera *et al.*[18] in a novel study in which samples were extracted directly from a forearm vein of a volunteer. In this study, an automated flow system was used. A more concentrated acid digestion mixture together with a longer conduit in the microwave field was employed in an attempt to increase the digestion efficiency. Furthermore, a membrane gas-liquid separator was incorporated downstream of the microwave to de-gas the digested samples. Despite these measures, the authors still reported incomplete digestion and it remained necessary to use matrix matched standards. The on-line microwave digestion studies described so far both used flame AAS, but the technique is by no means restricted

to this detection system. In two detailed publications, Tsalev *et al.*[19,20] described the development of an on-line microwave digestion system which used vapour generation AAS, for the determination of hydride forming elements. This system incorporated a small, focused microwave cavity operated at 40 - 50 W power, which enabled aqueous samples, flowing at 10 ml min^{-1}, to be heated to around 90°C within 4 seconds of entering the microwave. This system was used to measure As, Bi, Pb, Sn and Hg in urine and environmental waters, after pre-treatment off-line with either a bromide / bromate acid or persulphate acid oxidizing reagent mixture, depending on the analyte to be measured.[21] Using this approach, quantitative recoveries of eight separate inorganic and organic Hg species in urine were obtained using aqueous inorganic Hg standards[22]. The system was later modified to include a 1m long, 0.3 mm i.d. section of tubing, located downstream of the microwave. This tubing acted as a flow restrictor which increased the pressure in the system during digestion and hence improved the digestion efficiency. The system was used to measure As in blood samples, diluted 1:10 with dilute HCl and Triton dispersant. After the digestion step, the sample was passed through a cooling coil and finally, in order to allow the As vapour to be generated, the system also incorporated an on-line reduction step. This complex manifold operated at a relatively slow sampling frequency of 7 - 10 per hour, but nonetheless was found to yield good recoveries against aqueous standards, from samples spiked at the outset with As(V). In a separate study, Guo and Baasner[23] developed an on-line microwave digestion system coupled to a cold vapour AAS detector, to measure Hg in blood samples. In this case, the samples (diluted 1:1) were treated off-line, before digestion, with a bromate-bromide oxidant mixture. The authors found it necessary to merge the sample stream with permanganate after digestion to ensure complete oxidation. Using this system, quantitative recoveries for Hg in four organomercury compounds in 1:10

diluted blood were obtained at a respectable sampling frequency of 45 per hour. The on-line studies discussed so far were concerned with liquid samples, which despite being susceptible to incomplete digestion, are not expected to cause blockage problems in the flow system. On-line digestion of slurries, in which the sample matrix consists of a powder dispersed in a liquid, is inherently more difficult. Analysis of such samples generally requires more extreme digestion conditions and use of wider bore tubing to prevent flocculated sample particles obstructing the flow. With these factors in mind, Haswell and Barclay[24] constructed an on-line microwave digestion system coupled to an AAS detector, for the determination of trace metals in organic matrix samples. The slurried samples (0.005 to 0.05% m/v) were prepared in 5% HNO_3 and transported at 6 ml min^{-1} through a 20 m long, 0.8 mm i.d. conduit located within a microwave cavity operated at 525 W. The system also incorporated a 5m loop immersed in an anti-freeze bath, to cool the digested sample, and a length of narrow bore tubing to act as a back pressure regulator which maintained a high pressure in the system, thereby increasing the digestion efficiency. With this system, the authors illustrated that accurate quantitative results could be obtained for the samples using simple aqueous standards, at a sampling rate 4 times better than a batch microwave digestion procedure.

In contrast to the work of Haswell and Barclay described above, Carbonell *et al.*[25] adopted an alternative approach to on-line microwave digestion of solids. In this study, recirculation of the samples through the microwave field was employed to improve the digestion efficiency. After passing the samples (in concentrated HNO_3) through the microwave a first time, the resulting digest was directed through a cooler and then through a gas trap to remove the gases evolved. After this stage, the sample was redirected through a loop and the sample then re-injected into the microwave to repeat the digest process. This recirculation was continued for 5 minutes to ensure that the

digestion was complete, then the final sample captured in the loop was injected into a flame AAS detector. Using this approach, a sampling frequency of 12 per hour was achieved. The accuracy of the technique was illustrated by the good agreement obtained between the measured and certified values of Pb in solid reference materials, using calibration solutions prepared in nitric acid.

In addition to coupling microwave digestion systems to flame AAS detectors, investigations have been made into using electrothermal atomisation (ETAAS) as the detection system. To this end, Burguera and Burguera[26] developed an on-line microwave digestion system coupled to an ETAAS detector for analysing lead in blood and solid biological samples. The samples were mixed on-line with an HNO_3 / HCl acid solution before passing into the microwave. The system was operated such that the sample and acid solutions were injected into separate water carrier streams and mixed by merging at a Y-piece. In this way, cross contamination and memory effects were minimised. After passage through the microwave, the sample was degassed, cooled and collected in the fixed volume loop of a valve. This volume was then injected into the graphite tube of the ETAAS instrument. With this system, good agreement between the certified and measured values of lead in blood, pig kidney and bovine liver were obtained. However, olive leaf and pine needle samples gave lower values than expected presumably because of incomplete digestion.

The work discussed up to this point in this section was centred on the use of closed microwave digestion systems, generally operated at elevated pressures. However, the the use of open vessel microwave digestion systems, for low pressure on-line analysis has been reported.[27] In this work, a HPLC manifold, coupled to an open vessel microwave digestion device, was developed for separation and speciation of SeIV and SeVI in water samples, using hydride generation with AFS detection. The samples were transported

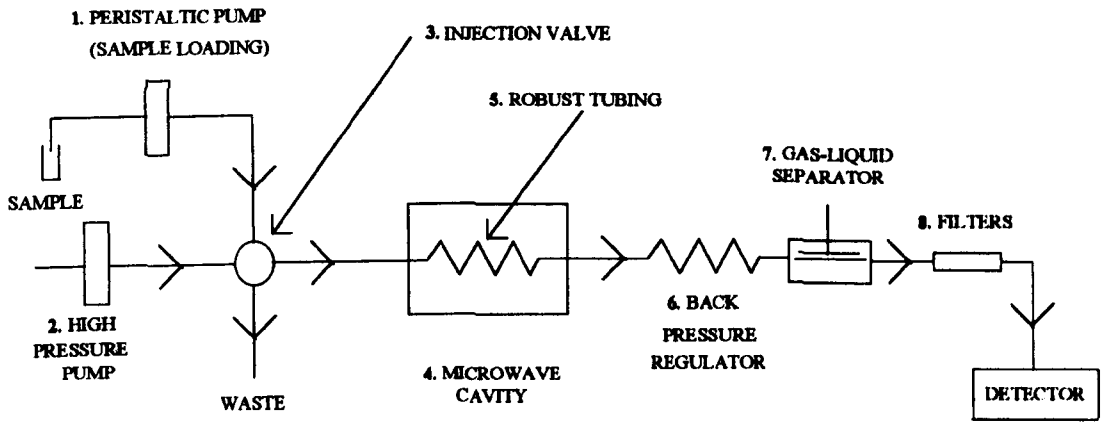
through a coil of PTFE tubing inserted into the open microwave cavity, where focussed microwave irradiation was carried out. The irradiation process reduced SeVI to SeIV, so that the all the separated Se species could be converted to selenium hydride. Using this approach, these authors were able to conclude that Se in the water reference material NST 1643d was present as 10.6 ng ml^{-1} SeIV and 2.8 ng ml^{-1} SeVI, giving a total Se concentration of 13.4 ng ml^{-1} versus a certified value of 12.7 ± 0.7 ng ml^{-1}.

