Sample Preconcentration and Matrix Removal Techniques for Ultra-Trace Determinations by Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

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This project is submitted in fulfilment of Higher Education and Training Awards Council requirements for the Degree of Doctor of Philosophy

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Submitted to the Higher Education and Training Awards Council,
September 2003

Dedication

In appreciation of the personal sacrifices of my parents and family,
I wish to dedicate this work to
John and Mary Casey,
my grandmother, Hannah Casey and
my brother and sister, Declan and Jean.

To all my friends both new and old,
Thank you for your help,
The laughter and some sadness we shared during the last year of my work will be an everlasting memory.

"*No ay memoria a quien el tiempo no acabe, ni dolor que nuerte no le consuma*"
Don Quixote (III, 1), Miguel de Cervantes Saavedra (1547-1616).

"*Planyeta yest' kolybyel razuma, no nyelzya vietchno zhit' v kolybyeli*"
Konstantin E. Tsiolkovsky (1857-1935).

"*Forgiveness is the key to action and freedom*"
Hannah Arendt (1906-1975)

"*Cogito ergo sum*"
René Descartes (1596-1650).

Sample Preconcentration and Matrix Removal Techniques for Ultra-Trace Determinations by Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

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Abstract

This thesis describes the design and application of a laboratory-based matrix removal/preconcentration system using an iminodiacetate chelating ion exchange reagent. The chelating ion exchange reagent selectively isolates trace elements in solution from the matrix (macro levels of Ca, Mg, Na and various anions in solution) of analytical samples. This leads to reduced matrix and spectral interferences and improved detection limits for atomic spectrometric determinations. The system is used in conjunction with a low-flow microconcentric nebuliser and Inductively Coupled Plasma Mass Spectrometry (ICP-MS). The analytical features of the system are described. These include the pH dependence of analyte recovery, the reproducibility and detection limits achievable by this technique.

The system is evaluated for the determination of trace elements in bivalve shells, mineral waters and certified water reference materials. Results of an extensive study profiling trace element constituents in bivalve shells around the Irish coast are presented. The results provide baseline data on the impact of trace element pollution on the Irish marine environment. In particular, the anthropogenic impacts of trace element pollution from the dumping of sewage sludge and dredge material at sea is dicussed.

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Declaration

The work reported in this Thesis was carried out solely by Noel Casey during the period September 1998 to September 2003. This Thesis has not previously been submitted to any other institution or awarding body for consideration for the award of any degree.

Signed: Noel Casey
Date: 9/10/03 Jean Bartlett

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Acknowledgements

I wish to acknowledge the following sources of financial assistance for my research

The Graduate Training Programme (GTP) and also the Biosolids Research Programme through Cycle 2 of the Higher Education Authority (HEA) Programme for Research in Third Level Institutions (PRTLI) part of the National Development Plan (NDP).

I wish to thank:

My supervisor, Dr. Ted McGowan for guiding me throughout my entire experience of research in the Instititue of Technology, Sligo.

Dr. Evin McGovern of the The Irish Marine Institute and Dr. Bret Danilowicz (Department of Zoology, University College Dublin) for providing the bivalve shell samples.

The staff of the Instititue of Technology, Sligo for both their technical and personal assistance during my work.

I would also like to extend my personal gratitude to the following people

John Anderson, M.Sc, of Anderson and Associates Consulting Forensic Engineers and Scientists, for both his technical and personal assistance.

To my fellow research students: Pamela McDonnell, Percy Foster, Ericka Murray and Javier Montero Serrano for sharing an office with me during the writing up phase of my research.

To Jose, Leticia and Pablo for providing both sustenance and friendship.

To my friend Sarah Gubbins for providing personal and technical assistance during my research in the Instititue of Technology, Sligo.

Table of Contents

IT Sligo
An Institiúid Teicneolaíochta, Shligeach

List of Figures

List of Tables

IT Sligo
An Institiúid Teicneolaíochta, Sligeach

Chapter One

1.0 Sample Introduction in Atomic Spectrometry.

The techniques of Atomic Absorption Spectrometry (AAS), Inductively Coupled Plasma Emission Spectrometry (ICP-AES) and Inductively Coupled Plasma Atomic Mass Spectrometry (ICP-MS) incorporate sample introduction system as a common component of their basic instrumentation. The modular layout of each instrument system with sample introduction is shown in **Fig. 1.1.**

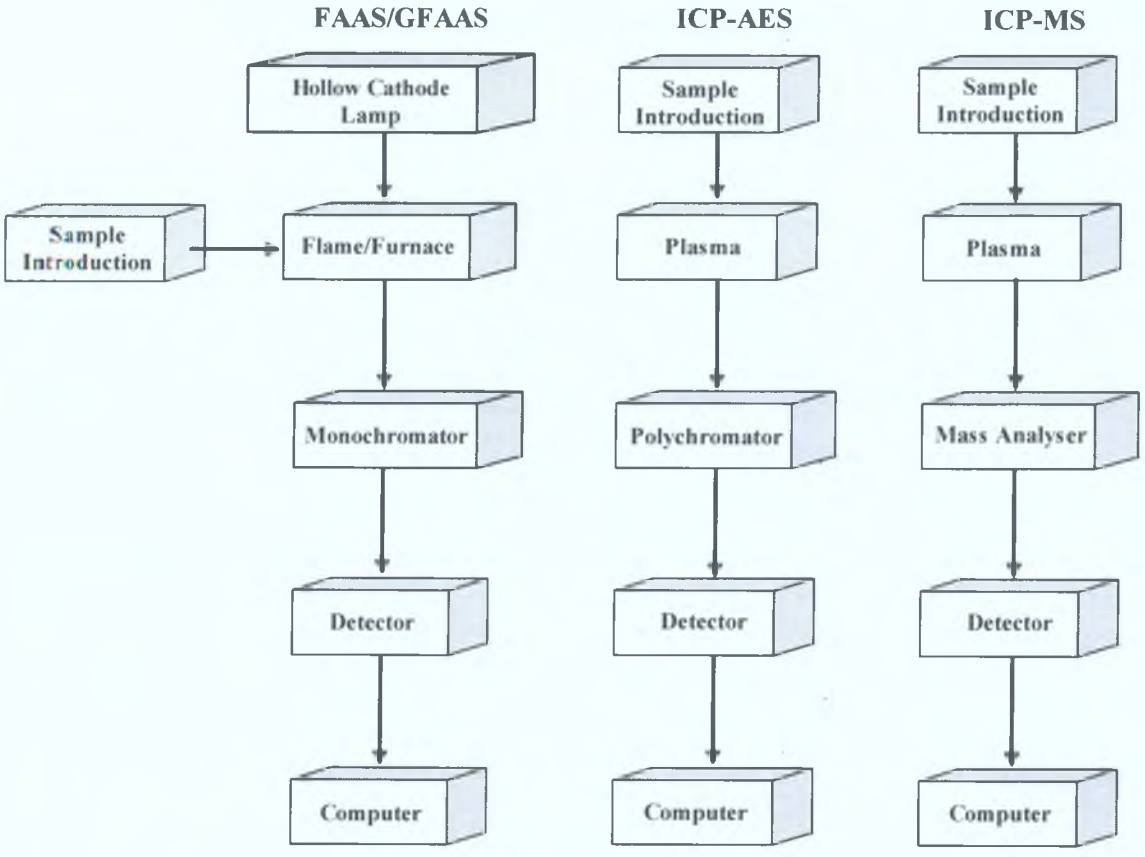


Fig. 1.1 Atomic spectrometry systems.

Samples for trace element analysis can be in the form of liquids, solids or gases. However modern instrumentation requires samples to be in a liquid form prior to sample introduction. The general methods for introducing solution samples into flame and plasma atomisation devices are summarised in **Fig 1.2**.

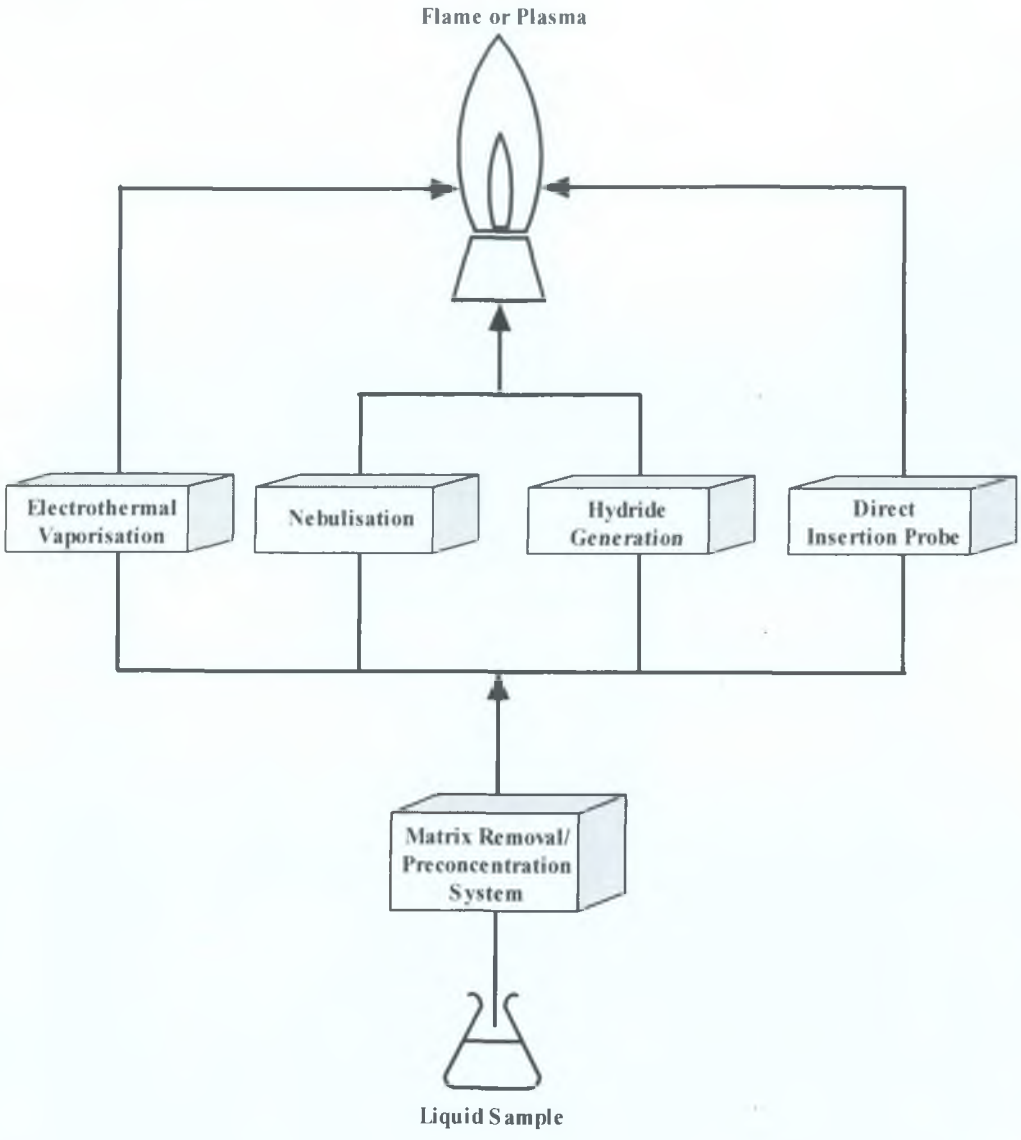


Fig. 1.2 Solution based sample introduction for atomic spectrometry.

Various systems have been developed for the introduction of solids, liquids and gases into plasma and flame sources. The introduction of samples into atomisation devices can be affected by a wide variety of physical and chemical interferences due to the sample itself or arising from features of the instrumental technique. Some examples of typical physical and chemical based interferences are shown in **Table 1.1**.

Table 1.1 Factors that affect sample introduction in atomic spectrometry

Physical Limitations	Chemical Limitations
Sample Viscosity	Sample Acidity
Dissolved Solids/Particulates	Memory Effects
Instrumentation design	Organic or Aqueous Samples

For all atomic spectroscopic methods, the analyte must be converted into the appropriate form in order to either absorb/emit radiation or generate charged ions suitable for mass spectrometry. This involves the application of heat to break up molecules into vapour phase atoms or ions [1]. Therefore, the conversion of sample analytes in solution into vapour-phase free atoms is not a simple task, and research is still continuing in this area.

The demand for lower detection limits with complex sample matrices has led to the development of sample pretreatment techniques prior to sample introduction. Sample pretreatment includes the use of sample dissolution techniques and matrix removal/preconcentration techniques. The main aim of this research involves the development and use of a matrix removal/preconcentration system to be used in association with the ICP-MS technique for the determination of trace elements. An introduction to matrix removal/preconcentration techniques and a brief description of the current sample introduction methods used in atomic spectrometry is given in this chapter.

1.1.1 Solution Nebulisation.

Solution samples are most commonly introduced into flames or plasmas directly via a continuous nebuliser, e.g. pneumatic nebuliser for FAAS and the Meinhard nebuliser for ICP-MS. A typical pneumatic type nebuliser is illustrated in **Fig 1.3**.

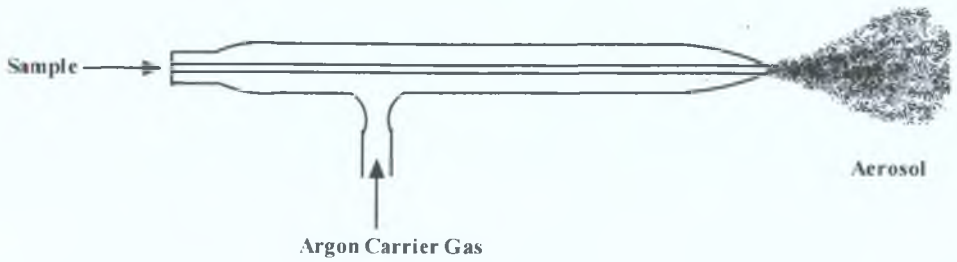


Fig. 1.3 Solution-based Meinhard nebuliser

The argon carrier gas exits the outer glass shell at high speed and carries the liquid sample from the nebuliser capillary tip. This generates a fine sample aerosol, which can be efficiently atomised/ionised in a plasma source.

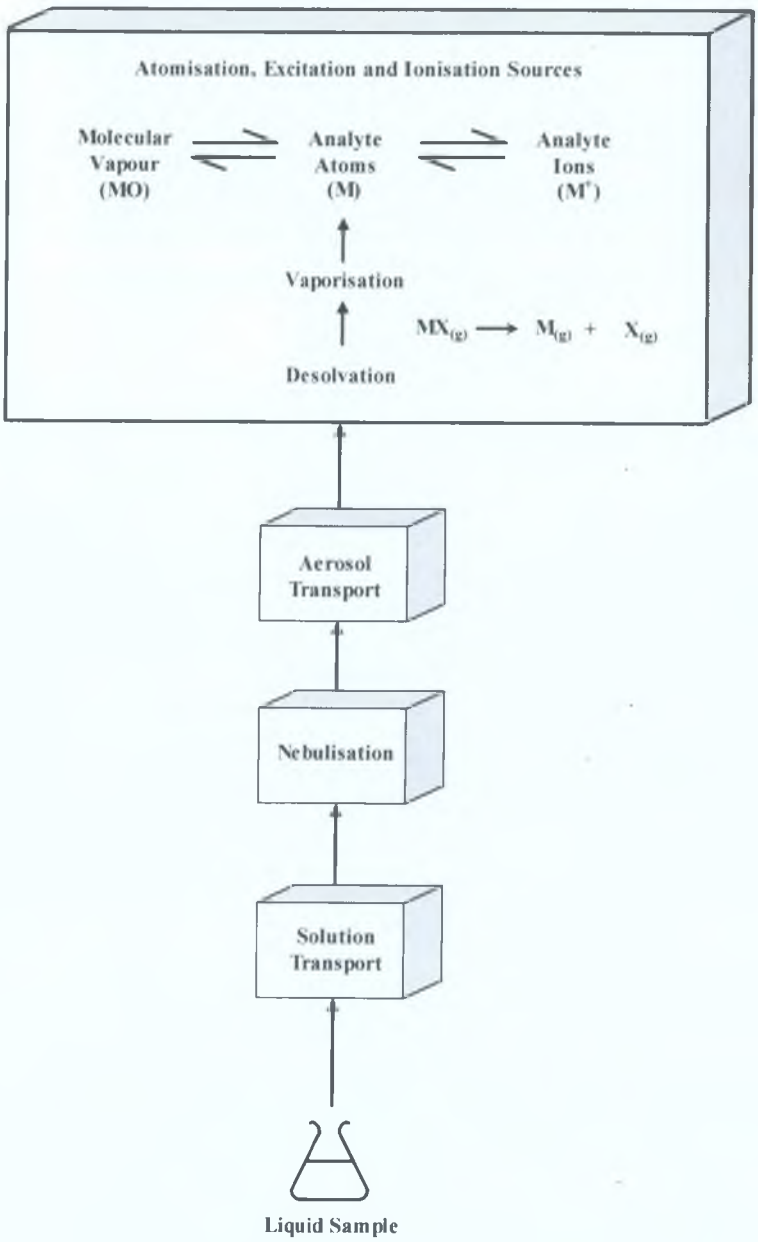


Fig 1.4 Overview of solution based nebulisation [2]

The steps involved in solution nebulisation are shown in **Fig 1.4**. The liquid sample is transported to a nebuliser where an aerosol of fine droplets is generated. The aerosol is then transported to the flame/plasma source. Heat from the source first removes the solvent and produces aerosol particles. Further heating leads to the vaporisation of the particles and the production of atomic, molecular and ionic species of the trace analytes. These different species are usually in thermodynamic equilibrium in confined regions of the flame/plasma source [1].

Solution based nebulisation is a complex process which can be affected by a high dissolved solids content, acidity and the viscosity of the sample. In order to overcome the limitations of the nebulisation process, matrix removal/preconcentration techniques have been developed to be used prior to solution nebulisation. For example, matrix removal/preconcentration prior to solution-based nebulisation ICP-MS was used for the analysis of rare earth elements (REE's) in seawater [3, 4] and fish otoliths [5]. An iminodiacetate-chelating reagent was used to remove the seawater and otolith sample matrix in addition to preconcentrating the analytes. The removal of the sample matrix reduced the physical and spectral interferences caused by these sample types.

Conventional nebulisers are very inefficient in terms of sample use since only 1-2% of the sample is introduced into the flame or plasma source. There has recently been much improvement in this area due to the design of low flow high efficiency nebulisers for ICP-MS instruments. A low flow Microconcentraic nebuliser (Cetac Technologies, Omaha, NE, USA) was used in conjunction with a matrix removal/preconcentration system for analysis of trace elements in water [6]. The nebulisers efficiency increased the overall efficiency of the system in terms of chemical reagent use and sample volume analysed.

1.1.2 Hydride Generation (HG).

Most elements are determined by atomic spectrometry after nebulisation of a solution into a flame or plasma. However, for some elements it is possible to generate a volatile hydride of the element by a chemical reaction in solution followed by transfer of this vapour into an instrument for subsequent measurement.

A number of elements form volatile hydride vapours by reaction with sodium borohydride and hydrochloric acid, these elements include arsenic, antimony, bismuth, lead, selenium, tellurium, tin and mercury. Hydride generation can be carried out in a continuous flow system and the generated metal hydride carried to the flame or plasma source by argon gas, **Fig. 1.5.**

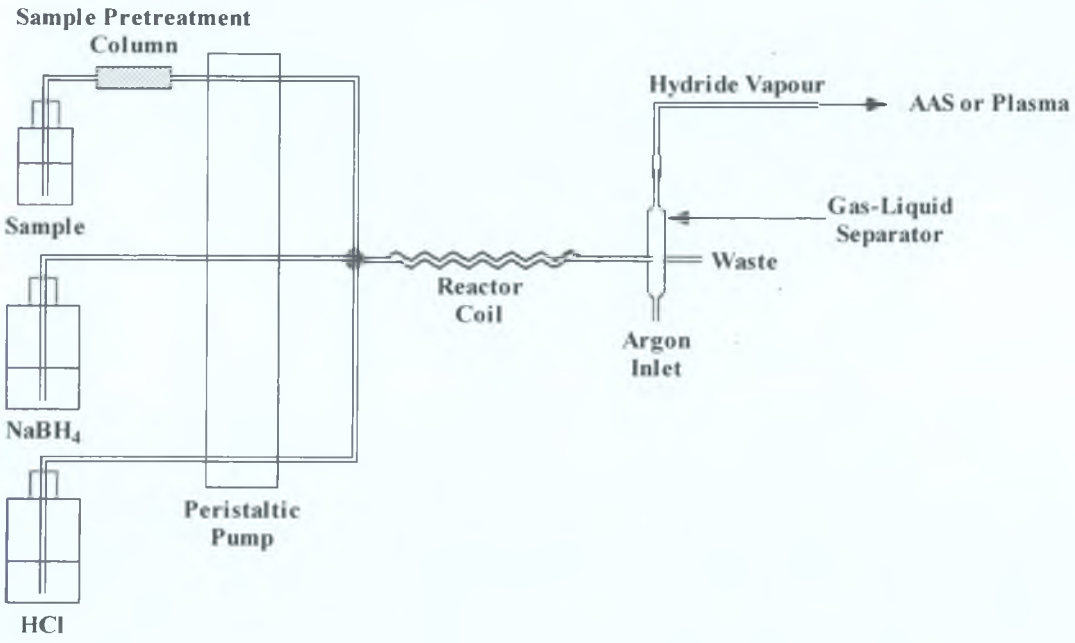


Fig. 1.5 Schematic of Hydride Generation system using column based sample pretreatment.

The major advantage when the element is transferred to the vapour phase is that it is removed from the sample matrix with an improvement in detection limits by a factor of ten or more. The generation of a hydride vapour bypasses the need for a solution-based nebuliser. This leads to increased sensitivity and eliminates sample introduction interferences arising from the solvent. When compared to conventional nebulisation techniques, hydride generation ensures almost 100% of sample analyte reaches the plasma source compared to approximately 1-2% for a conventional nebuliser.

The chemical equation for conversion of a metal analyte into a hydride is shown below.

$$NaBH_4 + 3H_2O + HCl \longrightarrow H_3BO_3 + NaCl + 8H^+ \xrightarrow{M^{n+}} MH_{n(g)} + H_{2(g)}$$

This technique, however, is not immune to interferences from the sample matrix and transition metals present in the sample can inhibit the formation of the hydride. A matrix removal/preconcentration step can be used to remove trace element interferences prior to hydride generation. One approach is to use activated alumina as an anion exchanger to quantitatively recovery the arsenic hydride while removing the interfering sample matrix [7, 8].

In another approach, interferences caused by transition elements were removed prior to hydride formation using an iminodiacetate chelating reagent. The chelating reagent removes the transition elements from the sample while the hydride forming species passes through the column to the hydride generation system [9, 10].

1.2 Discrete Sample Introduction.

Discrete samples are introduced as vapours using a variety of devices including electrothermal vaporisers, by direct insertion of probes into the flame or plasma and more recently by the highly successful method of laser ablation.

1.2.1 Electro Thermal Vaporisation (ETV).

Electrothermal vaporisation (ETV) has become a widely accepted method of sample introduction and is characterised by electrical resistance heating of the sample. The most common electrothermal vaporisation method is based on the graphite furnace, **Fig 1.6**. This furnace is a similar device to that used in furnace atomic absorption however its main function is to generate analyte vapour rather than ground state atoms prior to introduction to an ICP-MS instrument.

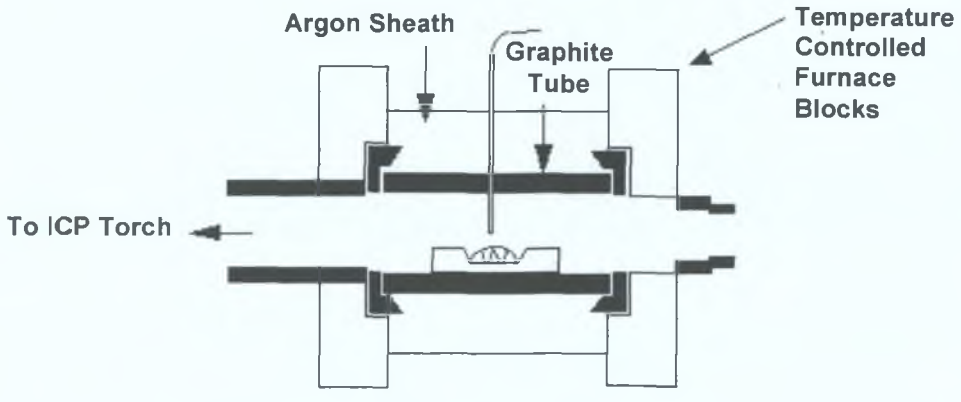


Fig 1.6 Schematic of ETV sample introduction system [11]

Liquid samples are introduced into the furnace, which is then heated to provide the thermal energy to break chemical bonds within the sample and to produce free ground-state atoms. The amount of light absorbed by the ground-state atoms is measured by the method of GFAAS. The amount of light energy absorbed increases as the concentration of the trace element increases. GFAAS is a more efficient method of atomisation because the entire liquid sample is atomised via electrothermal heating of the furnace.

The vapour can be transported to an ICP-MS and its trace element composition determined. ETV is most useful where sample sizes are small and ultra-trace analysis is required. The advantages, which ETV provides for atomic spectrometric techniques when compared to sample introduction by solution nebulisation are

(1) Microsample volumes can be analysed.
(2) Higher transport efficiency when compared to solution nebulisation.
(3) The ability to analyse organic samples.
(4) Matrix separation using temperature control and matrix modification.
(5) Significant reduction in matrix induced interferences.
(6) Very low absolute detection limits.

ETV is affected by interferences in the sample matrix and background spectral interferences are also a problem. The matrix affects the analyte atomisation process and reduces the overall sensitivity of the technique. Matrix removal/preconcentration systems have been used to overcome matrix and spectral interferences in ETV based sample analysis methods. The reduction of ETV based interferences achieved when using the Chelex 100 chelating ion exchange resin for matrix removal has been extensively studied by Marco Grotti [12-14].

A review of flow injection based matrix removal/preconcentration techniques carried out by Zhaolun Fang [15] also highlights the importance of sample pretreatment prior to trace element analysis by ETV based techniques.

1.2.2 Direct Sample Insertion (DSI).

The technique of direct sample insertion was first described by Horlick [16, 17] . Direct sample insertion involves the sample being placed onto or into a sample probe which is then inserted directly into the core of a flame or plasma, **Fig 1.7**. This technique can handle both solid and small volume solution samples.

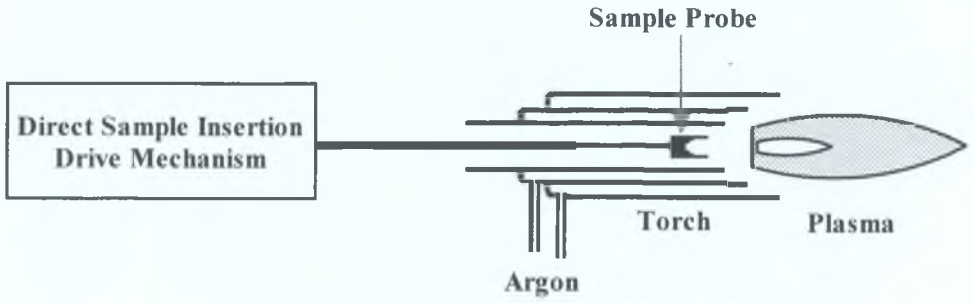


Fig. 1.7 Direct sample insertion probe.

The advantages of DSI when compared to other sample introduction techniques are based on the fact that, unlike nebulisation and external vaporisation devices, direct insertion of the sample into the flame or plasma achieves almost 100% transport efficiency. This results in improved detection limits. By directly analysing solid samples, sample dissolution and dilution are avoided thereby reducing the risk of sample contamination and increasing sample throughput.

The Delves Cup FAAS technique was specifically developed for the analysis of lead in micro volume samples [18]. It involves the direct insertion into a flame of sample using a nickel crucible or tantalum sampling boat. This method has been superseded by the development of graphite furnace based atomic spectrometry.

A number of reviews in this area of sample introduction for ICP-OES have been carried out although the use of the technique in ICP-MS has been limited [19, 20]. Disadvantages of this technique include the difficultly of obtaining good reproducibility and precision placement of the probe in the flame or plasma is essential. Direct insertion of the sample in the flame or plasma has not become a common sample introduction technique due its instrument-based complexity.

Matrix removal/preconcentration techniques have been used in conjunction with direct sample insertion. These include simple evaporation of the sample solvent prior to introduction to the plasma [21], insertion of eluent from a chelating reagent [22] and also direct insertion of the preconcentrating reagent with the chelated analytes, as a solid, directly into ICP plasma [23].

1.2.3 Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS).

The direct trace element analysis of solids can be achieved by using the technique of Laser Ablation ICP-MS (LA-ICP-MS) [24]. The basic overview of a laser ablation system is shown in **Fig 1.8**.

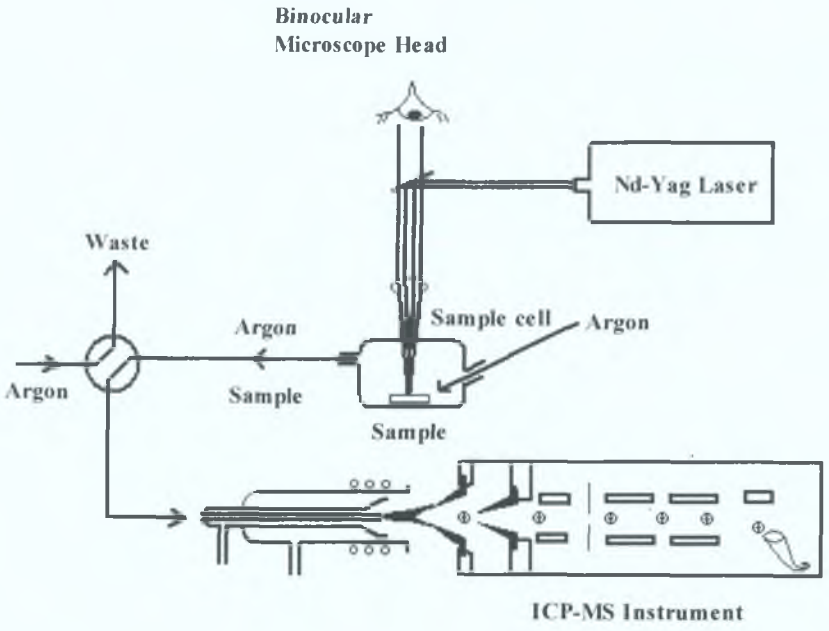


Fig. 1.8 Solid based laser ablation sample introduction technique [25].

A sample is placed inside the sample cell. The Nd-Yag laser is focused onto the surface of the sample using the binocular microscope head. The pulsed laser beam is used to remove very small amounts of material from the sample and this process is called laser ablation. Argon carrier gas transfers the ablated sample to the Inductively Coupled Plasma. Here, the sample plume is disassociated into atomic species and the atoms are ionised. The ionised atoms enter the mass spectrometer and are analysed to determine the concentration of analytes in the sample. This type of analysis avoids the use of sample dissolution techniques and does not require the use of nebulisation based ICP-MS. The introduction of this dry sample directly into the plasma avoids the formation of polyatomic interferences caused by the reaction of water and acid with the argon plasma. However, matrix and spectral interferences caused by solid sample are not eliminated.

1.3 Hyphenated sample introduction techniques.

In order to extend the capability of an analytical method, two or more analytical techniques that are traditionally used separately may be combined.

A Hyphenated technique is the common term for combining two techniques into a single system. An example of such a system would be a matrix separation/preconcentration system combined with atomic spectrometry as the detection system. Hyphenated techniques, which are used prior to sample introduction include: Ion chromatography, High performance liquid chromatography and Flow injection based instrumentation (**Fig 1.9**). All of these techniques have been used in conjunction with nebulisation or hydride generation sample introduction [8, 26-28]. The coupling of these techniques means that the flame or plasma based system is being used as a trace element detector for the separation technique.

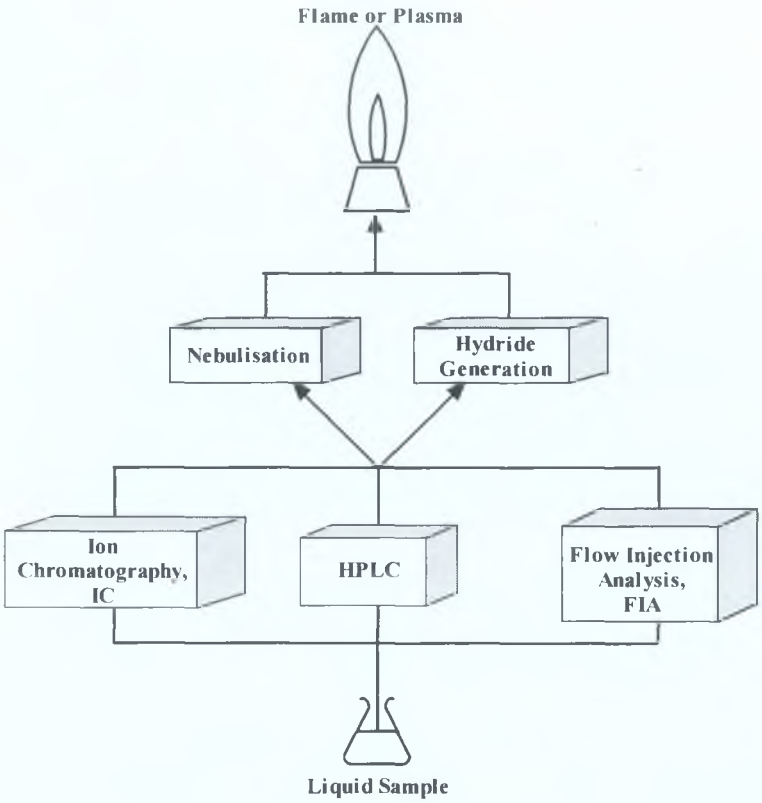


Fig. 1.9 Hyphenated techniques prior to sample introduction.

1.4 Matrix Removal/Preconcentration

Under ideal conditions, the analysis of trace elements would not require any initial preconcentration or separation stages. However, direct instrumental analysis is often not possible because the concentration of analytes are below the limits of detection of the method or the matrix provides an interference, which must be removed.

Often both problems occur simultaneously. Therefore it is still frequently necessary to use chemical based methods that separate and preconcentrate the analyte(s) from a particular sample in order to obtain a quantitative measurement. Separation of an analyte from a sample matrix removes interferences that can cause negative or positive errors on the measurement.

These separation techniques are usually time consuming and prone to a wide variety of experimental errors. In fact, the use of just a separation technique may not reach required detection capability, which is required for the measurement of most samples.

Preconcentration increases the concentration of the analyte(s) of interest in solution, or more precisely the mass ratio of the analyte(s) to sample matrix. It also has the advantage of either partially or fully removing any interference caused by the sample matrix. Usually the interferences requiring removal are high concentrations of other elements in the sample matrix.

Preconcentration techniques can be categorised in three general classes [29, 30]: Relative, Absolute and Selective. Relative preconcentration: Increases the amount of trace analytes relative to that of the main interfering matrix elements. The aim of relative preconcentration is to exchange the matrix for a suitable matrix to eliminate the interference from the sample matrix. Coprecipitation and ion exchange techniques are an example of relative preconcentration methods.

Absolute preconcentration: Trace elements are transferred from the sample of larger mass into the sample of smaller mass, so that the concentration of the trace elements is increased. Examples include the decrease in solvent volume during evaporation [31] and the transfer of trace elements from an aqueous solution into a smaller volume of organic solvent by extraction [32].

Selective preconcentration occurs when trace elements are selectively isolated from a sample. It is used when the simultaneous presence of several components in the concentrate may interfere with the results of the analysis. It is suitable for single element chromatographic based methods, e.g. Arsenic speciation by IC-ICP-MS [33].

A matrix removal/preconcentration method is judged by (1) its ability to preconcentrate the analyte(s) of interest and (2) the minimisation or total removal of interferences. This can be expressed in a mathematical equation

$$P = (R \cdot D)/100$$

Where **P** is Preconcentration factor (the concentration ratio of the analyte(s) in two different phases e.g. liquid-liquid extraction, for a recovery yield of less than 100%); **R** is Recovery yield (the efficiency of analyte recovery expressed in per cent);

D is Interference removal ratio, the ratio of the proportion of matrix interference-to-analyte before sample treatment to the proportion after treatment.

Matrix removal/preconcentration techniques have a number of advantages over direct instrument analysis.

(1) Improves (lowers) detection limits.
(2) Enhances accuracy.
(3) Simplifies calibration since the need to match matrices in the sample and standards is reduced or eliminated.

Although all the above points are advantages of preconcentration, there are some cases where one advantage may cancel out the other. An example would be when using a coprecipitation preconcentration procedure; an excess of the coprecipitating agent yields a higher recovery but also leads to higher limits of detection due to a larger signal blank. This means that a compromise should be reached between these factors. This is always dependent on the type of preconcentration method and the analytical instrument used to obtain the quantitative measurement.

The disadvantage of using matrix removal/preconcentration techniques is that any extra step in an analytical method is undesirable. It increases instrument complexity and the overall cost of the analysis. The need for a preconcentration step is now becoming a standard part of new analytical methods and since these techniques are increasingly automated some of the disadvantages of preconcentration methods (e.g. labour intensive and a low sample throughput) are being gradually overcome.

Evaporation, coprecipitation, solvent extraction, and ion exchange techniques are the most widely used methods of matrix removal/preconcentration. Therefore an overview of these methods is given in the following sections.