In this chapter, the use of microwave digestion for sample preparation for ICP-MS will be discussed. The performance of a batch digestion system is evaluated for the decomposition of three reference soil / sediment materials (MESS-1, SO-3 and BCR 142) and compared with that of an on-line digestion system. For the on-line system, results for digested samples collected off-line from the ICP-MS are compared with data obtained by directly coupling the on-line digestion system to the ICP-MS. This latter approach, which has not been reported for ICP-MS to date, is assessed in terms of its practical feasibility, together with its potential advantages and disadvantages.

6.4 Design considerations for on-line microwave digestion systems.

The inherent difficulties surrounding the science of on-line microwave digestion have complicated its development into a robust, commercially available analytical technique. The practical problems associated with the procedure, as discussed in section 2.2, lead to a requirement to incorporate specific physical features into the instrumentation design. The schematic diagram below (Figure 6.2) depicts the main components of a basic on-line microwave digestion system.

Figure 6.2. Principle components of an on-line microwave digestion unit.



(1) Sample loading pump (generally of the peristaltic type).

(2) High pressure development and control. This is necessary to ensure efficient, reproducible digestion within the microwave cavity. Since high pressures are generated, it can be necessary to use a high pressure pump to maintain flow through the system.

(3) Sample injection valve.

(4) Uniform microwave irradiation. Within the microwave cavity, the radiation level should be uniform throughout, to ensure even heating of the sample.

(5) Robust, flexible tubing. The material of the tubing required to pass samples through the system needs to be strong enough to withstand the high pressure and temperature (up to 150°C) developed during digestion. On the other hand, since long lengths of tubing are required to ensure sufficient residence time of the sample in the microwave cavity, the tubing material also needs to be flexible enough to form into a coil. In practice Teflon tubing (typically 1 mm i.d.), encased in a strengthening coat of Kevlar, is used.

(6) Back pressure regulator. This is required to maintain pressure within the microwave cavity and basically consists of a length of narrow bore (e.g. 0.3 mm i.d.) Teflon tubing, which constricts the flow and hence increases the pressure.

(7) Gas-liquid separator. This is needed to efficiently separate the gas bubbles from the liquid of the digested sample, before it passes into the detector system.

(8) On-line filters. These may be required if small particles, such as silica grains, survive the digestion process. If these are not filtered out, the system can block up. When filters are incorporated into the system, a means of back-flushing them must also be included.

6.5 Instrumentation.

6.5.1 The ICP-MS.

For this work a VG PlasmaQuad 2 Plus ICP-MS (VG Elemental, Winsford, Cheshire, UK) was used. The instrument was configured and tuned as described earlier (Chapter 3, section 3.4.2). In this study, the instrument was operated in both its standard data acquisition mode and its time resolved data analysis mode. The data acquisition parameters used throughout this work are described below (Table 6.1).

Table 6.1. Standard data acquisition and TRA operating parameters for the ICP-MS.

Standard data acquisition parameters		**Time resolved data acquisition parameters**	
Parameter	Value	Parameter	Value
Isotopes selected	^{59}Co, ^{60}Ni, ^{63}Cu, ^{67}Zn, ^{111}Cd, ^{204}Pb	Isotopes selected	^{59}Co, ^{60}Ni, ^{63}Cu, ^{67}Zn, ^{111}Cd, ^{204}Pb
Mode of data acquisition	Peak jumping, 3 points per peak	Mode of data acquisition	Peak jumping, 3 points per peak
Dwell time (ms)	10.24	Time per slice (s)	1
Total acquisition time (s)	60	Total acquisition time (s)	180

6.5.2 The static microwave digestion system.

For this study, a CEM MDS-81D batch digestion microwave system was used (CEM, High Wycombe, Bucks., UK). This system consisted of Teflon digestion vessels (100 ml) encased in a strengthening Kevlar jacket. During digestion, these vessels were capped with a Teflon lid which incorporated a Teflon safety rupture disk, machined to rupture above 120 psi, and finally sealed with a screw fit collar. The pressure evolved during digestion was monitored using one of the digestion vessels, specially designed to incorporate a pressure sensor. When the pressure reached a preset value of 100 psi in the monitor vessel, the microwave automatically switched off, thereby stopping the heating process and allowing the pressure to fall below 100 psi again. As soon as the pressure was registered to fall below this value, the microwave restarted. In this way, careful control of the digestion pressure could be maintained at a safe level, below the maximum pressure tolerated by the Teflon rupture safety disk. The microwave was programmed to carry out the digestion stepwise, starting at a relatively low power setting and ending at a high power level.

6.5.3 The on-line microwave digestion system.

For this work, the commercially available CEM SpectroPrep on-line microwave digestion system (CEM, High Wycombe, Bucks., UK) was used. The main components of the SpectroPrep system are described below:

(1) Sample uptake system. This consisted of an autosampler arm incorporating a stirrer and a peristaltic pump located downstream of a 4-way valve fitted with a 1 ml loop. The stirrer was required to ensure that the slurried samples were homogeneous immediately

before being drawn into the sample loop. The SpectroPrep could be programmed to move the uptake arm to the sample, start the stirrer and then start the peristaltic pump. The system automatically detected when the sample loop was full and then switched the sampling valve to direct the sample into the microwave. The automatic "sample loop full" detection feature was achieved by the incorporation of a capacitance detector located downstream of the sample loop. This detector registered the fall in capacitance caused by the acid in the samples as the sample solution passed through. When the capacitance fell to a level set in the SpectroPrep software, the sampling valve was automatically triggered.

(2) Sample digestion system. This comprised a high pressure pump to push the carrier stream and the sample through the long tubing of the microwave system, a microwave cavity, filters and a backpressure regulator to maintain high pressure in the system during the digestion process. A coil of tubing approximately 30 m long was located within the microwave cavity. This length of tubing was required to ensure that the residence time of the sample in the microwave field was sufficiently long to allow complete digestion to occur. The tubing of the coil was strengthened with a coating of Kevlar to ensure that it would not rupture under the conditions of high temperature and pressure reached during sample digestion. Filters were fitted at the outlet port of the microwave cavity to remove fine particles which escaped digestion. The backpressure regulator, located downstream of the filters, consisted of a 1 m long coil of 0.3 mm i.d. Teflon tubing. Since the tubing diameter of this coil was considerably smaller than that of the rest of the flow system (0.8 mm i.d.), the flow was restricted, thereby allowing the pressure in the system to be maintained at a level of around 200psi.

(3) Sample collection system. After the digested sample passed through the backpressure regulator of the SpectroPrep system, the output tubing was directed

through a cooling chamber to condense the acid vapour and cool the digest. The cooling chamber was constructed from Peltier coolers around which the output tubing (1 m) was coiled. On exiting the cooling chamber, the cooled digest was directed through a second capacitance detector which triggered a second valve to switch from the waste position (between analyses) to the collection position. The sample then passed through the autosampler collection arm and collected in a sample tube.

6.6 Experimental.

6.6.1 Reagents.

For static microwave digestion, concentrated nitric acid (69% (m/v)) (Aristar grade, BDH, Poole, Dorset, UK) was used. For on-line microwave digestion using the SpectroPrep system, nitric acid (10% (v/v)), prepared by diluting concentrated nitric acid with de-ionised water, was used. The sediment reference materials (MESS-1, SO-3, BCR 142) were obtained from Bureau of Certified Reference materials (BCR, Geel, Belgium) and used as supplied. De-ionised water (18 MΩ cm quality) was used throughout the study for the preparation of solutions. Elemental stock solutions (100 $\mu g\ ml^{-1}$, SpectrosoL, BDH, Poole, Dorset, UK) were used in the preparation of calibration mixtures.

6.6.2 Off-line digestion studies using ICP-MS detection.

Prior to investigating the viability of on-line microwave digestion coupled to ICP-MS, some initial studies were carried out using an off-line approach. Two procedures were

studied, based on static sample digestion using the CEM MDS-81D microwave digestion system and on-line digestion using the SpectroPrep system with sample collection off-line from the ICP-MS. The microwave digestion procedures developed in this study were validated by analysis of the soil and sediment reference materials MESS-1, SO-3 and BCR 142.

6.6.2.1 Static microwave digestion.

Sediment samples (0.5g) were accurately weighed into Teflon digestion bombs. Concentrated nitric acid (10 ml) was added and the samples left to stand for 1 minute to allow any effervescence arising from decomposing carbonates in the sample to subside. The vessel was then sealed as described above (section 6.5.2). The samples were then placed in the microwave and subjected to microwave irradiation according to the digestion program given in Table 6.2 below.

Table 6.2. Digestion program for the static microwave digestion study.*

Step	Microwave power (%)	Time (min)
1	40	5
2	60	10
3	80	15

* for safety purposes, the digestion process was allowed to reach a maximum pressure of 100 psi in each vessel. This was achieved through automatic off/on switching of the microwave power when the pressure reached the 100 psi level.

Once the digestion program was complete, the samples were left to stand for 1 hour to allow the pressure in the vessels to subside and the contents to cool. During this time, the vessels were not removed from the microwave because of the risk that the pressure within the vessels could explosively rupture the safety disk, especially if the vessels were moved or shaken whilst still at high pressure. After this cooling period, the vessels were carefully opened and the contents transferred to volumetric flasks (50 ml). The digests were diluted to the 50 ml mark, spiked with ^{103}Rh internal standard (10 ppb) and finally analysed by ICP-MS, using a fully quantitative approach.

6.6.2.2 On-line microwave digestion with off-line ICP-MS detection.

For this work, the CEM SpectroPrep system (described in section 6.5.3) was used. Samples (0.5g) were mixed with nitric acid (10%, 50ml) containing ^{103}Rh as internal standard at a concentration of 50ppb, in graduated polythene tubes. The samples were stirred vigorously using the stirrer attachment on the microwave system before aliquots of each (10ml) were drawn into the sample loop using a peristaltic pump. Once loaded, the slurried samples were injected into the microwave cavity. The samples were pumped through the system under pressure, using a water carrier stream, and allowed to digest within the microwave field, before passing through the cooling chamber of the system. After passing through the unit, the digested samples (15 ml) were collected and analysed by ICP-MS, using an external calibration approach. The collection volume was larger than the sample volume because (a) a degree of dilution occurred through dispersion of the sample into the leading and trailing edges of the water carrier stream and (b) the exit time of the sample was not identical in consecutive runs, so it was possible to 'lose' most

of the sample by collecting too small a fraction. The operating parameters for the on-line microwave digestion system are given below (Table 6.3).

Table 6.3. Operating parameters for the SpectroPrep on-line microwave digestion system.

Parameter	Value
Sample volume (ml)	10
System flow rate (ml min^{-1})	10
Carrier stream	Water
Total microwave power (W)*	600
Teflon digestion tubing length (m)	30
Teflon digestion tubing i.d. (mm)	1.0
Digestion time (min)	6
Cooler temperature (°C)	25
Cooler tubing length (m)	10
Collection volume (ml)	15

* During digestion, the microwave was operated at 50% power, for safety reasons.

6.6.3 Coupling the on-line microwave digestion system to the ICP-MS.

This final part of the study aimed to evaluate the performance and potential of coupling the SpectroPrep on-line microwave digestion system directly to the ICP-MS instrument. In the recent paper by Beary *et al.*[11], this analytical combination was proposed to be potentially feasible since, in the authors words, the flow rates and sampling volumes of the on-line microwave digestion system and the ICP-MS were compatible. For the microwave system to operate at an acceptable sampling rate, whilst still providing efficient sample digestion, it was necessary to run the system at a flow rate of 10ml min^{-1},

which was incompatible with the ICP-MS uptake rate of around only 1 ml min^{-1}. In order to interface the two systems, it was therefore necessary to incorporate a switching valve in the output line of the microwave to 'capture' a fixed volume aliquot of the digested sample before injecting it into the ICP-MS. This was necessary because of fluctuations observed in the output flow of the microwave system, related to pressure variation and bubble formation during the digestion process. Since this flow injection approach was intended to be adopted for the on-line digestion / on-line ICP-MS analysis, some preliminary work was performed in which pre-digested samples of the reference materials were analysed using flow injection as the method of sample introduction. In this way a set of data was generated which could be compared with the results obtained for the complete on-line system.