1.4.1 Evaporation

One of the simplest methods of preconcentration for analytes may be the evaporation method. The sample is acidified and the solvent is allowed to evaporate. Relatively high preconcentration factors can be achieved with this method. Since there is no matrix removal step, this method is primarily used for relatively clean samples where the matrix does not interfere with the method of analysis. The preconcentration of trace elements of electronic grade water was carried out by the technique of evaporation [31]. The sample was then redissolved in nitric acid and its trace element composition analysed using ICP-MS instrumentation.

The technique of evaporation has also been used in conjunction with other sample pretreatment techniques such as chelation ion exchange [34]. A chelation ion exchange step was carried out and the acid eluent was then evaporated to dryness. The analytes were then redissolved in a smaller volume of nitric acid matrix prior to introduction to the ICP-MS instrument.

1.4.2 Coprecipitation.

Coprecipitation has been defined as the incorporation of impurities, which are usually soluble in the liquid phase, into a precipitate. The precipitate can be thought of as the carrier of the impurities. In the field of analytical chemistry it has been utilised to carry out the preconcentration of many trace elements by forming a precipitate in a sample matrix. The precipitate can then be separated from the sample and redissolved in a smaller volume of liquid thus achieving preconcentration of the trace elements originally in the sample matrix [35-36].
There are two mechanisms by which coprecipitation can occur [37].

1. Isomorphous mixed crystal formation: This is where one of the ions in the crystal lattice of the precipitate is replaced by the ion of a trace element to form a mixed crystal. This occurs if the crystals are isomorphous and the ionic radii of the ions involved are similar.

2. Adsorption: Unlike molecules or ions within the crystal, which are surrounded by other molecules or ions, those on the surface interact with neighbours underneath. Consequently the species on the surface can attract molecules or ions from the solution, adsorbing them onto the surface of the precipitate. The adsorption process can occur after the precipitate has formed or during its formation. Trace elements can be adsorbed on the precipitate surface (surface adsorption) or on growing crystals of the precipitate (internal adsorption).

There are two main groups of coprecipitants, inorganic and organic. The most common inorganic coprecipitants used are hydrous oxides such as: $Fe(OH)_3$, MnO_2, $La(OH)_3$, $Mg(OH)_2$ and $Y(OH)_3$. This is due to the fact that most metals form insoluble hydroxides at certain pH values. The use of inorganic coprecipitants is a common preconcentration technique for natural dilute solutions such as fresh water, seawater and treated waste solutions. Major elements (Na, K, Mg and Ca) in solution do not coprecipitate to any significant extent while the trace elements are coprecipitated. The type of coprecipitant used depends on its properties and the potential interferences that it has on the method of determination.

Many methods of coprecipitation of trace elements with organic compounds have been developed. These usually involve the use of two reagents, which are added to a sample matrix. The first is a complexing agent which complexes the trace elements of interest while the other is an organic solvent, which is sparingly soluble in aqueous solution thus forming a precipitate of the complexed trace element in solution. The most commonly used organic coprecipitants are oxines (such as 8-Hydroxyquinoline), Diphenylthiocarbazone and Dithiocarbamates.

Separation and preconcentration of trace elements (Cu, Fe, Ni, Co, Pb, Cd, Mn and Cr) in urine, sediment and dialysis concentrates was carried out by coprecipitation with Samarium hydroxide with Flame Atomic Absorption Spectrometry (FAAS) [38]. The simultaneous determination of Pd, Pt and Rh in environmental airborne and road dust samples was carried out by ICP-MS using Tellurium hydroxide coprecipitation [39]. These are examples of trace element coprecipitation using inorganic carriers. The trace elements in the samples were redissolved in the appropriate solvent and analysed by FAAS and ICP-MS respectively.

A flow injection on-line coprecipitation system coupled to an ICP-MS was applied to the determination of Cr, Mn, Fe, Co, Ni and Cu in certified estuarine water [40]. Sodium diethyldithiocarbamate (NaDDTC) was used as the organic based coprecipitant. The determination of trace amounts of molybdate in natural waters was carried out using thionalide-ammonium pyrrolidinedithiocarbamate as the organic coprecipitation reagent and subsequent neutron activation analysis [41].

1.4.3 Solvent extraction

Solvent extraction is another method widely used for preconcentration and separation. Most trace metals can be extracted with a chelating agent into organic solvents. The method is based on the relative solubility of a chelate in two immiscible liquids, e.g. water and Methyl Isobutyl Ketone (MIBK). This technique has been used to remove interferences, preconcentrate trace elements and improve detection limits for different atomic spectrometry techniques [42-44].

The distribution coefficient is an equilibrium constant that describes the distribution of a metal chelate, A, between two immiscible solvents can be written as the following equilibria.

$$A(aq) \rightleftharpoons A(org)$$

$$K_d = \frac{[A]_{org}}{[A]_{aq}}$$

The distribution coefficient, K_d, is approximately equal to the ratio of the solubility of the metal chelate, A, in two immiscible solvents. The chelate is usually nearly insoluble in water. In general chelating agents are often quite soluble in organic solvents but have limited solubility in water.

Ideally for efficient matrix removal/preconcentration a distribution coefficient, K_d of >>1 is required, **Fig 1.10**. This leads to the complete transfer of chelate from the aqueous to the organic layer.

If the distribution coefficient for the solvent extraction is equal to one, $K_d \sim 1$, then the separation of the matrix from the metal chelate is incomplete. This results in poor recovery of the analyte in the extracting organic solvent.

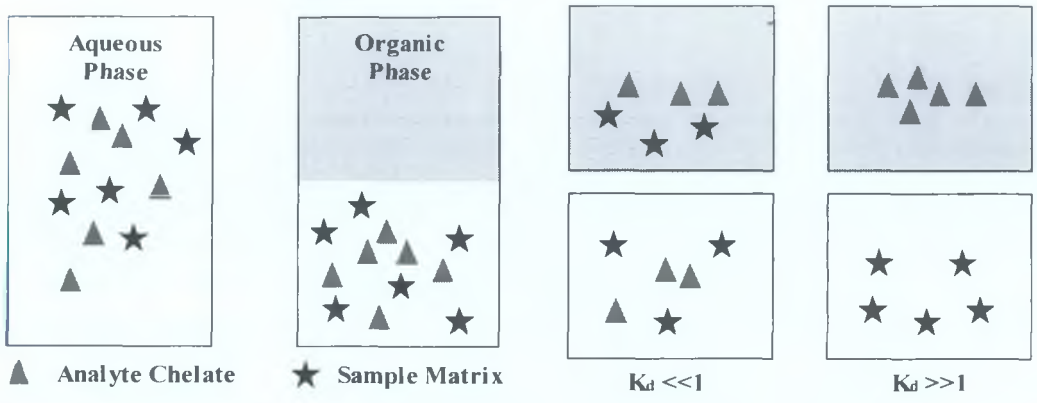


Fig. 1.10 Solvent extraction with a distribution coefficient

Organic solvents used for the extraction of chelated trace elements require the following properties

1. Immiscible with water (non-polar)
2. High solubility for metal chelate compounds
3. Relatively low boiling point (removal)
4. Non-toxic

Solvents such as ketones, esters, ethers, alcohols and other oxygen containing hydrocarbons are suitable for atomic spectrometry techniques.

Methyl Isobutyl Ketone (MIBK) is an example of an organic compound used in solvent based extraction procedures and is widely used with chelating agents such as Oxine (8-Hydroxyquinoline), Diphenylthiocarbazone and Ammonium pyrrolidine dithiocarbarmate (APDC). The chemical structures of these chelating agents illustrating metal complexation are shown in **Fig. 1.12**.

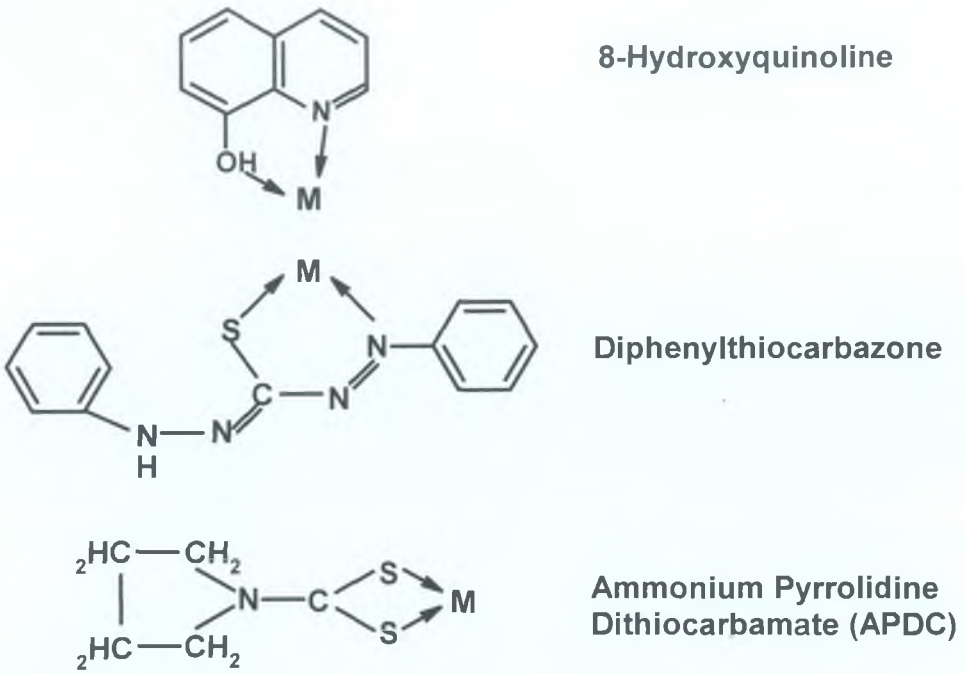


Fig 1.11 Typical chelating agents used for solvent extraction. M; metal ion.

The main applications of solvent extraction method are for the preconcentration of trace elements and also separation from interferences in the sample matrix. However, it is a more labour intensive procedure when compared to ion-exchange based techniques. This makes the technique less feasible for routine analysis.

The following are some examples of the application of solvent extraction in association with atomic spectrometry for trace element analysis. Trace elements (Cd, Co, Cu, Fe, Ni, Pb and Zn) in seawater were determined by ICP-MS using an off-line dithiocarbamate based solvent extraction technique. The simultaneous trace element analysis capability of ICP-MS instrument reduced analysis time and improved detection limits for the technique [45].

An automated on-line solvent extraction/back extraction procedure was developed for the determination of cadmium in soils, plant and water samples by electrothermal atomic absorption spectrometry (ETAAS) [32].

A flow injection based technique was developed for the analysis of hexavalent chromium by using solvent extraction preconcentration and its subsequent determination by electrothermal atomic absorption spectrometry (ETAAS) [46].

The Cr (VI) is complexed by reaction with ammonium pyrrolidine dithiocarbamate (APDC), and the chelate formed is extracted into MIBK in a knotted reactor made from PTFE tubing. The organic solvent is separated from the aqueous phase and delivered to the ETAAS instrument.

1.4.4 Ion Exchange.

An ion exchange reaction may be defined as the reversible interchange of ions between a solid phase (the ion exchanger) and a solution phase; the ion exchanger is insoluble in the medium in which the exchange is carried out.
Cation exchange can be described as follows

$$R\text{-}H^+ + M^+ \rightleftharpoons R\text{-}M^+ + H^+$$

R Functional group
H^+ Hydrogen ion
M^+ Metal ion

The positively charged metal ion (M^+) exchanges with the hydrogen ion (H^+) present on the cation exchanger functional group. Anion exchange can be described as follows

$$R^+A^- + B^- \rightleftharpoons R^+B^- + A^-$$

R Functional group
A^- Counter ion
B^- Metal anion

The negatively charged anion (B^-) exchanges with the counter ion on the anion exchanger functional group.

All ion exchangers consist of a substrate and ion exchanger group. These groups are covalently bonded to the substrate. Modern synthetic ion-exchangers use polystyrene divinylbenzene (PSDVB) as the substrate. PSDVB provides a chemically stable three-dimensional network on which the ion exchanger can be attached [47].

Cation exchangers contain acidic functional groups i.e. sulphonic, carboxylic and phosphoric acid groups. Anion exchangers contain basic inorganic groups such as primary, secondary and tertiary amine groups. Based on the dissociation ability of the ion exchanger group, a further classification is possible; i.e. strong, medium or weakly acidic (or basic) ion exchange resins. In **Table 1.2**, examples of ion exchangers including their classification are shown [48-49].

Table 1.2 Ion exchanger types

Ion exchanger	Type	Functional Group
Cation	Strongly Medium Weakly	Sulphonic Phosphoric Carboxylic
Anion	Strongly Medium Weakly	Quarternary amine Primary, Secondary, Tertiary amines,
Chelating	Weakly Strongly	Iminodiacetic Amino phosphonic

Iminodiacetic acid (IDA) is a weakly acidic compound (pK_1 = 2.98, pK_2 = 9.89) comprised of two carboxylic acid functional groups and also a secondary amine group. A major feature of IDA is that it can act as both an ion exchanger as well as a chelating agent and its metal selectively is highly dependant on pH. When bonded to the resin substrate the IDA incorporates a tertiary mine structure.

Most ion exchangers used for the preconcentration of trace elements are cation exchangers however anion exchange resins have also been used for matrix removal/preconcentration methods for the determination of Chromium [50], Arsenic [51] and Selenium [52-53]. These elements primarily exist as anions in solution.

1.4.5 Chelation Ion Exchange

Chelates are metal-ligand complexes where the ligand attaches to the metal at more than one position, or with more than one functional group. Typically, ligands with multiple bonding capabilities display a high affinity for metals and form very stable complexes.

A chelate consists of a metal ion (an electron acceptor) and a ligand (an electron donor). The coordination sites of a metal ion in aqueous solution are filled with water. As the water of solvation of the metal ion is replaced by a ligand a metal complex or a chelate compound is formed **(1)**.

$$M(H_2O)_x^{n+} + L^{m-} \rightleftharpoons [M(H_2O)_{x-1}L]^{n-m} + H_2O \qquad (1)$$

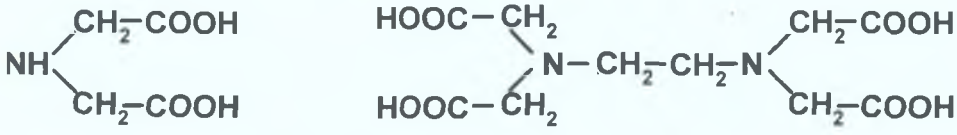
The number of electron donor atoms available for complex formation classifies ligands.

1.4.6 Iminodiacetate complexation

Chelating ion exchange resins are particularly well suited for the removal of trace metal ions from samples with complex matrices. Important characteristics for suitable resins include:

1. The resins have high capacities for ions that are found in trace concentrations.
2. The resins are stable and readily regenerated.
3. Temperature effects are for the most part negligible.
4. Resins should have a high selectively for the trace elements of interest.

As is obvious from the structure of iminodiacetate, IDA, it is the chemical analogue of the Ethylenediaminetetraacetic acid (EDTA) molecule, **Fig 1.12**. EDTA itself is the most important and flexible chelate currently used for trace element determinations.



Iminodiacetic acid — Ethylenediaminetetraacetic acid

Fig. 1.12 Chemical Structure of Chelating Compounds.

Therefore, the behaviour of the IDA chelating resin is analogous to the behaviour of EDTA, both in terms of metal selectively and behaviour versus pH [54].

In general, IDA based chelating resins behave similarly to weak acid cation resins but also exhibit a high degree of selectively for trace element cations. The high degree of selectively for specific trace elements permits separation of these metals from solutions containing high background levels of the major elements (e.g. sodium, potassium, calcium and magnesium) that do not form stable complexes with IDA. Regeneration properties are similar to those of a weak acid resin; the chelating resin can be converted to the hydrogen form with slightly greater than stoichiometric doses of acid because of the tendency of the trace element complex to become less stable under lower pH conditions.

1.4.7 Influence of pH on Iminodiacetate.

The major species of iminodiacetic acid in solution of varying pH are shown in **Fig. 1.13**.

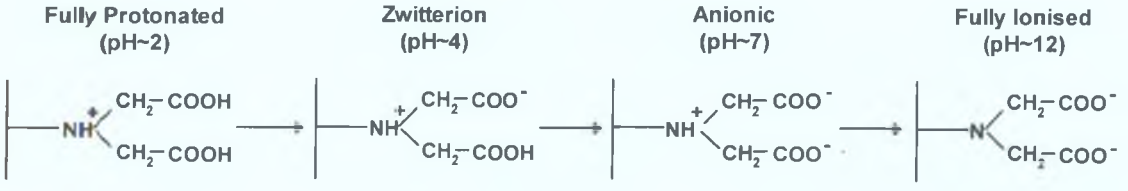


Fig. 1.13 The chemical structure of Iminodiacetate with changing pH.

At pH 4-7 iminodiacetate mainly functions as a chelating agent and at a pH >12 its acts as a weak anion exchanger and strong complexing agent.

A metal ion in solution does not exist in isolation, but in combination with ligands (such as solvent molecules or simple ions) or chelating groups (such as IDA), giving rise to complex ions. All metals form complexes although the extent of formation and the nature of these are dependent on the electronic structure of the metal. The stability of a complex (chelate) in solution depends on the degree of association between the metal and ligand species involved. The stability or formation equilibrium constant quantitatively expresses this stability. For the metal ion, M^+, and the neutral ligand, L, the following equations can be written

$$M^{2+} + L \rightleftharpoons (ML)^{2+} \qquad k_1 = \frac{[(ML)^{2+}]}{[M^{2+}]\,[L]} \qquad (1)$$

$$(ML)^{2+} + L \rightleftharpoons (ML_2)^{2+} \qquad k_2 = \frac{[(ML_2)^{2+}]}{[(ML)^{2+}]\,[L]} \qquad (2)$$

Where k_1 and k_2 are called stepwise stability constants. While the overall stability constant, K, is:

$$M^{2+} + 2L \rightleftharpoons (ML_2)^{2+} \qquad K = k_1\,k_2 = \frac{[(ML_2)^{2+}]}{[M^{2+}]\,[L]^2} \qquad (3)$$

The reaction between IDA (L^{2-}) and a divalent metal ion (M^{2+}) can be represented by the following equilibrium **(4)**

$$L^{2-} + M^{2+} \rightleftharpoons ML \qquad (4)$$

Therefore the formation constant for metal complex formation is given in equation **5**. Where **ML** is the metal complex

$$K_f = \frac{[ML]}{[M^{2+}]\,[L^{2-}]} \qquad (5)$$

Stability constants are expressed in a logarithmic form as they can vary from small to exponential figures.

Iminodiacetic acid (IDA) is a weak acid with two ionisable protons (H_2L). The acid-base equilibrium and dissociation constants are represented below

$$H_2L \rightleftharpoons HL^- + H^+ \qquad K_1 = \frac{[H^+]\,[HL^-]}{[H_2L]} \qquad (6)$$

The pK_1 for IDA is 2.98 and therefore it is a weak acid and it will exhibit a strong pH dependence. The pK_2 of 9.98 represents a very weak second dissociation (**7**).

$$HL^- \rightleftharpoons H^+ + L^{2-} \qquad K_2 = \frac{[H^+][L^{2-}]}{[HL^-]} \qquad (7)$$

The stepwise formation of an iminodiacetic acid metal chelate involving two iminodiacetate molecules is shown in **Fig. 1.14**.