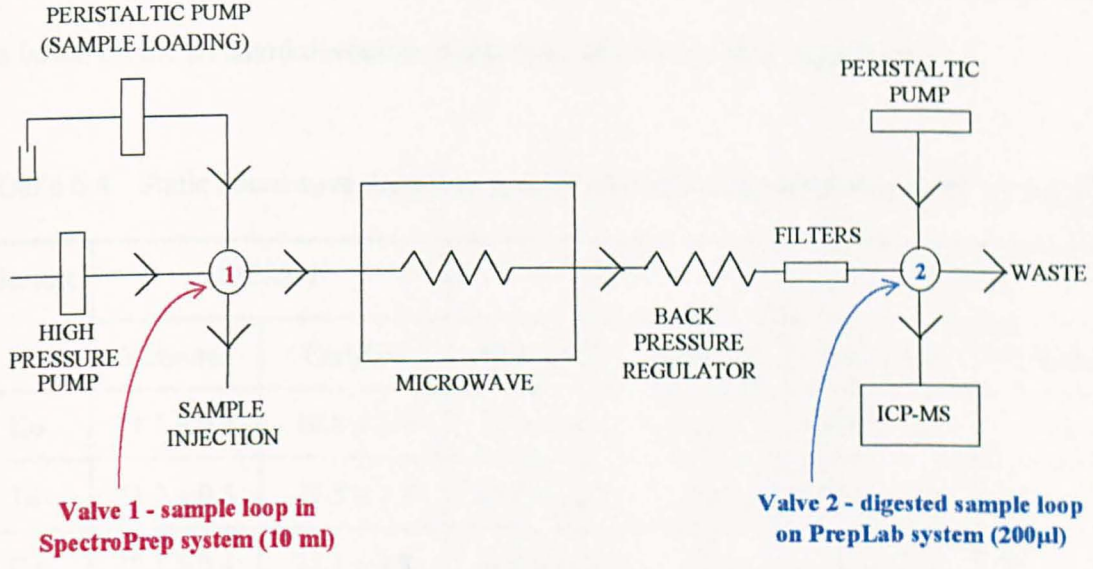
6.6.3.1 Flow injection analysis of samples digested using the static microwave system.

In this work, the samples prepared for the batch microwave digestion study (section 6.6.2.1) were used. These samples were loaded into a 200µl injection loop, located across the front port of the dual 6-port injection valve on the PrepLab system. By using the PrepLab system, sample loading, injection and analysis could be automated. The analyte concentrations were quantified using an external calibration approach, by injecting the calibrant solutions in the same way as the samples. Time resolved data acquisition was used to acquire data for the analysis peaks, using the parameters given earlier (Table 6.1). After acquiring the data, the files were exported to a third party software package (MassLynx, VG MassLab, Cheshire, UK) for peak processing.

6.6.3.2 Coupling the on-line microwave digestion system to the ICP-MS.

As indicated earlier, to interface the microwave system directly with the ICP-MS it was necessary to insert a valve into the output flow line of the microwave digestion unit, because of the high and variable output flow of this system. This was achieved by using the front port of the dual 6-port valve of the PrepLab system. The valve was fitted with a fixed volume loop (200 µl) so that a reproducible fraction of the digested sample could be collected while the valve was aligned with the microwave output flow. On switching the valve the collected sample was carried by a separate carrier stream, pumped at 1 ml min^{-1}, into the ICP-MS. The valve arrangement is displayed in detail in the schematic diagram below (Figure 6.3).

Figure 6.3. The on-line microwave digestion - ICP-MS manifold.



For this work, the SpectroPrep was operated with the same parameters used in the off-line ICP-MS study (see Table 6.3). It was necessary to operate the ICP-MS in time resolved analysis mode, since the digested sample was transported to the ICP-MS in the form of a transient peak. The time resolved data acquisition parameters used were

presented earlier (Table 6.1). After acquiring the data, the files were exported to a third party software package (MassLynx, VG MassLab, Cheshire, UK) for susbsequent peak processing. The concentrations in the digested samples were calculated using external calibration with a set of calibration solutions injected into the ICP-MS via the same 200µl loop used to capture the output from the SpectroPrep.

6.7 Results and Discussion.

6.7.1 Microwave digestion studies with off-line ICP-MS detection.

6.7.1.1 Static microwave digestion..

The results obtained for the reference materials digested under batch conditions are presented below (Table 6.4). The range quoted for the measured analyte concentrations is based on the standard deviation of the mean of two separate digestions.

Table 6.4. Static microwave digestion data for the reference material analysis (in $\mu g\ g^{-1}$).

Element	MESS-1		SO-3*		BCR 142	
	Measured	Certified	Measured	Certified	Measured	Certified
Co	11.1 ± 0.2	10.8 ± 1.9	5.7 ± 0.4	12 ± 8	8.7 ± 1.0	---
Ni	24.9 ± 0.5	29.5 ± 2.7	16.6 ± 1.1	16	27.8 ± 3.2	29.2 ± 2.5
Cu	20.1 ± 0.4	25.1 ± 3.8	11.6 ± 1.3	17	21.3 ± 2.6	27.5 ± 0.6
Zn	173 ± 0.4	191 ± 17	51.5 ± 3.1	52	93.6 ± 12.9	92.4 ± 4.4
Cd	0.64 ± 0.01	0.59 ± 0.10	0.15 ± 0.01	0.14 ± 0.08	0.27 ± 0.05	0.25 ± 0.09
Pb	28.1 ± 0.8	34.0 ± 6.1	9.4 ± 0.13	14	29.9 ± 4.8	37.8 ± 1.9

* For some certified results, range information was unavailable (see also later tables).

It is clear from Table 6.4 that some agreement was obtained between the measured and certified concentrations of the elements analysed. From these results it is apparent that of the three reference materials analysed, SO-3 shows the poorest agreement. This is undoubtedly due to incomplete dissolution of the silicate material, which makes up a significant percentage of this sample. In order to ensure complete digestion of silicate material, a small amount of HF is required in the digestion mixture. This was not performed in this preliminary study as the intention was to compare the different digestion approaches using only nitric acid, on the grounds of safety. Using the batchwise method of microwave digestion, a total of 6 samples could be conveniently digested in a single run (i.e. 25 minutes + 1 hour cooling) (Table 6.2). The time taken to prepare each sample for microwave digestion was approximately 5 minutes and a further 2 minutes was required to transfer the digested samples to volumetric flasks and dilute them. Combined with an ICP-MS analysis time of around 3 minutes per sample, this amounted to an overall average analysis time of around 24 minutes per sample (including the cooling time) for the batch microwave digestion system. The batch microwave digestion system was relatively straightforward to use and was effective, within the limits of this work, for digesting the sediment and soil reference materials studied. The only drawback noted for this technique was the fairly lengthy sample preparation time. In addition, it was observed that occasionally, a particular sample vessel could reach a higher pressure than the monitor vessel. This ultimately lead to a rupturing of the safety disk in the cap of the vessel and subsequently ejection of the pressurised sample from the vessel into the microwave. In this event, it was necessary to stop the microwave, carefully remove the offending sample and replace it with a freshly prepared one. This lead to an increase in the overall analysis time.