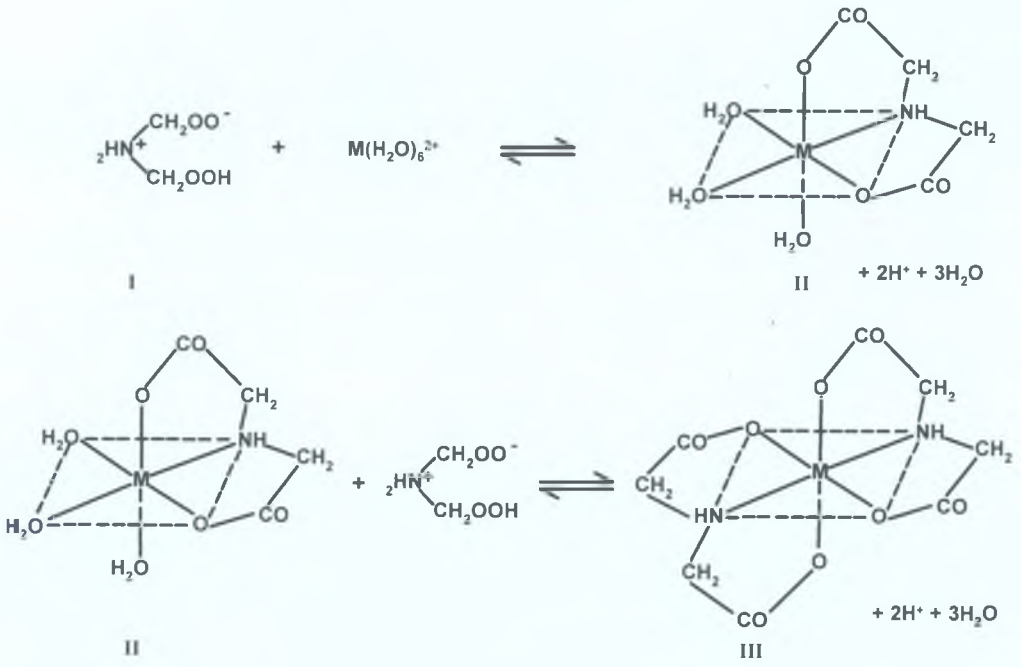


Fig. 1.14 Stepwise formation of an Iminodiacetic acid metal chelate.

The complexation reaction is a typical Lewis acid-base reaction. The neutral ligand forms coordinate covalent bonds with the metal ion by donating lone pairs of electrons to the unoccupied p or d orbital of the central metal ion. One of the products is the hydronium ion, H^+, displaced from the weak acidic ammonium group of the ligand and from the acidic carboxylic group [55-56].

Complexes containing five and six heterocyclic membered rings are the most stable, while four or seven membered rings are less stable. Iminodiacetic acid in solution forms a highly stable six membered heterocyclic ring (**Fig 1.14**). This is very similar in behaviour to EDTA based chelates and this is not surprising as it is a chemical analogue of the EDTA molecule. Stability increases with the number of attachment points so multiligand (polydentate) complexes are more stable than monodentate complexes.

This is called the chelate effect. When a ligand forms complexes with two metal ions at equal concentration in a solution, the ligand will form complexes preferentially with the metal ion that produces a complex with the larger stability constant.

1.5 Complex Stability Theories.

Several models for understanding metal-ion complex stability in small molecules have been developed. These include the Irving-Williams series, Pearson Hard-Soft Acid Base Rules and the Gibbs-Donnan model. All of these methods have been used for predicting the relative stabilities of metal-ion complexes with varying degrees of success.

1.5.1 The Irving-Williams Series.

The Irving-Williams series depicting the stability of small metal complexes was derived by measuring their stability constants [57]. The order of metal complex stability is shown below

$$Mn^{2+} < Co^{2+} < Ni^{2+} < Cu^{2+} > Zn^{2+} \sim Pb^{2+}$$

As one moves across the periodic table, the nuclear charge increases and the ionic radii decrease. The increasing nuclear charge leads to stronger metal-ligand bonds. The decreasing ionic radii lead to shorter and therefore stronger metal-ligand bonds. These factors partly explain the increasing stability of metals complex in the Irving-Williams series.

The Crystal Field Stabilisation Field Energy (CFSE) theory has also been used to explain the order of the Irving-Williams series [58]. The series does not have a general theory explaining its origins but it may be related to the electron population of the d orbitals of the various ions dictates the order of metals in the series. The Irving-Williams series is primarily used to predict trends in the comparative strength of the interactions of various metal-ions with their ligands. However, as usual, even small chelators do not follow these trends in a uniform way.

1.5.2 Pearson Rules

While the Irving-Williams series does not take into account the chemistry of the coordinating ligand the Pearson rules do [59]. These rules state that soft i.e. highly polarizable, acids will tend to more strongly associate with soft bases and hard, low polarisablility, acids will tend to more strongly associate with hard bases. After determining the affinity of various metal-ions for a variety of ligands classified, as hard or soft, the metal-ions were placed into three different classes; hard, soft or borderline.

Hard acids prefer to bind to hard bases and soft acids prefer to bind with soft bases. Examples of hard acids include: alkaline metal, alkaline earth metals, lighter transition metals (Ti^{4+}, Cr^{3+}, Fe^{3+}, and Co^{3+}) and the hydrogen ion (H^{+}). Typical soft acids include: heavier transition metals and lower oxidation states (Cu^{+}, Ag^{+}, Hg^{+}, Hg^{2+}, Pd^{2+} and Pt^{2+}). Bases can be categorised according to their groups as follows: (hard to soft): N>>P>As>Sb; O>>S>Se>Te, F>Cl>Br>I.

Hard acids/bases tend to be small and slightly polarizable while soft acids/bases tend to be larger and more polarizable. These divisions are useful since most chelating ligands have oxygen, sulphur or nitrogen in their structure and are ranked as hard, soft and borderline respectively.

Unfortunately most of the transition metal series are classed as borderline and this means that they can act as both a hard metal and occasionally as a soft metal. Attempts have been made to rank these borderline metals into some sort of order but to no avail and this is, in effect, the greatest weakness of the Pearson rules [60]. Complex stability can be predicted in a general way by the hard soft acid base system. The basic premise of Pearson rules is that an individual cation "prefers" to bond with one type of ligand over another. The type of cations that can complex with ligands can be classified under the following headings

(1) Type A metal cations: These cations are spherically symmetrical, with an inert gas configuration and are known as hard spheres or hard acids e.g. Alkalis; Na, K (weakest interaction with ligands and are rarely considered) and Alkaline Earths; Ca, Mg.

This correctly predicts the behaviour of the iminodiacetate chelating resin with these metals, and provides an explanation as to why this resin is very efficient at removing these elements from samples.

(2) Type B metal cations have more easily polarised electron shells, which form covalent complexes and are termed soft acids (Ag, Zn, Hg, Pb and Sn).

These cations tend to form complexes with soft ligands containing sulphur, phosphorus and nitrogen. Within these groups of metal ions, complex stability is proportional to the charge of the metal ion.

1.5.3 Gibbs-Donnan Model

Ion-exchange chelating resins have been widely used for matrix removal/preconcentration techniques. But, few attempts have been made to describe in detail the behaviour of the trace metal chelation reaction. The reason may be that the exchange coefficients of chelating resins vary largely with changing experimental conditions, so that it seems to be not of practical use to determine a quantity which changes under different conditions. Recently it has been demonstrated that the Gibbs–Donnan model is helpful for predicting the chelation of metals at trace levels on ion-exchange chelating resins [61], when the resin is present in large excess, under different controlled conditions. The Gibbs–Donnan model has been used to characterise solid ion-exchangers and in particular their protonation characteristics.

According to the Gibbs–Donnan model the resin is represented as an aqueous phase, separated from the external solution by an interface through which a potential difference, called the Donnan potential, is set up, due to the different mobility of the charged species. Chelating resins when compared to normal ion exchange resins contain donor groups, which are able to form stable complexes with specific metals. The metal can enter into the ion-exchange chelating resin by different mechanisms, i.e. ion-exchange, diffusion, and complexation by the donor groups. The first two mechanisms are expected from the Gibbs–Donnan model, which considers the resin phase as a real solution phase, separated from the external solution, in which the molecules or ions are at different concentrations.

Therefore, for metals that do not form complexes with chelating resins, e.g. alkaline metals, only the first two mechanisms are important [62].

In terms of peer reviewed papers, the Gibbs-Donnan model has been extensively used to try to explain the behaviour of the iminodacetate chelating ion exchange resin with trace metals [63-64].

The iminodiacetate chelating resin has been chosen for these studies because of its well-defined properties. Its behaviour has been extensively studied both empirically and theoretically by thermodynamic based calculation methods such as the Gibbs-Donnan model. Different successful applications of this model have been published including studies of protonation equilibria [64] and the sorption behaviour of bivalent metals with iminodiacetate chelating resins [65].

1.5.4 Critical Stability Constants for Iminodiacetic acid.

Critical stability constants are derived from extensive analysis of the behaviour of different metals with ligands [66-67]. They are used to determine the properties of metal-ligand reactions in solution.

The method of glass electrode based potentiometry is used to determine the critical stability constants in systems where very stable complexes are formed. The constants provide an indication of the strength of the iminodiacetate chelate formed with a particular metal under specific experimental conditions, **Table 1.3**.

Table 1.3 Critical stability constants of iminodiacetic acid chelates.

Element	Log k_1	Element	Log k_1	Element	Log k_1
Na^+	0.36	Fe^{2+}	5.8	H^+	9.34
Li^+	0.96	La^{3+}	5.88	Th^{4+}	9.69
Ba^{2+}	1.67	Ce^{3+}	6.18	Sc^{3+}	9.8
Sr^{2+}	2.23	Y^{3+}	6.78	Cu^{2+}	10.56
Ca^{2+}	2.6	Zn^{2+}	7.15	Fe^{3+}	10.72
Mg^{2+}	2.98	Pb^{2+}	7.36	Cr^{3+}	10.9
Ag^+	3.56	Al^{3+}	8.1	VO_2^{2+}	11.7
Mn^{2+}	4.72	Ni^{2+}	8.3	Hg^{2+}	11.8
Cr^{2+}	5.01	UO_2^{2+}	8.78	Be^{2+}	13.95
Cd^{2+}	5.71	VO^{2+}	9	Co^{3+}	29.6

25°C, Ionic strength 0.1M

These constants provide an indication of the lower stability of alkali/alkaline earth elements when compared to the higher stability trace elements iminodiacetate chelates

1.5.5 Selectivity factors for Iminodiacetic acid

A list of selectivity factors for divalent cations with iminodiacetate resin is given in **Table 1.4**. The selectivity factor is a quantitative measure of the affinity that the resin displays for a particular cation compared to its affinity for a reference cation, in this case Zn^{2+}.

Table 1.4 Iminodiacetate selectivity for divalent cations using Zn as a reference.

Element	Selectively Factor
Hg^{2+}	1060
Cu^{2+}	126
UO^{2+}	5.7
Ni^{2+}	4.4
Pb^{2+}	3.88
Zn^{2+}	1
Co^{2+}	0.615
Cd^{2+}	0.39
Fe^{2+}	0.13
Mn^{2+}	0.024
Ba^{2+}	0.016
Ca^{2+}	0.013
Sr^{2+}	0.013
Mg^{2+}	0.009
Na^{+}	0.0000001

Selectivity values for any particular system (i.e. conditional stability constants) depend on a wide variety of factors, which include pH, ionic strength, and the presence of other complex-forming species such as humic/fulvic acids. Thus Mercury, Hg^{2+}, appears high in the selectivity series in the presence of nitrate ions, but low in the series in the presence of chloride ions, with which it forms a stable complex. The approximate order of selectivity for cations in nitrate or chloride solutions is

Cu^{2+} >>Pb^{2+} >Fe^{3+} >Al^{3+} >Cr^{3+} >Ni^{2+} >Zn^{2+} >Ag^{+}>Co^{2+} >Cd^{2+} >Fe^{2+} >Mn^{2+} >Ba^{2+} >Ca^{2+} >>>Na^{+}

A selectivity series for cations in an acetate buffer system at pH 5 is

Pd^{2+}>Cu^{2+} >>Fe^{2+} >Ni^{2+} >Pb^{2+}>Mn^{2+} >>Ca^{2+} = Mg^{2+} >>>Na^{+}

The selectivity for various cations in aqueous solutions at pH 4 is

Hg^{2+} >Cu^{2+} >Pb^{2+} >>>Ni^{2+} >Zn^{2+} >Cd^{2+} >Co^{2+} >Fe^{2+}>Mn^{2+} >Ca^{2+} >>>Na^{+}

The selectivity at pH 9 in the presence of 1.5M $(NH_4)_2SO_4$ is

Co^{2+} >Ni^{2+} >Cd^{2+} >Cu^{2+} >Zn^{2+} >Ca^{2+} >>>Na^{+}

These selectively series illustrate how the presence of other cations and anions in solution can affect the order of the series [68]. However these changes do not affect that the alkali/alkaline earth metals have the lowest affinity for the iminodiacetate resin when compared to other trace elements.

1.5.6 Limitations of Complex Stability theories.

The Irving-Williams series, Pearson Hard-Soft Acid Base Rules and the Gibbs-Donnan model provide a useful insight into the formation of metal chelates with iminodiacetate and thousands of other ligands. However, none of these theories individually answer all the questions regarding the formation and stability of metal chelates. These theories only apply to simple systems under controlled experimental conditions and therefore their application is limited by this fact. Critical stability constants provide a measurement of the stability of trace metal complexes when compared to matrix elements such as calcium and magnesium. However, it is of limited use when trying to explain the chelating behaviour of iminodiacetate in complex environmental samples e.g. seawater.

1.6 Chelating Ion Exchange Reagents

A number of commercially available iminodiacetate based chelating reagents have been developed over the last 25 years. The following table is an example of some the commonly available chelating ion exchange reagents, **Table 1.5**.

Table 1.5 Iminodiacetate based chelating reagents.

Name	Manufacturer	Capacity	Particle Size
Chelex 100	Bio-rad Labs	0.4meq/ml	150µm
Metpac CC1	Dionex	0.4meq/column	18µm
Prosep Chelating I	Bioprocessing	Cu^{2+} > 50mmol/ml	75-125µm
AF 650M Chelate	Tosohaas	20mmol/ml	65µm
Empore Disks	3M Empore	0.45mmol	10µm

Chelex 100 (Biorad Lab., Richmond, VA, USA) and Metpac CC-1 (Dionex Corp, Sunnyvale, CA, USA) are polystyrene based chelating reagents. The swelling problems associated with the use of polystyrene based substrates have been well documented [69].

Prosep Chelating I (Bioprocessing, Consett, Durham, UK) is a controlled pore glass (CPG) based iminodiacetate chelating reagent. This rigid substrate is not affected by swelling when used in matrix removal preconcentration systems but has a lower capacity when compared to polymer chelating resins [70].

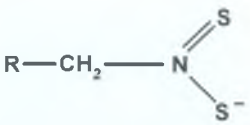
AF 650M Chelate (Tosohaas, Montomeryville, PA, USA) is a methacrylate polymer based iminodiacetate chelating reagent [71]. It doesn't suffer from the swelling problems of polystyrene substrates and has a higher capacity than CPG substrates.

Finally, Empore chelating extraction disks represent a novel development in this area and are 47mm diameter filtration disks with iminodiacetate chelating group immobilised onto its PSDVB substrate (3M Empore, St. Paul, MN, USA).

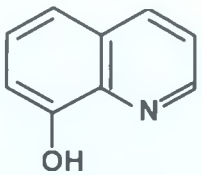
The disk provides a simple method of matrix removal/preconcentration and is suitable for small numbers of sample analysis [72].

Some laboratories carrying out a particular analysis prepare their own chelating resins, particularly in situations where there is no commercial source of these reagents.

Examples include dithiocarbamate [73] and 8-hydroxyquinoline [74] functional groups being either immobilised onto a polymer resin or controlled porosity glass, **Fig 1.15**.



Dithiocarbamate



8-Hyroxyquinoline

Fig. 1.15 Laboratory synthesised chelating functional groups.

Natural polymer ion exchange resins such as cellulose and chitosan are available with iminodiacetate chelating functional groups attached. Both cellulose and chitosan have a polysaccharide based polymer substrate and are similar in chemical structure. Cellulose based iminodiacetate chelating reagents have been synthesised in laboratories using aminoethyl cellulose as a starting material [75]. A Chitosan based chelating reagent is available commercially as Chitopearl Cl03 (Fuji Spinning Co. Ltd., Japan) [76].

1.7 Chelating Ion Exchange Substrates.

A wide variety of substrates have been used to chemically immobilise chelating functional groups

1. Polystyrene divinylbenzene.
2. Acrylic polymer (methacrylate).
3. Controlled pore glass.
4. Naturally-occurring polymers (Cellulose and Chitosan).

The type of substrate that a chelating group is chemically immobilised onto can play an important role in the overall behaviour of the reagent in a matrix removal/preconcentration system. The substrate used can affect the chelating reagents capacity, flow-rate behaviour and swelling properties.

1.7.1 Polystyrene Divinylbenzene (Polystyrene-DVB)

Polystryrene divinylbenzene is the most commonly used substrate in a variety of ion exchange techniques (cation, anion and chelating ion exchange).

The polystyrene-DVB needs to be chemically bonded with iminodiacetic acid to perform as a chelating ion exchange material. The styrene-divinylbenzene polymer is treated with chloromethyl ether as in the preparation of strong anion exchangers [37]. The product is then treated with ammonia and chloroacetic acid, which produces the iminodiacetic acid group, attached to the benzene ring. Each benzene ring does not acquire a chelating group. The number of groups present varies form batch to batch so that the capacity of the resin varies from batch to batch, but averages around 0.4 meq/ml for Chelex 100 (Biorad Lab., Richmond, VA, USA).

The Iminodiacetate chelating functional groups are covalently bonded to the resin, **Fig. 1.16**.

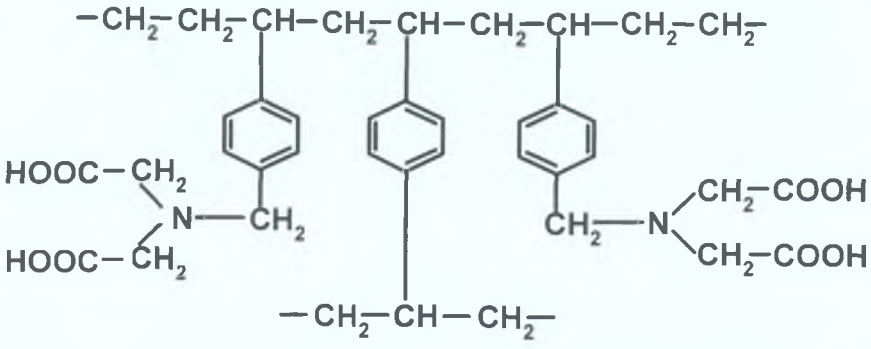


Fig. 1.16 Chelating resin with a polystyrene-DVB substrate.

It is important that the chelating sites are formed throughout the resin. The chelating ion exchange process is not a surface phenomenon, with more than 99% of the metal capacity of the material being found in the interior of the resin [77]. This type of resin can have a high chelating capacity. Chelex 100 (Biorad Lab., Richmond, VA, USA), Metpac CC-1 (Dionex Corp, Sunneyvale, CA, USA) and Muromac A1 (Muromachi Kagaku Kogyo Kaisha, Ltd., Japan) are examples of polystyrene-DVB based chelating resins with an iminodiacetate functional group.

The resin can differ in the level of cross linkage in the polymer structure, (1-20%). A more highly cross linked resin leads to a lower capacity. Lower cross-linking polystyrene-DVB resins swell when converted from its salt to ionic form. The iminodiacetate based polystyrene DVB resin can undergo significant volume changes of up to 100% of its original column volume [68] and this represents a major disadvantage when considering its use in a matrix removal/preconcentration system. This leads to back pressure problems in column based systems due to repeated contraction and expansion of the resin. It requires a buffer conditioning step to return the resin to its original volume prior to loading the next sample. This is an additional analytical step in the method, which increases both analysis time and degrades detection limits

1.7.2 Acrylic

Acrylic based resins are straight-chain hydrocarbons based on methacrylate monomer, **Fig. 1.17**. Divinylbenzene is still used as a cross-linker in this type of resin but does not have the same swelling properties associated with polystyrene based chelating resins.

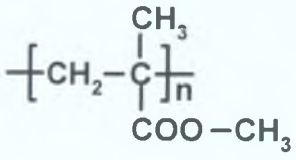


Fig. 1.17 Methacrylate polymer substrate.

Toyopearl AF-Chelate 650M (Tosohaas, Montomeryville, PA, USA) is an iminodiacetate resin with a macroporous methacrylate backbone. It's use in matrix removal/preconcentration systems was first reported by S.N. Willie [78].

1.7.3 Controlled-Pore Glass.

An alternative support, which is not susceptible to dimensional changes with changing solution composition, is Controlled-Pore Glass (CPG).

Controlled-Pore Glass (CPG) is silica based inorganic support for use with chelating functional groups. CPG is produced from a borosilicate base material, which is heated to separate the borates and the silicates. The borates are leached out from the material, leaving the silica glass with uniform, controlled pores [79].

CPG possesses a reactive surface that can be easily functionalised with chelating groups and can be conditioned rapidly between samples, leading to shorter analysis times. Numerous matrix removal/preconcentration systems using laboratory or commercially produced CPG have been used in matrix removal/preconcentration systems during the last 10 years [70, 80]. An iminodiacetate CPG (Bioprocessing, Consett, Durham, UK) is an example of a commercially available chelating reagent, **Fig 1.18**.

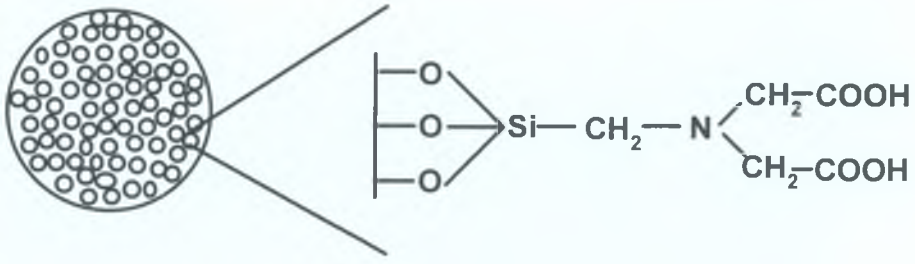


Fig. 1.18 CPG Iminodiacetate chelating reagent.

While 8-hydroxyquinoline CPG (**Fig 1.19**) is an example of a laboratory produced chelating reagent [81].

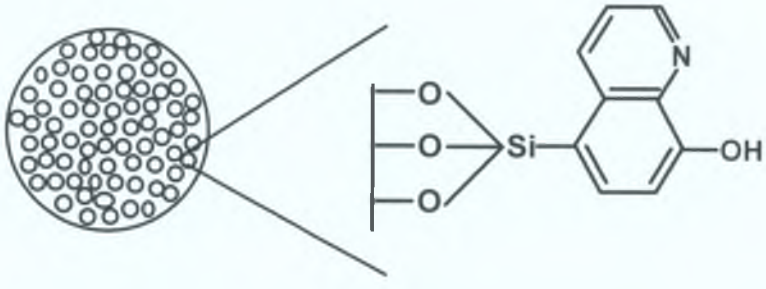


Fig. 1.19 CPG 8-hydroxyquinoline chelating reagent.

1.7.4 Naturally-occurring polymer substrates: Cellulose/Chitosan.

Cellulose/Chitosan are naturally occurring polymers [82], which have been chemically modified to be used as a substrate for iminodiacetate chelating reagents.

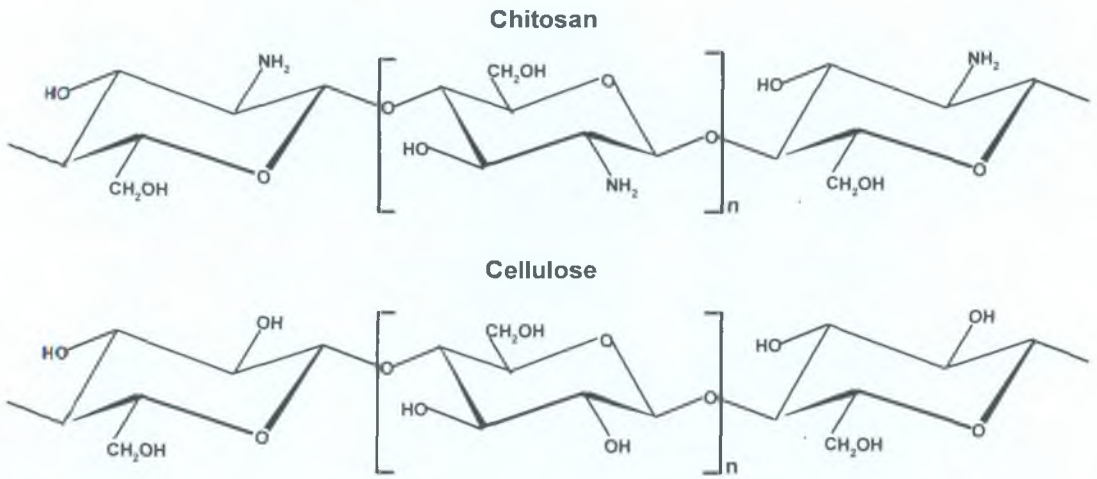


Fig. 1.20 Naturally occurring polymer substrates for chelating reagents.

The chemical structures of chitosan and cellulose are shown in **Fig 1.20**. As can be seen, chitosan is a chemical analogue of cellulose. An iminodiacetate chelating reagent based on a chitosan substrate has also been successfully used for matrix removal/preconcentration [34]. These naturally occurring polymers do not suffer from swelling when exposed to different solutions types, have fast reaction kinetics and high chelating capacity when compared to controlled pore glass reagents.

Overall there has been a gradual move away from polystyrene-DVB based iminodiacetate chelating reagents (Chelex 100, Muromac A1 and Metpac CC-1) to more flexible substrates such as methacrylate, controlled pore glass and cellulose. These alternative chelating reagent substrates have been successfully adapted for use in matrix removal/preconcentration systems. The swelling property of polystyrene-DVB has essentially ensured that its use in mini/micro preconcentration columns has been limited.

Further miniaturisation of matrix removal/preconcentation columns is now possible, as newer substrates do not suffer from the swelling problems of polystyrene-DVB based chelating reagents. This will increase the use of the matrix removal/preconcentration systems in automated sample pretreatment systems for sample introduction in atomic spectrometry.

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Chapter Two

2.0 Design of a Matrix Removal/Preconcentration System.

2.1 Introduction.

The design and operation of sample matrix removal/preconcentration systems for atomic spectrometry has been the focus of intensive research efforts. The chelating resin, Chelex 100 (Biorad Lab., Richmond, VA, USA), has been the most commonly used ion exchange reagent for matrix removal/preconcentration of trace elements over the last 25 years. A number of publications have highlighted the use of this resin in simple off-line column systems [86-88] and in on-line column systems [69, 89, 90] with subsequent trace element analysis using atomic spectrometric detection.

The complexity of matrix removal/preconcentration systems increased as improvements in column, pump and valve design were incorporated into newer systems. These equipment improvements combined with studies of the behaviour of chelating resins in column systems led to changes in analytical methodology. Overall, this led to faster analysis times, reduced matrix interferences and lower detection limits for trace element analysis using atomic spectrometric techniques.

The most comprehensive overview of flow injection based separation and preconcentration has been provided by Zhaolun Fang [91]. A large number of sample pretreatment systems have been influenced by this work.

2.2 Batch methods.

Matrix removal/preconcentration techniques can be described as falling into two main categories, which are generally described as batch or column based methods. Batch based preconcentration methods have been successfully developed for sample pretreatment prior to trace element analysis by ICP-MS. These include the analysis of trace and rare earth elements in river water [92, 93], seawater [94] and blood serum [95]. All of these methods involved the addition of Chelex 100 to isolate the analytes from the sample with subsequent trace element analysis by ICP-MS.

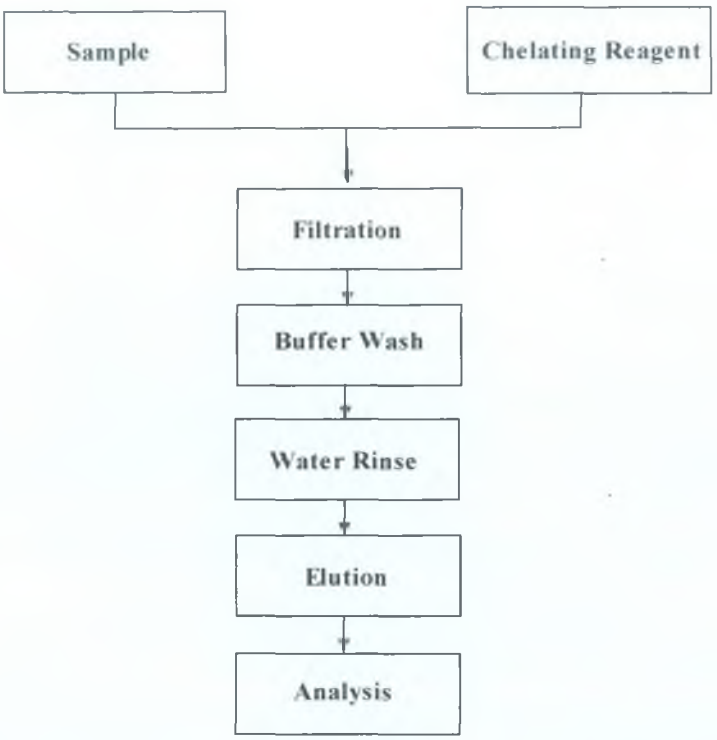


Fig. 2.1 Schematic representation of batch based matrix removal/preconcentration.

The method of batch sample pretreatment is illustrated in **Fig. 2.1**. A quantity of chelating reagent is added to a buffered sample and the mixture is then equilibrated for a specified time by stirring. When the experimental conditions are optimised, the analytes of interest will complex with the chelating reagent.

The reagent is then separated from the sample solution by filtration. A buffer wash step is required to remove any residual sample matrix retained in the reagent. The water rinse step prevents buffer and sample matrix being eluted with the analytes. The analytes are then eluted from the reagent by mixing with nitric acid eluent.

The batch method suffers from a number of limitations including [37]

1. The efficiency with which the analytes binds to the chelating reagent is often low because of equilibrium effects.
2. The large sample and quantity of chemical reagents required.
3. The reaction time must be adjusted whenever the composition of the sample changes.

4. The chelating reagents must be cleaned thoroughly to eliminate cross contamination between samples thereby greatly increasing the required sample manipulations.
5. Higher risk of airborne contamination.
6. Sample throughput is limited when compared to column-based techniques.

Despite the above disadvantages of the batch technique its simplicity of operation can be an advantage for certain types of analysis particularly when a limited number of samples are involved.

The costs involved in implementing the batch methods are minimal when compared to the capital costs of commercial column based preconcentration systems. Finally, minimal technical skills are required to carry out the batch method.

2.3 Column based methods

The limitations of batch mode techniques have led to the development and use of sample pretreatment by column mode methods. Column based methods have therefore proved to be the most popular ways of matrix removal/preconcentration using chelating resins [69, 78, 88].

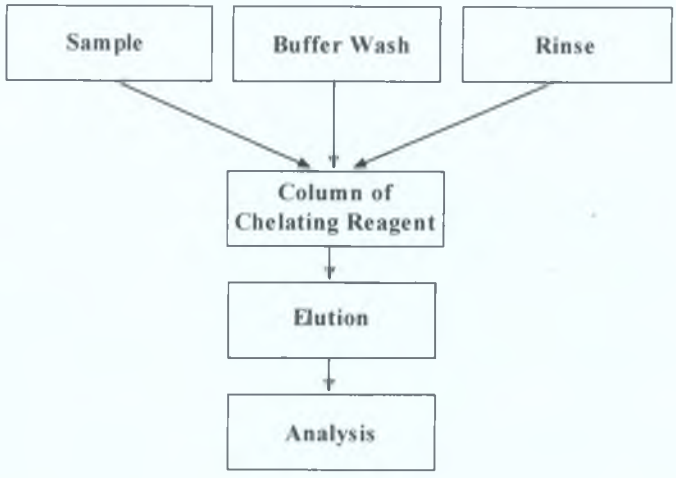


Fig. 2.2 Schematic representation of column mode preconcentration.

The steps involved in column-based sample pretreatment are shown in **Fig. 2.2**. The chelating reagent is packed into a suitable column and a measured volume of sample is passed through it. A buffer wash step is used to remove residual matrix in the column and water is then used as a rinse step to remove buffer from the column prior to elution. The analytes are eluted from the column using an acid eluent and analysed by the appropriate atomic spectrometric method.

The dimensions and type of columns used vary depending on the type of sample or analyte being determined. The physical and chemical properties of the resin also affect the columns design, which in turn affects the type of pumping system required to be used in the matrix removal/preconcentration technique.

The Metpac CC-1 column (Dionex Corp, Sunnyvale, CA, USA) provides an example of how resin properties affect column design. The small particle size, 18μm, leads to high pressures when samples are pumped through the column. This requires the use of high-pressure pumps to pump the sample through the resin. Therefore the body and fittings of the column must generally be made of PEEK or metal to withstand the high pressures developed by the pumping system.

Column design can range from very simple devices, such as a laboratory-based columns made from tygon tubing to proprietary columns, which are commercially available. Typical commercial systems include Mini columns (Valco Instruments Co. Inc., USA), Microbore columns (Omnifit Ltd, Cambridge, UK), Metpac CC-1 (Dionex Corp, Sunnyvale, CA, USA) and the FIAS column (Perkin Elmer Inc, Boston, MA, USA).

Column based systems can be operated either off-line or on-line. In the former case, the sample is manually loaded onto a suitable column where the analyte is preconcentrated. The preconcentrated analyte is then eluted from the column and analysed at a later time by the appropriate procedure. In the on-line method, the pretreatment column is coupled directly to the analytical instrument so that sample pretreatment, elution and analysis steps are carried out automatically.

Column based methods of separation and preconcentration have favourable characteristics when compared to batch or even continuous flow (hydride generation) techniques. These characteristics can be summarised as

1. High preconcentration factors (PF). PFs of 10-50 can be obtained.
2. High sample throughput and shorter analysis times when compared with batch procedures.
3. Lower sample consumption reduces cost of analysis. This is particularly useful when micro-volume sample analysis is required.
4. High reproducibility; typically in the range 1-3% RSD.
5. Automation of these systems is possible, which is ideal for use in online sample analysis.
6. The risk of contamination is reduced owing to the closed and inert design of the separation system used. This is a particularly important factor in trace-element analysis.

Low pressure pumping systems can be incorporated into the design and this allows the possibility of miniaturisation when compared to other high-pressure based systems [6].

2.4 Overview of commercial matrix removal/preconcentration systems.

Various commercial and laboratory based sample pretreatment systems have been designed to automate a wide variety of analytical operations including solvent extraction, coprecipitation, hydride generation and various types of ion exchange. Laboratory based solvent extraction [96] and coprecipitation [97] systems have been developed but none are commercially available. Column based sample pretreatment has proved to be the most popular and reliable method of trace element analysis using flow injection systems [98, 99].

The Dionex Sample Concentration Module system uses its proprietary ion chromatography technology incorporating the Metpac CC-1 chelating column. High pressure pumping (1500psi) achieving flow rates of 4 ml min^{-1} are used to transport solutions through the system.

The Metpac CC-1 resin is comprised of a highly crossed linked polystyrene divinylbenzene polymer with a covalently bonded iminodiacetate substrate. The physical and chemical properties of this macroporous iminodiacetate chelating resin require the use of a conditioning step prior to sample loading. Typically, the analysis time using this type of system is 25 minutes per sample.

The Dionex preconcentration module has been used in conjunction with an ion chromatograph prior to analysis by ICP-AES trace element detection [100]. This design leads long analysis times and increased costs associated with operating the extra instrumentation. The instrument has been used as an online sample pretreatment system for matrix removal/preconcentration but appears to be unsuitable for off-line applications [101].

The Flow Injection Analysis System (FIAS) is a low-pressure flow injection system developed by Perkin Elmer [101, 102]. It uses two low-pressure peristaltic pumps with flow-rates of 4 ml min^{-1} and one flow injection valve. The entire system is computer controlled and can be used with an ICP-MS instrument.

The analysis is typically 5-10 minutes per sample. The conical columns used can be loaded with different types of reagents for applications including anion exchange, chelating ion exchange and hydride generation. Overall this system has proved itself to be a more versatile sample pretreatment system when compared to the Dionex sample concentration module.

2.5 On-line versus Off-line Systems.

Depending on the application, online sample pretreatment systems have a number of advantages when compared to their off-line counterparts. The main advantage of on-line systems is that they provide a fully automated sample analysis system, which requires minimal analyst intervention. The combination of an auto-sampler with these online systems ensures a high sample throughput.

The problem with online systems lies with the economics of the analysis. The coupling of these techniques to ICP-MS instruments means that the instrument can be idle for significant periods of time during the sample pretreatment step. Long-term instrumental drift can also be a problem for this approach. Automation has overcome the technological and practical issues of online systems but the overall cost and precision of analysis for hyphenated ICP-MS systems is still a problem. If the cost of the analysis is not significant in terms of the overall cost of the project then online systems are the most feasible option available to large-scale analytical laboratories.