6.7.1.2 On-line microwave digestion, with off-line ICP-MS detection.

Use of the on-line microwave digestion system was expected to increase the sample throughput compared to the batch wise approach, whilst maintaining the accuracy and precision of the analysis. From the papers referenced in section 6.3.2 it is apparent that there are contradictory opinions in the literature as to the practicality of on-line microwave digestion, with some workers encountering few problems[10] whereas others catalogue a series of difficulties.[15,16] On the basis of these reports, it was anticipated that the on-line digestion approach could lead to some practical difficulties, which did indeed prove to be the case. The problems encountered whilst attempting to digest the sediment and soil reference materials with the on-line microwave digestion system were as follows:

(1) Blockage of the sample valve entrance and exit ports, caused by accumulation of particles in the sample slurries.

(2) Blockage of the sample loop itself through the same particle flocculation mechanism.

(3) Rapid bubble formation in the tubing in the microwave cavity as the sample began to digest. This in turn caused (i) a considerable increase in pressure which, on several occasions caused the unit to switch off as the safety level was exceeded and (ii) serious flow instability at the microwave output. In fact, after the initial pressure increase and subsequent flow increase, the flow was observed to dwindle to nothing until the input high pressure pump built the pressure back up again, at which point the flow suddenly restarted. This feature of the system renders it impossible to efficiently interface the unit directly to the ICP-MS.

(4) Blockage of the in-line particle filters and the back pressure regulator.

(5) Contamination and washout problems due to sample contact with metal flange fittings within the valves and the long tubing lengths in the system. Flanged fittings were required to withstand the pressure within the system.

(6) Pressure problems at the points where plastic screw fittings were attached in the system. This mainly caused leaks, but also increased the risk of a tube rupture occurring, with the associated release of hot acid (>100°C) under pressure.

Despite these practical difficulties, a set of data were obtained for on-line microwave digestion of the sediment reference samples, with off-line ICP-MS detection, as presented below (Table 6.5). The range presented for the measured analyte concentrations shows the standard deviation of the mean values of two separate digestions.

Table 6.5. On-line microwave digestion results for the certified reference material analysis using off-line ICP-MS measurement (in $\mu g\ g^{-1}$).

Element	MESS-1		SO-3		BCR 142	
	Measured	Certified	Measured	Certified	Measured	Certified
Co	41.3 ± 2.7	10.8 ± 1.9	30.0 ± 7.9	12 ± 8	34.6 ± 1.7	---
Ni	34.4 ± 3.4	29.5 ± 2.7	40.9 ± 8.0	16	44.4 ± 2.8	29.2 ± 2.5
Cu	34.4 ± 4.3	25.1 ± 3.8	25.1 ± 13.4	17	39.8 ± 1.4	27.5 ± 0.6
Cd	1.05 ± 0.13	0.59 ± 0.10	0.27 ± 0.06	0.14 ± 0.08	0.41 ± 0.01	0.25 ± 0.09
Pb	39.2 ± 3.6	34.0 ± 6.1	15.2 ± 4.0	14	39.1 ± 0.9	37.8 ± 1.9

Results for Zn are not given in this table since contamination problems arising from the injection valve prevented acceptable calibration and sample data being obtained. It is clear from Table 6.5 that there is generally poor agreement between the expected and the measured values, especially for Co and Ni. Since ICP-MS suffers from well documented Ar and Ca polyatomic interferences on these elements (e.g. $^{44}Ca^{16}O$, $^{42}Ca^{16}O^{1}H$ on ^{59}Co and ^{60}Ni respectively), the elevated concentrations measured for these elements could be attributed to this cause. However, the same problem was not observed in the batch digestion with off-line ICP-MS detection study (section 6.7.1.1) so it appears more likely that either contamination or a problem with internal standard correction or with the Co and Ni calibrations has occurred. For the on-line digestion system, the time taken to prepare each sample was approximately 12 minutes (including preparation, uptake and collection time), with a further 3 minutes being required for ICP-MS analysis of the digest. This amounted to an overall analysis time of around 15 minutes per sample. The on-line microwave digestion system with off-line ICP-MS detection therefore offered a time saving of 9 minutes per sample compared to the batch system.

6.7.2 On-line microwave digestion coupled to the ICP-MS.

As described above (section 6.6.3), in order to interface the on-line microwave digestion system with the ICP-MS, it was necessary to incorporate a switching valve in the output line of the microwave to 'capture' a fixed volume aliquot of the digested sample before injecting it into the ICP-MS. This approach utilised a separate carrier stream, operating at a fixed flow rate of 1 ml min^{-1}, to carry the aliquot of sample reproducibly to the ICP-MS. This was necessary because of fluctuations observed in the output flow of the microwave system, related to pressure variation and bubble formation during the

digestion process. Since this flow injection approach was intended to be adopted for the on-line digestion / on-line ICP-MS analysis, some preliminary work was performed in which pre-digested samples of the reference materials were analysed using flow injection as the method of sample introduction. In this way a set of data was generated which could be compared with the results obtained for the complete on-line system.

6.7.2.1 Flow injection analysis of samples digested using the static microwave system.

The results obtained for the flow injection analysis of the pre-digested reference materials are presented below (Table 6.6). The range given for the concentrations measured is based on the standard deviation of the mean of two separate digestions.

Table 6.6. Flow injection analysis results obtained for the reference materials (in $\mu g\ g^{-1}$).