Off-line systems can be used as dedicated sample pretreatment systems and the pretreated sample can be analysed independently, e.g. the analytical instrument can be dedicated to continuous analysis rather than being limited by the time constraints of the pretreatment system. A larger number of samples can be processed offline without incurring the cost of continually running the instrument. This approach can offer a significant throughput advantage particularly for a busy environmental laboratory. The system used in this research is an off-line system that can be readily adapted for on-line operation when appropriate.

2.6 Matrix Removal/Preconcentration System Description

The equipment used in sample pretreatment system will be described in the following sections. The factors influencing the choice of the various components and modules used in the system will be described and the rationale influencing the choice of system components is discussed. The main system components are as follows

1. Peristaltic pump.
2. Transport tubing and connections.
3. Sorbent packed columns and connections.
4. Valve system and automation.

For trace element analysis using a chelating ion exchange reagent the system eventually developed into an off-line based matrix removal/preconcentration system. This system developed around a low-flow design concept incorporating a peristaltic pump.

In matrix removal/preconcentration systems the sample may be processed through the system by two methods. A time based sample-loading system where the time interval for sample loading into the system is fixed using a predefined flow-rate. A volume based system, which involves the injection of a fixed volume of sample into a mobile carrier stream.

Although both approaches have been used successfully, time based sample loading systems are easier to design and operate in that these system do not require the use of sample loops and flow injection valves. For these reasons it was decided to design a time based matrix removal/preconcentration system that could in the future be adapted to a volume based design with the addition of flow injection valves.

2.6.1 Peristaltic Pumps.

The use of liquid propulsion devices is the most basic piece of equipment in all sample pretreatment systems. The majority of systems are based on the use of a low-pressure peristaltic pump.

However, a commercial system that uses high-pressure pumps for liquid delivery is the Sample Concentration Module (SCM) from Dionex Corp. They developed the SCM for use with their own ion chromatograph, which uses high-pressure pumps to transfer liquids through its system. The use of high-pressure pumps in sample pretreatment has the following disadvantages:

1. Necessitates complex system design.
2. Requires higher capital and maintenance costs.
3. The coupling of a high-pressure system to the low-pressure solution sample introduction system normally used in atomic spectrometry is difficult.

As a result of the above impediments, and after a thorough literature review of published systems, it was decided to opt for a low flow sample preconcentration system. The choice of a low-pressure system influenced the choice of the pumping system used. The main requirements of an ideal liquid propulsion device for the low-pressure sample pretreatment system are

1. Reproducible flow-rates both on a short to long-term basis.
2. Multi-channel pumping capability to facilitate pretreatment of several samples simultaneously.
3. Pulseless liquid delivery.
4. Chemical resistance to the liquids that will be pumped through the system.
5. Adjustable flow-rates.
6. Low capital and maintenance costs.

For the most part, a low-pressure peristaltic pump meets most of the above requirements [91]. The principle of the peristaltic pump is based on alternating contraction and relaxation of Tygon tubing, operating in a similar way to the biological behaviour of the oesophagus **(Fig. 2.3)**.

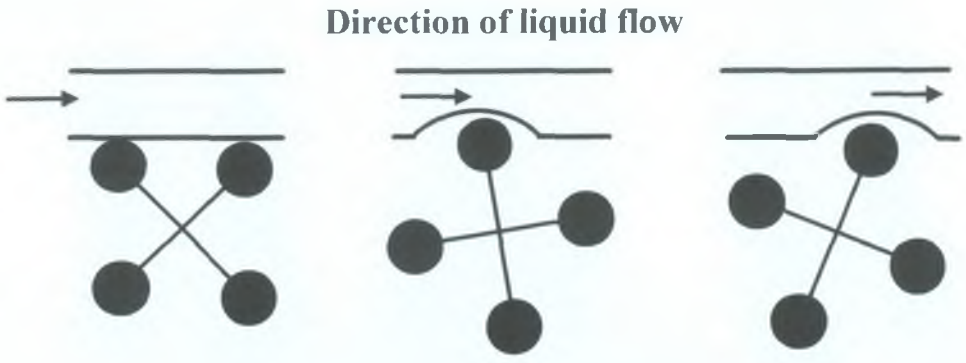


Fig. 2.3 Operation principle of Peristaltic pumps.

The alternating compression and relaxation of the tube generates a vacuum. This forces the liquid through the tube at a constant flow-rate. A number of simple two channel peristaltic pumps have previously been used to control separate liquid streams in sample pretreatment systems [104]. Computer control of these simple pumps allows the development of complex sample pretreatment systems [105]. The individual control of separate liquid streams has the advantage of reducing the waste of continuously running reagents. Multichannel pumps control all liquid streams at once. Although this is a simpler design, it has no individual control of the liquid streams, which can lead to waste of chemical reagents.

Despite the above disadvantages it was decided, due to its ready availability, to initially use a multichannel peristaltic pump (Gilson Minipuls, Gilson Inc. Wisconsin, USA) in the prototype system. As already mentioned the peristaltic pump operates at low pressures, however enough pressure should be produced to push the liquid through the system. This obviously requires that care must be taken in the choice of chelating resin and other components so as not to cause a pressure build-up in the system.

The peristaltic pump is capable of pumping four different liquid streams at various flow rates depending on the application. The first stream is for the sample solution and is used to load the sample onto column. A buffer rinse stream removes residual matrix from the column. A water rinse stream removes any remaining buffer and residual matrix from the column. Finally, an acid eluent stream elutes the analytes from the column.

2.6.2 Transport Tubing and Connections.

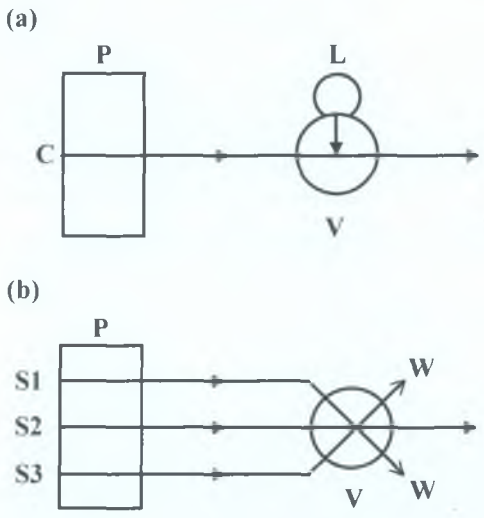
Although the simplest part of the sample pretreatment system, it does merit some discussion. The purpose of transport tubing and fittings is to connect the pump, valves, column and detector together. The length and diameter of the tubing can have a number of important effects on the operation of the system. Varying the length and diameter of the tubing can reduce sample analysis time, and trace element memory effects. The types of fittings used in the system are also important. They provide a leak free seal between all the components in the system and this helps reduce sample contamination and ensures steady flow-rates.

A number of companies have developed plastic based threaded fittings suitable for low-pressure sample pretreatment systems (Omnifit Ltd, Cambridge, UK and Valco Instruments Co. Inc., USA). This has helped make the construction of laboratory-based systems for research purposes more practical [106]. Threaded fittings are strongly recommended for use in on-line separation systems. A tygon tubing inner diameter of <0.5mm is used when the lowest dead volume is required in a length of connection, e.g. the connection between a valve and nebuliser of an atomic spectrometer. Although tubing with a smaller diameter would produce lower dead volumes, this is not recommended due to increased risks of blockage by small particles in the sample.

Tubing of inner diameter of 0.5-1mm is suitable for most sample pretreatment systems. These dimensions provide a reasonable compromise between low dead volume and low flow impedance. Large inner diameter tubing of >1mm are used in hydride generation systems that require high flow-rates [107]. The PFTE and Tygon tubing used in system were used as supplied by AGB Scientific Ltd, Dublin, Ireland.

2.6.3 Valve System and Automation.

The most complex part of the sample pretreatment system is the valve. Valves have a number of important functions including flow injection of sample or eluent into a carrier stream (**Fig 2.4a**) and also stream selection (**Fig 2.4b**).

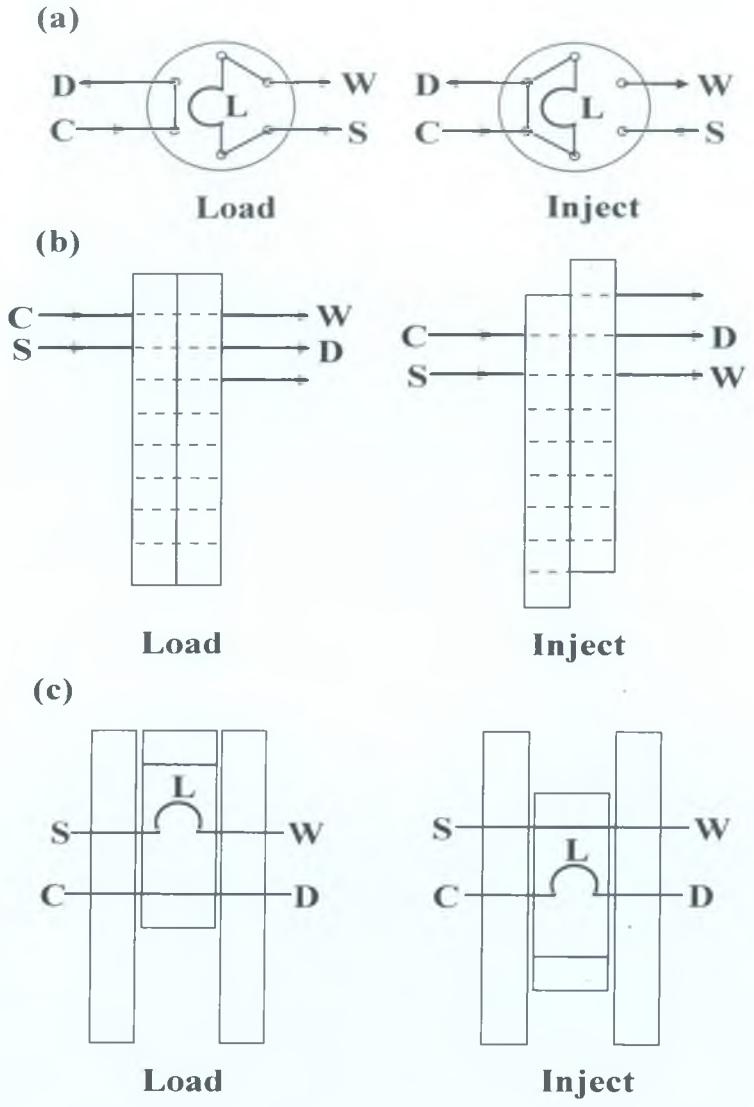


P-peristaltic pump; C-carrier; L-Sample loop; V-valve; S-stream; W-waste.

Fig. 2.4 Schematic diagram of (a) Flow injection valve (b) Stream selector valve.

Flow-injection valves inject a fixed volume of sample into a mobile carrier stream. Stream selector valves are used to select sequentially the different liquid streams required for the sample pretreatment technique. The requirements of low-pressure valves differ from those normally used in High Pressure Liquid Chromatography (HPLC). It is not necessary for the valves to withstand extremely high pressures as in HPLC applications, however low pressure valves used in sample pretreatment systems are often required to perform other functions in addition to sample injection. The valves are actuated more frequently than HPLC valves and therefore need to be very rugged in design. They should be able to provide a large number of leak-proof actuations using a wide range of chemical reagents during their lifetime.

Typical valves commonly used in sample pretreatment systems are the 6-port rotary valve, 8-channel multifunctional valves and the 3-layer commutator valve. The operation of these valves in flow injection systems is illustrated in **Fig 2.5**.



(a) 6-port rotary valve, (b) 8-channel multifunction valve, (c) 3-layer commutator valve; S-sample, L-sample loop, W-waste, C-carrier, D-detector.

Fig. 2.5 Schematic diagrams flow injection valve designs.

The 6-port rotary valve is adapted from conventional HPLC injection systems for use in matrix removal/preconcentration systems [108], **Fig. 2.5a**. The simple design of this valve however limits it ability to carry out the more complicated functions often-required in on-line systems. A number of these valves used in groups can be used to construct a matrix removal system but this renders the design of the system cumbersome to build and control.

A valve design that has shown great flexibility for use in sample pretreatment separation systems is the 8-channel multi-functional valve first developed by Fang in 1984 [69], **Fig 2.5b** and this is a time based valve design. Numerous commercial versions of this multi-purpose valve have been developed and used in commercial flow injection systems. The 3-layer commutator valve consists of a three-layer sandwich design.

The middle layer slides between the two outer layers during actuation (**Fig. 2.5c**) [109]. This valve is capable of performing a number of complicated functions in sample pretreatment systems. The combination of injection and stream selection valves in one system forms the basis of a pretreatment system capable of being coupled online to an ICP-MS instrument.

All of the valve designs discussed above have been used in the construction of sample pretreatment systems. Valves have been both controlled manually and also by computer. Computer control of the valve system is a prerequisite for fully automated online sample pretreatment systems. In general these valves have been adapted for trace metal analysis by the use of inert plastic coated components. The level of valve complexity is dependent on the requirement of the matrix removal system. If used in the correct way, a relatively simple valve system can achieve most of the aims of sample pretreatment. Off-line systems do not require the level of valve complexity of on-line systems and the number of valves is reduced. These systems are usually more economical to build and maintain.

The multiposition stream selector valve used in this research is illustrated in **Fig. 2.6**. The primary purpose of this valve design is to switch to different liquid streams during the pretreatment process e.g. switch from a column rinse step to analyte elution step.

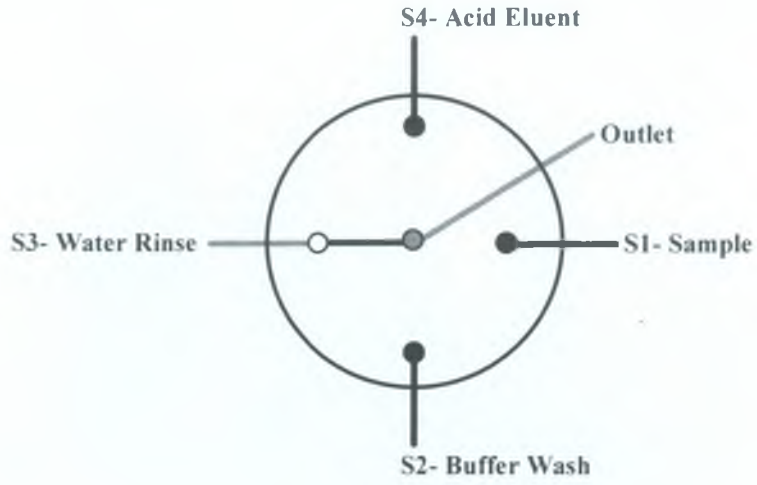


Fig. 2.6 Valco 4-Stream Selector Valve.

The valve used for this research is the Valco C25ZF-3184EMH model (Valco Instruments Co. Inc., USA). It is a 4 multiposition flow-through, micro-electric valve. These cheminert valves are used for stream selection, automated flow systems and multiple sample stream analysis. They consist of a simple, flat plate rotary design. The valves have polymer-threaded fittings with the option of 4-14 ports.

The valve's micro-electric actuator is a microprocessor-controlled model.
Some of the features of this valve include

1. High speed actuation.
2. Bi-directional (step either forward or backwards).
3. Manual control with LED position indicator, step and home functions, and clockwise and counter-clockwise functions.
4. RS-232 serial interface bi-directional communication.
5. Compact size.

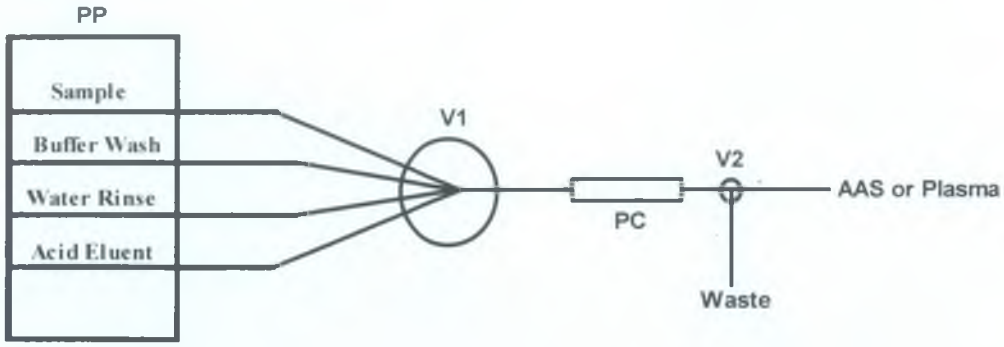
Multiposition valves move in continuous revolutions by incremental steps, unlike the back and forth switching of two positions, i.e. load-inject, valves. Each step selects one stream and directs it through the valve outlet. In standard models, the non-selected streams are dead-ended. However, this valve was ordered with an optional rotor that returns each stream to its source or waste. This valve although entailing an extra cost has cheminert flangeless fittings. These types of fittings provide an adequate seal therefore avoiding leakage.

The valve rotor is the internal part of a valve and contains the engraved slots, which connect the ports on the stator, and is made from a proprietary reinforced polymer. The valve stator is the stationary part of the valve and it contains the fittings and fluid sealing surfaces, which are made of a highly inert polymer. The RS-232 serial interface on the valve offers various commands like stream position access, direction control and shortest route.

Also, a timetable software program, which is run under MS-DOS, is supplied to enable easy control of the valve by computer. The valve is also capable of being connected in series to other valves. This allows the control of two stream selection valves and one flow injection valve. This valve design can therefore be used to develop a complex sample pretreatment system. A number of commercially developed software packages are available to control the valve including FlowTEK (www.GlobalFia.com) and MPValve (Diablo Analytical, Inc., USA) and vary in price from 500-1500 US dollars.

The valve is in effect the heart of the system as it switches between each stream, e.g. wash selection to sample selection to eluent selection, at a specified time in a fairly reproducible manner. The valve has four flow-through streams and one outlet stream, which is connected to the column. The current system is automated in terms of switching between different streams but is not yet suitable for online connection to a flame AAS/ICP-MS. All the streams exit through the column and therefore could enter the detector and this is clearly undesirable for an online system, as only the eluent stream should be allowed to reach the detector.

A simple switching valve (V2) connected in series to the stream selector valve with either manual or automatic control is required after the column in order to switch the sample, wash and conditioning stream to waste while only the eluent stream is connected to the detector (**Fig. 2.7**).



PP peristaltic pump; V valve; PC preconcentration column.

Fig. 2.7 Schematic of a basic matrix removal/preconcentration system

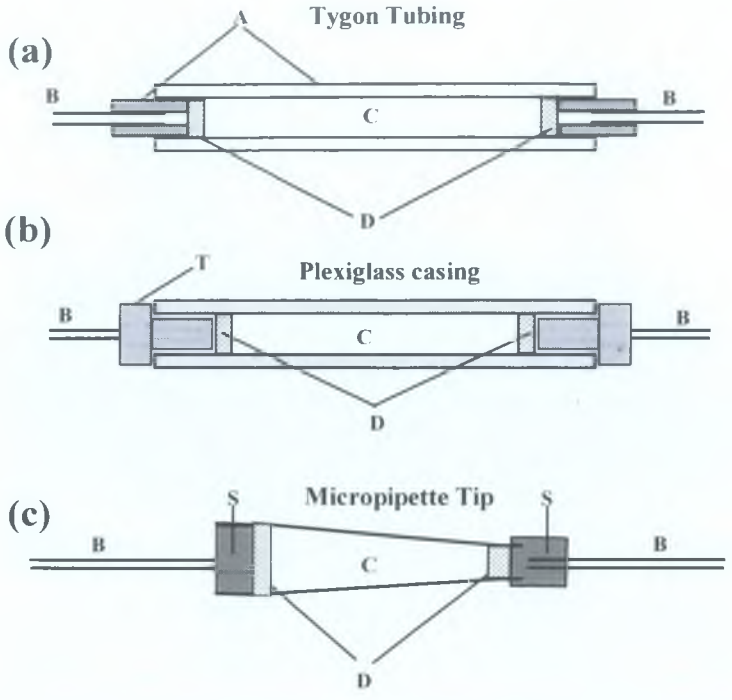
2. 7 Sample Pretreatment Manifolds.

A number of sample pretreatment system designs have been used to carry out sample pretreatment prior to introduction to atomic spectrometric detectors. These include sorbent packed columns [110], knotted reactors [111] and membrane sampling devices [112].

The main focus of this research was the use of a column based sample pretreatment system. A discussion of column designs used in this research will be described in the following section.

2.7.1 Sorbent Packed Column.

The use of column-based systems has become one of the most popular methods of sample pretreatment. The main column designs, which have been used are shown in **Fig 2.8**.



(a) Uniform-bore column with push-fit connections. (b) Uniform-bore column with threaded connections. (c) Conical column. A, Tygon Tubing. B, PTFE Tubing. C, Sorbent. D, Foam. T, Threaded Fittings. S, Thick-walled silicon rubber tubing.

Fig. 2.8 Sorbent column designs.

The use of push-fit connections to construct a column represents one of the simplest column design methodologies (**Fig 2.8a**). It basically consists of joining together tygon tubing of different inner diameters. Foam plugs are used to hold the sorbent inside the column. For some applications and provided care is taken, push-fit connections in columns can perform to the same standard as threaded fitting based columns used in low pressure sample pretreatment systems. However, columns with threaded fittings have a number of inherent advantages when compared to push-fit connections, (**Fig. 2.8b).**

1. Threaded fittings provide better long-term flow-rate stability, no sample leakage over time and the use of higher flow-rates is also possible.
2. The threaded column can be repacked with different types of sorbents (e.g., chelating reagents, anion exchange resins) to carry out a wide variety of separation tasks.

A variation of the uniform bore column is the conical-based column design (**Fig. 2.8c**). The Perkin Elmer FIAS flow injection system has always used conical shaped columns and has proved itself to be reliable over long periods of continuous use. When compared to uniform bore columns, conical-shaped columns allow greater loading of sample onto the column while minimising backpressure problems [91].

Uniform-bore type columns with threaded fitting connections have been successfully used in online and offline based systems for many years [6, 78]. Commercially available columns did not meet the requirements of our proposed matrix removal/preconcentration system both in terms of specification and cost.

Minicolumns available from Valco and Perkin Elmer were more suitable for flow injection based systems. The Dionex preconcentration column was designed for use in high pressure based chromatographic systems [100]. This column design when used in low-pressure systems leads to flow-rates of <1ml min^{-1} using this Metpac CC-1 resin (Dionex Corp, Sunnyvale, CA, USA) because of the resins small particle size (18μm). Omnifit columns (Omnifit Ltd, Cambridge, UK) were too large for the proposed sample pretreatment system and their use would have led to both flow-rate and backpressure problems.

A number of different column designs were investigated during the development of a suitable preconcentration column. The main problem was to design push-fit connections capable of withstanding the flow-rates and pressures developed by the laboratory system. Although conical shaped columns using micropipette tips were tried, making leak proof connections was not successful. The next preconcentration column used in this research is a uniform bore design constructed from pieces of tygon tubing having different inner diameters (**Fig 2.9**).

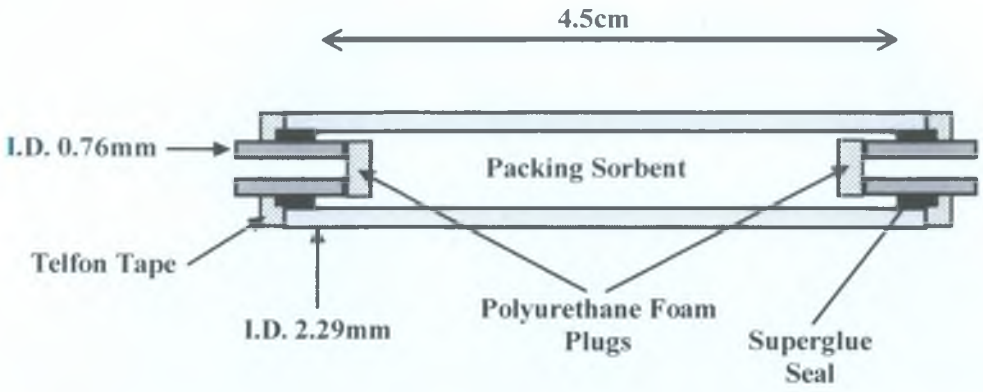


Fig. 2.9 Tygon tubing based column design.

Tygon tubing of 4.5 cm in length and 2.29 mm inner diameter was used to pack the chelating reagent. One end of the column was sealed with a plug of polyurethane foam and Tygon tubing of a smaller inner diameter, 0.76 mm. The fitting sealed using telfon tape (Heat Merchants, Sligo, Ireland) and superglue (Loctite, Ireland). When super glue was used on its own to seal the two tubes together leaking occurred within a short period as the pump speed was increased. The use of telfon tape in conjunction with the super glue formed a leak proof seal with the tube connections. Following several tests, this type of seal proved to be adequate for the low-pressure matrix removal system.

100 mg of chelating reagent was packed into the column with the aid of a vacuum to ensure a uniform packing of the material. The column was then sealed at the other end using the same telfon push fit connection. The main weakness of this push-fit column is the cumbersome connection of smaller inner diameter tubing to the wider diameter column tubing and also the connection to the peristaltic pump tubing itself.

Another disadvantage of this column design is that once the push fit connection is sealed then it cannot be reopened again. These columns can therefore not be repacked with fresh chelating reagent. It was decided to design a uniform bore column with threaded fittings, **Fig. 2.10**, to overcome the limitations of push-fit columns.

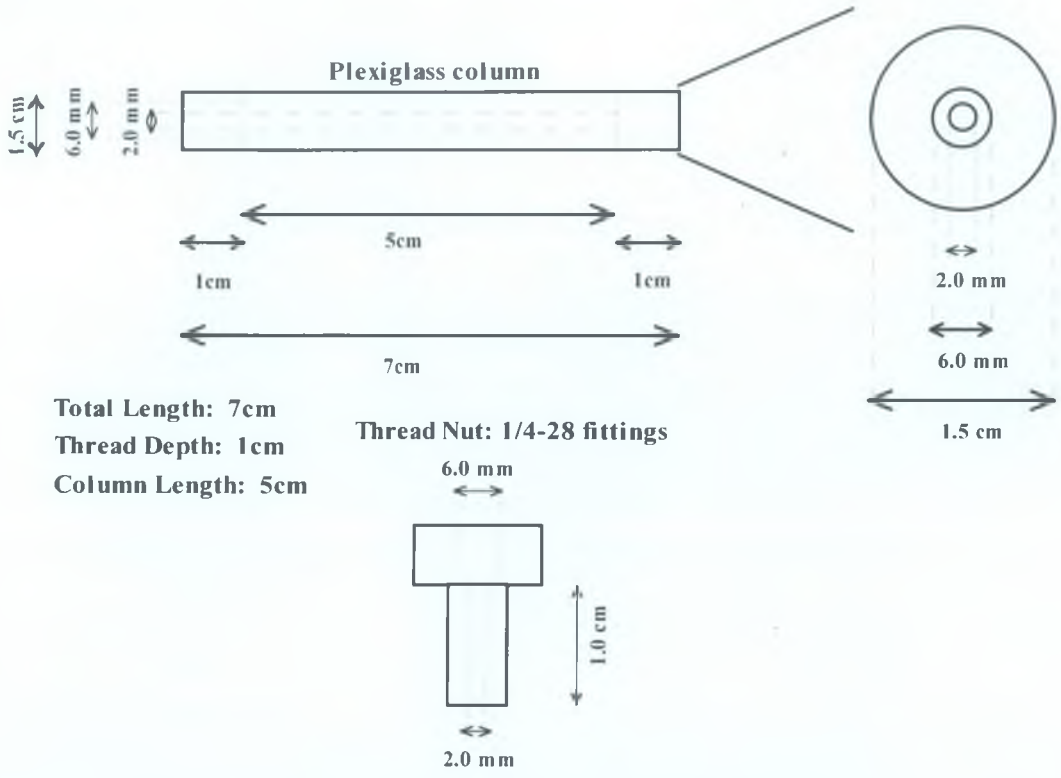


Fig. 2.10 Uniform-bore column using threaded fittings.

The design of the column is adapted from a column design supplied by Dr. Alan Newman, of Coventry University, U.K. The column is a uniform bore design with threaded fittings and is made from a plexiglass block. For higher flow-rates, the use of cheminert fittings (Valco Instruments Co. Inc., USA) ensures that there is a good seal between the column and the tubing connecting the column to the peristaltic pump. The peristaltic pump tubing is connected to the column using Perifit fittings (Valco Instruments Co. Inc., USA). This column is more rugged than the push-fit column design but it will not lead to the use of higher flow-rates.

Backpressure problems caused by the resin used and the limitations of peristaltic pump means that a flow rate of 4 ml min^{-1} is only possible. However this flow rate is acceptable for the intended purpose of this system.

2.7.2 Column Packing Material (Resin).

The first contact with an iminodiacetate-chelating reagent was with the now discontinued product CETAC DSX-100 preconcentration/matrix elimination system (Cetac Technologies, Omaha, NE, USA). The chelating agent consisted of a SPR (suspended particulate reagent) with iminodiacetate (IDA) immobilised onto polystyrene beads, which were approximately 0.2μm in diameter. An aliquot of the SPR suspension was added to the sample and adjusted to the appropriate pH using ammonia solution.

The sample was processed through the DSX-100 system, with a hollow fiber filter cartridge removing the SPR beads and any chelated elements (e.g. Cu, Ni, Zn, Cd and Pb) from the sample solution. Matrix elements such as Na, Ca and Mg were then washed from the filter to waste. The filter was back-flushed with nitric acid eluent and the SPR beads were released into an autosampler vial. The SPR beads with the chelated elements were then aspirated directly into the ICP-MS instrument using a solution-based nebuliser [113]. This is a high tech alternative to the batch technique but it still has a disadvantage of taking one hour to process a single sample. The high cost of the system and its complex design (3 peristaltic pumps, 3 injections valves) ensured that this product was eventually not a commercially viable method of matrix removal/preconcentration. The aspiration of the SPR reagent into the instrument resulted in an increased running cost associated with this technique when compared to column based techniques. The SPR reagent itself was not suitable for use in conventional batch or column based matrix removal/preconcentration system due its small inner diameter of 0.2 μm.

This led to the search for an alternative iminodiacetate reagent and to the use of Chelex 100 (Biorad Lab., Richmond, VA, USA). The resin has been extensively studied for sample pretreatment techniques and has been used both in batch and column based operations [92-96]. The manual provided with the resin provides a good basic overview of how the resin chelates trace elements in samples [68]. This resin was purchased in order to test the basic features of the resin for trace elements preconcentration. It was a readily available product at a minimal cost (25 grams of resin for less than €50).

A burette-based column system was constructed in order to evaluate the physical and chemical characteristics of the resin packing material (**Fig. 2.11**). The chemical behaviour of the resin is discussed in detail in the next chapter. The physical characteristics (i.e. swelling, impedance to solution flow-rate, etc.) were directly relevant to the choice of resin used in the matrix removal/preconcentration systems and some observations are described here.

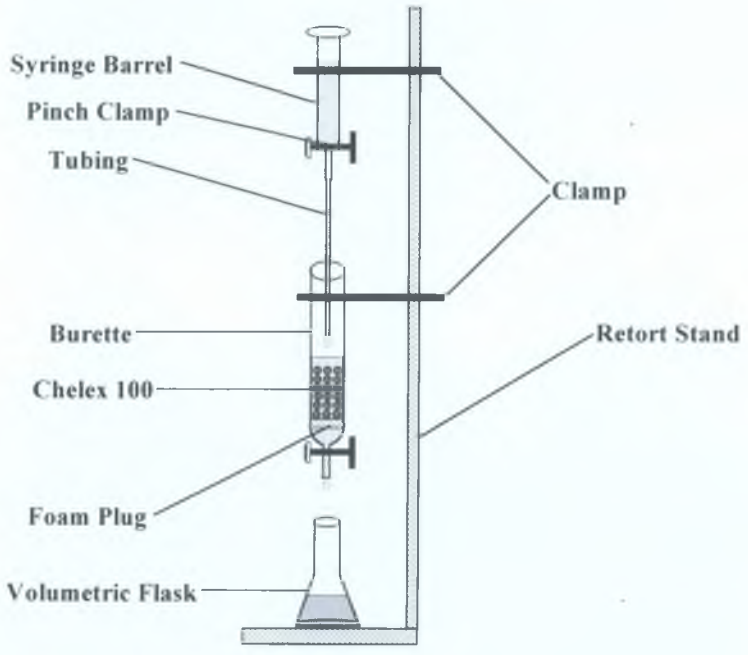


Fig. 2.11 Modified burette column using Chelex 100 chelating resin.

Five grams of chelating resin was placed in a modified 10ml graduated burette. A polyurethane foam plug was placed into the bottom of the burette to hold the resin in place. A volume of water covered the resin at all times to ensure that the resin was equilibrated with the surrounding solution and that no air entered the pores of the polymer resin. This simple experimental system was used to study the behaviour of copper and the matrix elements (calcium, magnesium and potassium) and also to observe important physical characteristics of the resin. Chelex 100 was observed to swell in solution by as much as 50% of its volume on being converted from its salt to acidic form in the modified burette system. Swelling of the resin, while acceptable in the burette-based system, has important negative implications regarding its use in fixed volume preconcentration columns.

The contraction and expansion of the resin by 50-100% of its original volume in a sealed column would lead to erratic blockages, leakages at fittings and flow-rate fluctuations caused by excessive changes in back-pressure which occur when the resin changes size [69]. Swelling and contraction also affects the performance of a unidirectional flow based system by progressively yielding tighter packing of the resin, which ultimately blocks the flow of liquid through a column. This problem can be partially overcome by altering the flow direction through the column by means of counter current elution, which reduces any blocking of the flow. This does not overcome the swelling changes that the resin undergoes during its use [88]. The large number of publications describing the problems of swelling with this type resin, led to the search for reagents, which are more suitable for use in miniaturised columns.

Prosep Chelating I (Bioprocessing, Consett, Durham, UK) was the next iminodiacetate reagent, which was investigated for use in a matrix removal/preconcentration system. It is a controlled pore glass (CPG) reagent with iminodiacetate immobilised onto its surface. The CPG reagent has some important advantages over its polymer-based counterparts. Its rigid structure ensures no swelling occurs when exposed to different solution types.

The iminodiacetate functional group is covalently bonded to the surface of the reagent and this eliminates the need for a buffer conditioning step in the analysis. Although CPG reagents have a lower capacity when compared to polymer based IDA reagents this is not a serious disadvantage when carrying out trace element analysis. The size of the CPG beads, <125 μm, when packed into the burette ensured a flow-rate of less than 1 ml min^{-1}.

The reagent packed into the burette in such a way as to make an acceptable gravity based flow-rate impractical. Therefore, no other chemistry based studied could be carried with the CPG reagent without the development of a column based flow system. A review of the literature using CPG reagents [70, 80, 105] indicated that the use of small quantities of reagent (50-100 mg) in an appropriately designed column was feasible.

2.7.3 Preliminary physical testing of matrix removal/preconcentration system flow rate.

Initially, the flow-rate characteristics of the laboratory-designed columns were evaluated, **Fig. 2.9**. The column was connected to peristaltic pump tubing (i.d. 1.09 mm) using push fit connections. Flow-rates greater than 4 ml min^{-1} are possible by the use of a larger inner diameter peristaltic pump tubing. However, higher flow rates led to back pressure problems, erratic flow-rates caused by pump pulsation and eventually the push fit connections started to leak and break apart.

The flow-rate characteristics of the column at different peristaltic pump settings (revolutions per minute) were studied using a Gilson Minipuls peristaltic pump (Gilson Inc. Wisconsin, USA). A fixed volume of water pumped through the column and the time was recorded. The flow-rate in ml min^{-1} was then calculated, **Table 2.1**. Each measurement was carried out in triplicate.

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Table 2.1 Laboratory column flow-rate study using a peristaltic pump

Flow-rate, ml min^{-1}	RPM	%RSD
1.01	9.5	2.18
1.50	15.2	1.50
1.74	18.0	1.35
2.02	20.9	1.17
2.31	23.7	0.91
2.61	26.6	2.64
2.85	29.4	0.21
3.05	29.4	0.79
3.20	32.1	4.24
3.27	35.1	4.37
3.49	37.0	0.31
3.60	38.0	0.29
3.68	37.3	0.20
3.69	37.4	0.10
3.70	37.2	0.75
3.70	38.0	0.48
3.70	37.5	0.26
3.73	37.8	1.11
3.82	37.1	3.50
3.93	40.8	3.96
4.09	43.6	0.14
4.37	46.5	0.79

The reproducibility in flow rate was in the range 0.1-4.37% and did not change significantly as the flow rate was increased. The flow-rate was steady over short and long term use. At these flow rates there was no observable pulsation of the tubing. The correlation coefficient of 0.9936 (**Fig 2.12**) indicates that an acceptable linear relationship exists between flow-rate and peristaltic pump setting.

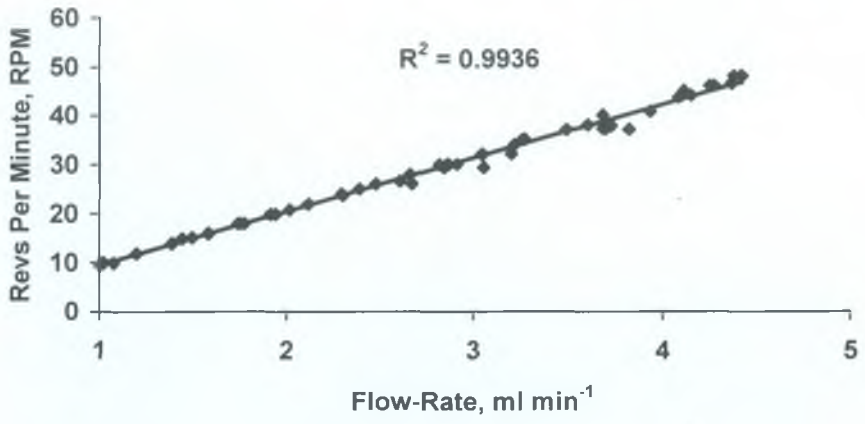

Fig. 2.12 Effect of peristaltic pump RPM versus system flow-rate.