Element	MESS-1		SO-3		BCR 142	
	Measured	Certified	Measured	Certified	Measured	Certified
Co	9.9 ± 0.4	10.8 ± 1.9	5.97 ± 0.01	12 ± 8	8.0 ± 0.1	---
Ni	24.3 ± 0.8	29.5 ± 2.7	19.0 ± 0.1	16	28.2 ± 0.7	29.2 ± 2.5
Cu	21.2 ± 1.2	25.1 ± 3.8	13.9 ± 0.2	17	22.9 ± 1.4	27.5 ± 0.6
Cd	0.60 ± 0.01	0.59 ± 0.10	0.13 ± 0.01	0.14 ± 0.08	0.23 ± 0.03	0.25 ± 0.09
Pb	29.8 ± 1.7	34.0 ± 6.1	9.2 ± 1.1	14	29.4 ± 1.3	37.8 ± 1.9

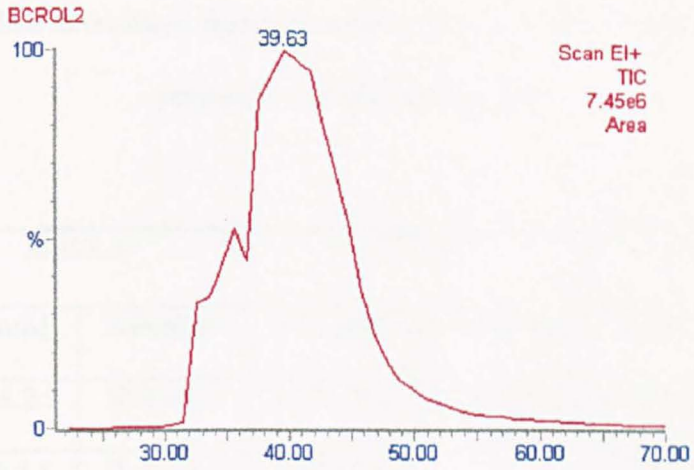
It is clear from Table 6.6 that the results obtained for most of the elements analysed compare relatively well with the accepted values. Results for Zn are not given in this table since contamination problems arising from the injection valve prevented acceptable

calibration and sample data being obtained. The acceptable accuracy and precision of the results obtained in this work illustrated that flow injection analysis was an appropriate approach for this analysis and furthermore, would be expected to be an effective method when applied to the on-line microwave digestion - ICP-MS direct coupling work. It is noteworthy to mention at this point that flow injection sample introduction offered the additional benefit of reducing the level of sample matrix reaching the ICP-MS interface thereby avoiding any possible clogging of the sampler and skimmer cones. In addition, the effective reduction in matrix concentration generated by the dispersion of the small sample slug lead to a reduction of matrix effects within the plasma itself. This feature also improved the washout characteristics of the system by essentially eliminating memory effects between the samples.

6.7.2.2 Direct coupling of the on-line microwave digestion system with the ICP-MS.

Physically interfacing the on-line microwave digestion system with the ICP-MS was in fact a straightforward exercise as indicated earlier (section 6.6.3.2). The coupling process simply involved linking the microwave output tubing via the PrepLab dual 6-port valve to the ICP-MS nebuliser. The difficulty lay in efficiently capturing aliquots of the sample leaving the microwave and reproducibly injecting them into the ICP-MS. This process was complicated by the presence of digestion gas bubbles in the microwave output and the fluctuating flow pattern referred to earlier (section 6.7.1.2, (3)). Nonetheless, it was possible to capture a 200µl aliquot of the digested sample from the microwave and divert this into the ICP-MS. A typical example of the sample peak shapes obtained in this way is illustrated below (Figure 6.4)

Figure 6.4. Typical peak response measured using the directly coupled on-line microwave digestion - ICP-MS system.



In Figure 6.4, it is evident that the peak resembles a typical flow injection trace except that it has spikes present. These spikes are a result of digest gas bubbles caught in the sample fraction injected into the ICP-MS. These gas bubbles prevent reproducible injections from being possible, because the quantity of gas in each sample cannot be controlled. Separating the gas from the liquid under the on-line conditions examined here was not found to be practical, since the technique was dependent on sampling directly from the microwave output stream. Furthermore, gas-liquid separator systems are generally designed to divert the vapour into the detector whilst sending the liquid to waste, rather than vice versa. Despite the difficulties encountered, a set of data were obtained for the three sediment reference materials, using external calibration based on injecting the calibrant solutions through the same valve used to divert the microwave output into the ICP-MS. To compensate for the inherent variability of the on-line microwave digestion system, the samples were spiked with ^{103}Rh internal standard at 50ppb before digestion. The calibrant solutions were similarly spiked with the same internal standard concentration. The results obtained in this study are presented below

(Table 6.7). The range attributed to the measured concentration of each analyte is the standard deviation of the mean value obtained from two separate digestions.

Table 6.7. On-line microwave digestion with on-line ICP-MS detection results for the reference materials (in $\mu g\ g^{-1}$).

Element	MESS-1		SO-3		BCR 142	
	Measured	Certified	Measured	Certified	Measured	Certified
Co	15.01 ± 2.7	10.8 ± 1.9	5.12 ± 0.2	12 ± 8	8.36 ± 0.01	---
Ni	33.7 ± 7.8	29.5 ± 2.7	16.7 ± 1.4	16	26.4 ± 0.1	29.2 ± 2.5
Cu	28.2 ± 1.5	25.1 ± 3.8	12.2 ± 2.2	17	22.3 ± 0.8	27.5 ± 0.6
Cd	0.72 ± 0.01	0.59 ± 0.10	1.04 ± 0.2	0.14 ± 0.08	0.23 ± 0.02	0.25 ± 0.09
Pb	42.2 ± 0.4	34.0 ± 6.1	11.1 ± 1.4	14	36.0 ± 1.4	37.8 ± 1.9

When the extensive complications associated with this analysis are considered, the results obtained for the complete on-line system compare favourably with the accepted values. As observed with the batch microwave digestion results, the data for SO-3 appear to be lower than expected for some elements. As noted previously (section 6.7.1.1) this is a consequence of the incomplete dissolution of the silicate material in this sample. For the on-line microwave / ICP-MS coupled system, the time taken to prepare and analyse each sample was approximately 7 minutes. This amounted to a reduction in the analysis time by 17 minutes per sample compared to the batch microwave digestion system and by 8 minutes per sample compared to the on-line digestion system with off-line detection. Directly coupling the on-line microwave digestion unit with the ICP-MS therefore offered a significant improvement in sample throughput over the alternative approaches.

However, the directly coupled system was more difficult to control in terms of the sampling reproducibility.

6.8 Conclusions.

The use of microwave digestion as a means of sample preparation for sediment materials has been explored. Microwave digestion systems based on batchwise digestion have been compared with an on-line microwave digestion system for the analysis of several trace metals in sediment and soil reference materials. The results of this study showed that the batchwise digestion approach, although time consuming, was effective for preparing samples for ICP-MS analysis. Using on-line microwave digestion with off-line collection lead to a decrease in the actual sample preparation time, but a catalogue of practical problems were discovered during this work. These difficulties, coupled with the relatively poor results obtained, were initially not particularly encouraging for the on-line microwave digestion approach. Interfacing the on-line microwave digestion system with the ICP-MS instrument was successfully achieved. The coupling was accomplished by simply diverting the microwave output through a valve, comprising a fixed volume loop, which captured an aliquot of the digested sample. This aliquot of sample could then be injected and transported to the ICP-MS using a carrier stream independent of the fluctuating output flow of the microwave. This technique offered the (theoretical) benefits of increased sample throughput and reduced risk of sample contamination, because the digestion and analysis processes both took place in the enclosed tubing of the system. Despite the numerous practical difficulties encountered with the on-line digestion system, injection of digested samples was achieved and some encouraging results were obtained.

6.9 References.

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CHAPTER 7

GENERAL CONCLUSIONS AND SUGGESTIONS FOR FUTURE WORK

7.1 On-line matrix separation and preconcentration for ICP-MS.

In the 14 years since its first commercial release, ICP-MS has rapidly evolved to become one of the premier techniques for trace and ultra-trace elemental analysis. ICP-MS offers rapid, multi-element analysis from pg ml^{-1} to μg ml^{-1} concentrations with the added potential of isotopic information. However, the technique has some limitations, notably the formation of polyatomic species which overlap with particular isotopes, high susceptibility to sample matrix effects and low tolerance to high concentrations of dissolved solids (generally up to a maximum of 0.2% (m/v)). The overlap of polyatomic interferences, which are formed by combination of the plasma gas with air gases and components in the sample solution, can be circumvented if another, interference-free isotope of the element is available. However, if the interference obscures an element for which only one isotope is available (e.g. $^{40}Ar^{35}Cl$ on ^{75}As), an interference correction must be employed, which can lead to inaccurate concentration calculations. However, the formation of certain argon related polyatomic interferences (notably $^{40}Ar^{35}Cl$ can be reduced or even essentially eliminated through the use of mixed gas plasmas based on the addition of small amounts of, for example, nitrogen or ethene to the nebuliser gas. Matrix related problems such as significant signal suppression in the presence of high concentrations of easily ionised elements (e.g. Na, K, Li) can often be effectively compensated for using internal standards, but the associated physical problems of torch

injector and interface cone blockage by the salt content of such samples can only be alleviated by diluting the sample. However, dilution of the sample may reduce the analyte concentration to a level below the detection limit of the instrument and will not prevent the formation of polyatomic interferences. The upshot of all this is that ideally the sample matrix should be removed in some way to improve the analysis.

The work in this thesis was principally concerned with developing a procedure for separating and preconcentrating trace elements from saline matrix samples, prior to analysis by ICP-MS. A rapid, manual on-line matrix separation system was constructed and interfaced directly to the ICP-MS instrument. This system incorporated a mini-column containing the chelating reagent 8-hydroxyquinoline (8-HQ) covalently immobilised onto a controlled pore glass support. The system was applied to the analysis of Ti^{4+}, V (as VO^{2+}), Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Ag^{+}, Cd^{2+}, Ce^{3+} and Pb^{2+} in saline matrix samples. Elements retained on the column were eluted into the ICP-MS in the form of transient peaks, which were successfully monitored using recently developed time resolved data acquisition software. For this manifold, a sample throughput of 15 h^{-1} was achieved with a relative precision of less than 3%. It was shown in this study that linear calibrations, which were equivalent in slope, could obtained from spiked pure and sea water solutions. This illustrated that external calibration, using pure water solutions to quantify analytes in more complex sample matrices, was a practical alternative to using matrix matching standards. The efficiency with which the sample matrix was eliminated using this manual system was determined by monitoring the effect of the $^{40}Ar^{23}Na$ interference on the ^{63}Cu : ^{65}Cu ratio. In the presence of Na, the measured ^{63}Cu : ^{65}Cu ratio is abnormally high, but after passage through the manual matrix separation system, the measured Cu ratios were in agreement with the natural value of 2.24, thereby proving that effective matrix elimination had been achieved. Using this

manual matrix separation manifold, results which agreed well with the certified values were obtained for the estuarine CRM SLEW-1 and the coastal CRM CASS-2.

An investigation into the use of a new chelating reagent, PROSEP® Chelating-1, using an automated matrix separation system was performed. This reagent, which contained iminodiacetate functional groups covalently immobilised onto the glass support, was demonstrated to be an ideal material for on-line matrix separation with ICP-MS detection. The rigid controlled pore glass support did not expand or contract with changing solution composition, in contrast to some polymeric materials, which prevented back pressure build up in the system. Furthermore, analyte retention on the material occurred rapidly, allowing high loading flow rates to be used. The automated flow injection manifold developed gave a sampling rate of 12 h^{-1} with relatively low sample consumption (3 ml) and was successfully applied to the analysis of V (as VO^{2+}), Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Ag^{+}, Ce^{3+}, Cd^{2+} $Pb^{2+}$and U (as UO_2^{+}) in saline matrix samples. The manifold design also gave a maximum preconcentration factor of 10. As with the 8-HQ system described above, linear calibrations of comparable sensitivity were obtained with both pure water and saline water matrices. This feature allowed accurate analysis of saline samples using a calibration procedure based on matrix-free water standards, a fact which also illustrated that analyte retention was unaffected by high salinity levels. The effect of residual matrix on the process was found to be negligible by the observation that the ^{63}Cu : ^{65}Cu and ^{48}Ti : ^{49}Ti ratios were unaffected by the $^{40}Ar^{23}Na$ interference and ^{48}Ca isobaric overlap respectively. Use of the PROSEP® Chelating-1 reagent over a period of around 240 hours lead to only slight decrease (1 - 5%) in analyte recovery, illustrating the stability of the material. In terms of analyte retention capacity (based on batch measurements), PROSEP® Chelating-1 was found to perform similarly to CPG - 8-hydroxyquinoline. However, the inflexibility of the CPG support meant that the

capacity of the reagent was lower than polymeric based reagents. On the other hand, the rigid nature of the material, together with its good chemical stability, resistance to dimensional change with changing solution composition and wide elemental application, were found to be highly beneficial features for the on-line matrix separation system developed in this study. With the development of high resolution, magnetic sector ICP-MS, some of the problems encountered with matrix related polyatomic interferences can be overcome. The high resolving power achievable with these systems (>10,000) means that it is now possible to resolve interferences such as $^{40}Ar^{16}O$ from ^{56}Fe, $^{40}Ar^{35}Cl$ from ^{75}As and $^{40}Ar^{23}Na$ from ^{63}Cu, whilst still retaining sufficient sensitivity to measure low pg ml^{-1} concentrations of these elements. The relative cost and physical complexity of these instruments has decreased considerably over the last 10 years, so routine high resolution ICP-MS is gradually becoming a reality. However, despite the technological advancements in ICP-MS instrumentation, the physical problems caused by the presence of saline samples in the plasma remain. For this reason, matrix separation prior to analysis will continue to be necessary for the accurate measurement of trace metals such as Cu, Co, Ni and V in saline samples.

In addition to fully evaluating the performance characteristics of the automated matrix separation manifold, the effects of high concentrations of humic material and a range of elements on the separation and retention process were examined. The concentration of humic material (as humic acid) in the samples was increased from 0 to 50 μg ml^{-1}, to determine the effect on the analyte retention efficiency. It was found that no significant effect was observed on the retention of Ti, V, Mn, Co, Ni, Cu, Zn, Pb and U, illustrating the strong complexation ability of the material. Of the elements tested, only Cu showed any measurable reduction in retention. This was expected since this element is known to readily form strong complexes with humic substances. The observed increase in

response for Mn was concluded to be due to residual humic acid on the column being released by the eluent and passing into the plasma to form the $^{40}Ar^{14}N^{1}H$ species, which overlaps with ^{55}Mn. The effect of increasing the concentration of a range of elements on analyte retention efficiency yielded some interesting results. Concentrations of Mn and U up to 100 μg ml^{-1} had little effect on analyte retention, because of the weak complex formation of these elements with the IDA groups of PROSEP® Chelating-1. In contrast, Cu had a significant effect on most of the elements analysed, because of its high complex formation constant with IDA. Analysis of three saline industrial effluent samples containing high levels of Mn (around 500 to 1000 μg ml^{-1}) showed little effect on the measurement accuracy (based on results obtained by sample dilution) for strongly retained elements, such as Cu and V. However, for more weakly retained elements such as Co and Pb, a significantly lower value than expected was measured.

7.2 On-line microwave digestion coupled to ICP-MS.

In order to analyse trace elements in solid samples using atomic spectrometric techniques, the sample must almost always be digested or dissolved prior to analysis, since few analytical techniques are available for direct solids analysis, and those that are tend to be insufficiently sensitive (e.g. XRF, laser ablation - ICP-MS). Traditionally, solid samples were worked up into solutions using acid digestion, based on an open hotplate approach, operated at atmospheric pressure. This procedure is time consuming, and can be insufficiently rigorous for samples which are resistant to acid digestion (such as certain minerals). In recent years, the advent of microwave assisted digestion has facilitated much faster and more rigorous sample digestion. Microwave digestion uses a combination of high temperature, and often high pressure (with the exception of open

vessel microwave digestion methods) to achieve efficient sample decomposition. In addition, the technique yields strongly oxidising species (such as NO_2Cl) in the digestion mixture, as a result of interaction between the digestion acids and the incident microwave radiation. The efficiency of microwave digestion and the variety of samples for which it is a suitable digestion method have made the technique popular for batch wise sample preparation. The search for faster sample preparation and analysis methods have led to research into on-line microwave digestion, and in recent years, this technique has been applied with varying degrees of success for analysis of samples ranging from whole blood to marine sediments (see Chapter 6, section 6.3.2). In this study, the performance of batch wise and on-line microwave digestion were compared for the analysis of trace metals in sediment and soil reference materials. Batch wise microwave digestion was found to be an effective, though time consuming, method for sample preparation for ICP-MS analysis. In contrast, on-line microwave digestion with off-line collection offered reduced sample preparation time, but resulted in serious practical problems, including blockages, incomplete digestion and contamination. At this stage it appeared that the on-line microwave digestion technique was an inappropriate method for sample preparation. Nonetheless, a an attempt was made to directly interface the on-line microwave digestion system to the ICP-MS instrument. This coupling was successfully achieved by using a valve, which contained a fixed volume loop, to trap an aliquot of the digested sample leaving the microwave. This aliquot of sample was subsequently injected into a separate carrier stream, independent of the varying output stream of the microwave, and transported to the ICP-MS. Using this approach, increased sample throughput and reduced risk of sample contamination, because of the completely enclosed nature of the flow system, were achieved. The on-line microwave digestion - ICP-MS system was used to successfully inject portions of digested samples and some

encouraging results were obtained. Despite this preliminary success, it should be noted that many practical problems were encountered with the on-line microwave digestion technique.

7.3 Suggestions for future work.

In this thesis, the subject areas of on-line trace metal enrichment and matrix separation and on-line microwave digestion have been explored. The results obtained so far indicate that on-line microwave digestion / ICP-MS has potential but more work needs to be done to tackle some of the practical issues. In future, it may be feasible to directly interface on-line microwave digestion with matrix separation systems to facilitate trace element analysis in saline digest samples, such as biological and sewage materials. The matrix separation systems reported in this thesis offered quite rapid sample analysis, but considering the expense of operating an ICP-MS, any additional time saving during sample preparation would amount to a substantial cost saving. With this in mind, a possible future project could involve the development of a multi-column system to allow preconcentration and matrix separation of one sample whilst another is eluted into the ICP-MS. A reduction in size of the matrix separation manifold could also lead to a significant reduction in the sample preparation time and would also permit the analysis of small sample volumes. This approach could be of benefit for the analysis of clinical samples, such as blood plasma and serum, for which only a small sample volume may be available. Reducing the matrix separation manifold dimensions could also ultimately allow the system to be operated with very low flow rates (of the order of $\mu l\ min^{-1}$) which would permit coupling the system to a microconcentric nebuliser.

APPENDIX

Conference and publications record pertaining to the work reported in this thesis.

Posters

June 1994 6th Flow Analysis Conference, Toledo, Spain

July 1994 Research and Development Topics in Analytical Chemistry, Hatfield, UK

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

Jan. 1995 Winter Plasma Conference on Spectrochemistry, Cambridge, UK

July 1995 Research and Development Topics in Analytical Chemistry, Hull, UK

July 1995 Society for Analytical Chemistry Meeting, Hull, UK

July 1996 Research and Development Topics in Analytical Chemistry, Nottingham, UK

Oral presentations

Oct. 1995 National User Group Meeting, VG Elemental, Winsford, Cheshire, UK

Oct. 1995 Federation of Analytical Chemistry Society Symposium, Cincinnati, USA

Dec. 1995 Institute for Reference Materials and Measurements, Geel, Belgium

Jan. 1996 Flow Injection Analysis Meeting, Northern Ireland RSC Division, Queen's University, Belfast, UK

Feb. 1996 Final Year Colloquium, University of Hull, Hull, UK

April 1996 Zeneca Specialities, Blackley, Manchester, UK

June 1996 ICI, Wilton, Cleveland, UK

July 1996 8th Biennial National Atomic Spectroscopy Symposium, University of East Anglia, Norwich, UK

Publications

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