The flow rate study demonstrated that the push fit connections are reliable over long periods of use at flow-rates of <4 ml min^{-1}. They are rugged enough in design and operation, i.e. stable flow-rates and no backpressure problems. Since the reproducibility of the flow-rate did not vary significantly, the optimum flow-rate was based on the requirements of high sample throughput, minimal backpressure and the capabilities of the peristaltic pump being used.

Considering the inner diameter of the tubing and type of preconcentration column used a flow rate of 4 ml min^{-1} was deemed acceptable for this laboratory system. The flow-rate also compared well to the majority of commercial and laboratory developed sample pretreatment systems, which operate also at flow-rates of ≤4mls min^{-1} [2, 3, 114].

The next stage of the research was to examine the various experimental parameters that affect the analytical performance of the system: pH, acid eluent volume and concentration, sample buffer concentration, buffer wash volume and matrix element concentration. Flame Atomic Absorption Spectrometry (FAAS) was used for the preliminary studies and multielement Inductively Coupled Plasma Mass Spectrometry (ICP-MS) technique was used in all additional studies.

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Chapter Three

3.0 Analytical Characteristics.

3.1 Introduction.

In this section, the analytical characteristics of the iminodiacetate matrix removal/preconcentration system will be discussed in detail. A number of experimental parameters were optimised and their effects on system performance examined. These parameters include:

1. pH.
2. Buffer concentration.
3. Buffer wash concentration and volume.
4. Nitric acid eluent concentration and volume.
5. Linearity.
6. Flow rate.

3.2 Analytical Methodology and Instrumentation.

3.2.1 Reagents.

Reagents used for trace elements analysis will be described in the following sections. Glassware and containers were soaked overnight with 10% nitric acid and rinsed several times with ultra pure water prior to use.

FAAS Reagents.

Single-element calibration standards for atomic absorption measurements were prepared in 2% SpA nitric acid (Romil Ltd, UK) by dilution of SPEX CertiPrep 1000 mg l^{-1} certified single element standards. A calibration blank was prepared with the same matrix as the standards i.e. 2% nitric acid. All samples and standards were background corrected using this blank.

ICP-MS Reagents.

All ICP-MS calibration standards, internal standards and spiking solutions were prepared from certified ICP-MS standards manufactured by SPEX CertiPrep, USA. Nitric acid SpA (Romil Ltd, UK) was used in the preparation of samples, calibration standards and all other solutions.

Ultrapure water from a Millipore 2 analytical grade water purification system (Millipore, Badford, MA) was used for all dilutions and for the preparation of standard solutions.

An internal standard solution of 10 mg l^{-1} (Be, Sc, Rh, In and Bi) in 2% Nitric acid was prepared from SPEX CertiPrep (SPEX CertiPrep, USA) single element 1000 mg l^{-1} standards. All standards, samples, and blanks were spiked to 100 μg l^{-1} with this solution.

Multielement calibration standards (0.5, 2.5, 5, 10, 25, 50, 100, 250 and 500 μg l^{-1} were prepared in 2% Nitric acid by dilution of SPEX CertiPrep 1000 mg l^{-1} certified single element standards. A calibration blank was prepared having the same matrix as the standards i.e. 2% nitric acid. All samples and standards were background corrected using this blank. A 50 μg l^{-1} Relsope standard is prepared by dilution of 10 mg l^{-1} mulitelement solution. The 50 μg l^{-1} multielement calibration check standard was prepared from 10 mg l^{-1} SPEX Claritas mulitelement mix solution. The 10 mg l^{-1} multielement spike solution was prepared by dilution of the 1000 mg l^{-1} certified single element standards. Instrument recovery was determined for selected samples by spiking to a concentration of 100 μg l^{-1}.

Matrix removal/preconcentration system reagents.

The 0.5M Nitric acid eluent was prepared by diluting concentrated Nitric acid (Romil-SpA, UK). The controlled pore glass iminodiacetate reagent (Prosep, Bioprocessing, UK) and Chelex 100 (Biorad Laboratories, USA) were used as supplied. The ammonium acetate buffer solution (4M) was prepared by slowly adding 260 ml of concentrated ammonia (Romil-SpA, UK) and 230 ml of glacial acetic acid (Romil-SpA, UK) to 510 ml of ultrapure water in a 1 L polyethylene bottle. Adjustments in pH of this buffer were made using acetic acid or ammonium hydroxide as appropriate.

Cleanup of Buffer solution.

A number of elements had high background levels in the ammonium acetate buffer. This degrades the detection limits achievable with this technique. This problem was overcome for some of the experimentation by continuous recirculation of ammonium acetate buffer through columns of Prosep iminodiacetate reagent in order to remove the trace elements. Two peristaltic pumps were used to pump the ammonium acetate through several columns of the iminodiacetate buffer for 6 hours or longer.

Spike solutions.

A 10 mg l^{-1} multielement spike solution was prepared by dilution of the 1000 mg l^{-1} certified single element standards. To test method recovery, selected samples were spiked to a concentration of 25 μg l^{-1} with this solution.

3.2.2 Description of Instrumentation.

The Perkin Elmer 2380 Atomic Absorption Spectrometer was used in both absorption and emission mode. Calcium, magnesium and several other elemental measurements were made using the technique of Flame Atomic Absorption Spectrometry (FAAS) while Flame Atomic Emission Spectrometry (FAES) determined sodium. Multielement measurements were made using the technique of Inductively Coupled Plasma Mass Spectrometry (ICP-MS).

3.2.3 Operation of the Atomic Absorption Spectrometer.

The samples were analysed with a Perkin-Elmer 2380 atomic absorption-spectrophotometer in absorption mode. After inserting the appropriate hollow cathode lamp (HCL) for the analysis, the lamp was allowed to warm up for a minimum of 15 minutes. During this period, the position of lamp was aligned, the monochromator set at the correct wavelength, slit width, and the hollow cathode current set to the recommended level. The flame was lit and the flow of fuel and oxidant adjusted if required.

A signal-check solution (4 mg l^{-1}) was then aspirated and the burner head position and nebuliser flow rate was adjusted to obtain the optimium absorbance reading of 0.2 and also signal stability. The instrument is zeroed prior to calibration using a 2% nitric blank. A series of standards of the analyte (1, 2, 4, 8, 10 mg l^{-1}) are aspirated through the instrument and the absorbance reading recorded. The calibration curve was constructed by plotting the absorbance against the concentrations of the standards. The samples absorbance was recorded and its element concentration was determined from the calibration graph.

3.2.4 Operation of the Atomic Absorption Spectrometer in Emission Mode.

The procedure for flame emission analysis is similar to flame absorption. The instrument was optimised for the selected element using a hollow cathode lamp (HCL) of the analyte being determined. The lamp is then unplugged, flame lit and the instrument is switched to emission mode. The instrument is calibrated using a series of single element standards in appropriate range (1, 2, 4, 8, 10 mg l^{-1} or 10, 25, 50, 100 mg l^{-1}).

The emission intensity range (0-100) was set by first aspirating the calibration blank and then the highest standard used. The instrument was zeroed prior to calibration using a 2% nitric blank. The emission intensity was plotted against the concentration of the standards and a calibration graph was constructed. The emission intensity of the sample was recorded and its elemental concentration was determined from the calibration graph.

3.2.5 Operation of the Beckman Flame Photometer.

The instrument was calibrated using a series of single element standards in appropriate range (1, 2, 4, 8, 10 mg l^{-1} or 10, 25, 50, 100 mg l^{-1}). The emission intensity range (0-100) was set by first aspirating the calibration blank and then the highest standard used. The instrument was zeroed prior to calibration using a 2% nitric blank. The emission intensity was plotted against the concentration of the standards and a calibration graph was constructed. The emission intensity of the sample was recorded and its elemental concentration was determined from the calibration graph.

3.2.6 Inductively Coupled Plasma Mass Spectrometer.

Elemental determinations were made using an inductively coupled plasma mass spectrometer equipped with a microconcentric nebuliser. The ICP-MS instrument used was the VG PlasmaQuad (VG Elemental, Winsford, UK) equipped with a high performance interface. The interface between the plasma and the mass spectrometer consists of nickel sampling and micro skimmer cones with orifice diameters of 1.0 and 0.75 mm respectively.

The sample introduction system consisting of a microconcentric nebuliser MCN-100 (CETAC Technologies, USA) was used with a standard double-pass Scott type spray chamber and surrounding liquid jacket.

Instrument optimisation.

Prior to analysis the ICP-MS instrument was evaluated for accurate mass scale calibration, short-term stability, isotopic resolution and low background count levels. Instrument response was optimised at ^{115}In by adjustment of ion lens potentials, plasma position relative to the sampling cone and gas flow rates (nebuliser, auxiliary and plasma gas). Typical optimised instrument operating conditions are specified in **Table 3.1**.

Table 3.1 ICP-MS operating parameters

Cool flow	12.5 l min^{-1}
Auxiliary gas flow	0.6 l min^{-1}
Nebuliser gas flow	0.973 ml min^{-1}
Incident power	1500 w
Reflected power	5 w
Channels/amu	20
Detector mode	Pulse counting
Sample flow rate, MCN 100	150 µl min^{-1}

ICP-MS analytical protocol and measurements.

The calibration standards were run initially followed by the samples. Each blank, sample and standard solution was measured using three by one-minute replicate acquisitions. After each aspiration, the sample introduction system was rinsed for one minute with 2% nitric acid solution to avoid sample-to-sample memory effects.

Quality Control.

The ion count at Indium-115 was monitored for each solution as a check on general solution preparation and instrument performance (e.g. nebuliser blockage). The method blank, calibration blank, 50 μg l^{-1} reslope standard and 50 μg l^{-1} calibration check standard were run after each batch of 15 samples in order to monitor instrument drift (if these quality checks indicated errors then analysis of the preceding samples was repeated or corrections are made to the analytical results). ICP-MS laboratory precision was measured by the repeat analysis of samples during two separate runs carried out on different days.

The limit of determination (LOD) for each element was based on a multiple of the standard deviation of a blank solution (at least 6 times the standard deviation of the blank) and results lower than this limit are reported as less than the determination limit (e.g. <5 μg l^{-1}).

Method Blank.

When using the matrix elimination/preconcentration system, a method blank is used to determine if analytes or interferences are present in the laboratory environment, the reagents or the instrument, which will degrade the detection limits of the measurement. This method blank is prepared from buffer solution and is carried through the entire analytical procedure, including exposure to all glassware, ammonium acetate buffer, nitric acid eluent, internal standards and finally the instrumental analysis step used with samples.

3.2.7 Matrix Removal/Preconcentration System.

A schematic diagram of the matrix removal/preconcentration system is shown in **Fig. 3.1**. This was constructed from PTFE tubing and the column was packed with 100 mg of Prosep. The valve used in this system is a four-stream selector valve (Model C25F-3184-EMH, Valco Europe).

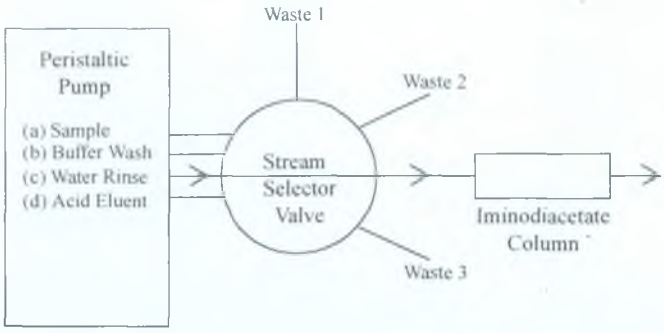


Fig. 3.1 Schematic diagram of matrix removal/preconcentration system.

Control over stream selection is achieved either manually using a manual control unit or automatically by computer via an RS 232 serial cable using an MS-DOS© based timetable program. The reagents were pumped through the column at 4 ml min^{-1} per channel using 1 mm i.d. Tygon® peristaltic pump tubing (VG Elemental, Winford, Chesire, UK) via a 4-channel peristaltic pump (Gilson Minipuls 2). A dual electrode pH meter (Model 691, Metrohm) was used throughout.

Operating Procedure.

The steps involved in the matrix removal/preconcentration procedure are illustrated in **Table 3.2**.

Table 3.2 Matrix removal/preconcentration operating procedure.

Step	Description	Solution	Time (min)
1	Column regeneration	Nitric Acid	1
2	Conditioning of column	Buffer Wash	1.5
3	Loading of sample	Sample	6.25
4	Matrix Removal	Buffer Wash	1.5
5	Rinse	Water	1.5
6	Elution start	Nitric Acid	1

Memory effects from a previous sample are removed in the initial acid washing step (step 1). The column is then conditioned by washing with the buffer solution (step 2) followed by loading of the sample for preconcentration and matrix removal (step 3). Another buffer washing step (step 4) removes the sample matrix.

The buffer itself is then washed from the system with a water rinse step (step 5). Finally the concentrated trace elements are eluted from the column with a nitric acid wash (step 6). Multielement analysis of the eluent solution is then performed by ICP-MS. A flow-rate of 4 ml min^{-1} was chosen for most studies for the reasons outlined in **section 3.7.6**.

3.3 Preliminary experiments using Chelex 100 resin.

The experiments were designed in order to become familiar with the basic procedures required for preconcentration and matrix elimination resins. In addition the system facilitated the study of the fundamental analytical characteristics of the iminodiacetate resin (i.e. pH dependence, buffer concentration etc.) prior to the development of the automated system.

Procedure

A buffered water sample was added to the modified burette with 5g of Chelex 100, **Fig. 2.11**. Preparation of the column was described previously, **section 2.7.2**. The retention of the test element copper was carried using this system for 21 replicate measurements. The experimental conditions used for this column system are shown in **Table 3.3**.

Table 3.3 Experimental conditions using the modified burette system.

Sample Buffer, M	0.25
Buffer Wash, M	0.25
pH	5.5
Acid Eluent, M	2

The initial concentration of buffer in the sample was 0.25M ammonium acetate with a pH of 5.5. 20 ml of sample was passed through the column, while continually ensuring that the liquid in the burette did not fall below the top of the resin packed in the burette.

A further 10 ml of 0.25M buffer was then used to wash the unretained sample components from the burette. A 20 ml water rinse step was used to wash the residual matrix and buffer from the column.

The retained analyte was then eluted from the resin using 10 ml of 2M nitric acid eluent, which was collected and analysed. The preconcentration factor (PF) obtained using this method was two.

The elements copper, magnesium and calcium were analysed by the technique of Flame Atomic Absorption Spectrometry (FAAS). Potassium was analysed by the technique of Flame Atomic Emission Spectrometry (FAES).

Results and Discussion.

The recovery of copper from Chelex 100 from the simulated sample is shown in **Table 3.4**. The recovery of copper from the simulated water sample was in the range 73-110%. These experiments indicated that the resin selectively chelated copper with high efficiency from the simulated sample under these experimental conditions. The precision of the analysis (1.6-6.7%) for replicate samples also showed how reliable the technique is even when using this simple experimental design.

Table 3.4 Recovery of copper from Chelex 100 using the modified burette.

Sample No.	% Recovery	% RSD
1	88	3.2
2	86	3.3
3	88	1.6
4	76	3.7
5	73	3.6
6	110	3.2
7	86	6.7

n=3

However, the variability in recoveries between samples is relatively high (73-110%). This variability indicates that the methodology used must be improved. The efficiency of matrix removal for the elements Ca, Mg and K is shown in **Table 3.5**.

Table 3.5 Matrix removal using Chelex 100 using the modified burette.

	Efficiency of matrix removal, %		
Sample No.	**Mg**	**K**	**Ca**
1	65	67	86
2	65	68	85
3	61	68	88
4	65	74	85
5	60	73	86
6	65	74	84
7	61	80	88
Mean	**63**	**72**	**86**
% RSD	**3**	**7**	**2**

n=3, pH 5.5

Since this was a preliminary experiment, these results were considered a successful demonstration of the capability of the chelating resin using these experimental conditions. The modified burette system successfully demonstrated some of the basic principles of using an iminodiacetate chelating resin. The procedures for using the system, buffer preparation and pH adjustment of the sample prior to introduction to the column, were studied and refined. The steps involved in loading the sample onto the resin and washing the column prior to elution of the analyte from the column using 2M nitric acid were also studied.

3.4 pH dependence of Iminodiacetate based Matrix Removal and Preconcentration.

The stability of iminodiacetate chelates is affected by changes in pH and therefore it is an important parameter to optimise.

3.4.1 Effect of ammonium acetate pH on the recovery of Co, Cd, Zn, Ni, Cu, Cr, Mn, Fe and Ca.

The pH dependence (pH 1-10) of the column system using the Prosep reagent was evaluated for a range of elements. A multielement solution was prepared in an ammonium acetate buffer system. The 0.25M ammonium acetate buffer was adjusted to the desired pH using either acetic acid or ammonium hydroxide.

The technique of Inductively Coupled Plasma Mass Spectrometry (ICP-MS) was used to determine the trace elements. The data results for Co, Cd, Zn, Ni, Cu, Cr, Mn, Fe and Ca are given in **Table 3.6**.

Table 3.6 Effect of ammonium acetate pH on element recovery.

	pH					
	2		4		5.5	
Element	% Recovery	% RSD	% Recovery	% RSD	%Recovery	%RSD
Co	1	n/a	81	9	96	3
Cd	7	n/a	94	4	95	3
Zn	0	n/a	87	5	100	7
Ni	1	n/a	97	6	100	3
Cu	86	3	97	4	97	2
Cr	0	n/a	3	58	3	8
Mn	0	n/a	55	53	44	27
Fe	90	4	85	1	80	1
Ca	0	n/a	20	39	17	9

Table 3.6 (continued) Effect of ammonium acetate pH on element recovery.

	pH			
	8		10	
Element	% Recovery	% RSD	% Recovery	% RSD
Co	93	5	85	4
Cd	88	2	89	4
Zn	74	3	79	3
Ni	98	3	93	5
Cu	98	4	95	3
Cr	84	4	42	2
Mn	94	5	90	29
Fe	91	15	94	18
Ca	89	6	95	4

n=3

The recovery of Co, Cd, Zn, Ni, Cu and Fe are shown graphically in **Fig 3.2a and b**. These elements show a similar same behaviour on the iminodiacetate reagent with maxmium recovery, >86%, in the region of pH 5.5. The precision of the measurements across the pH range was <10%.

The recovery of Mn is illustrated in **Fig.3.2b**. Manganese shows a different pH dependence to copper with optimium recovery in the region of pH 8.

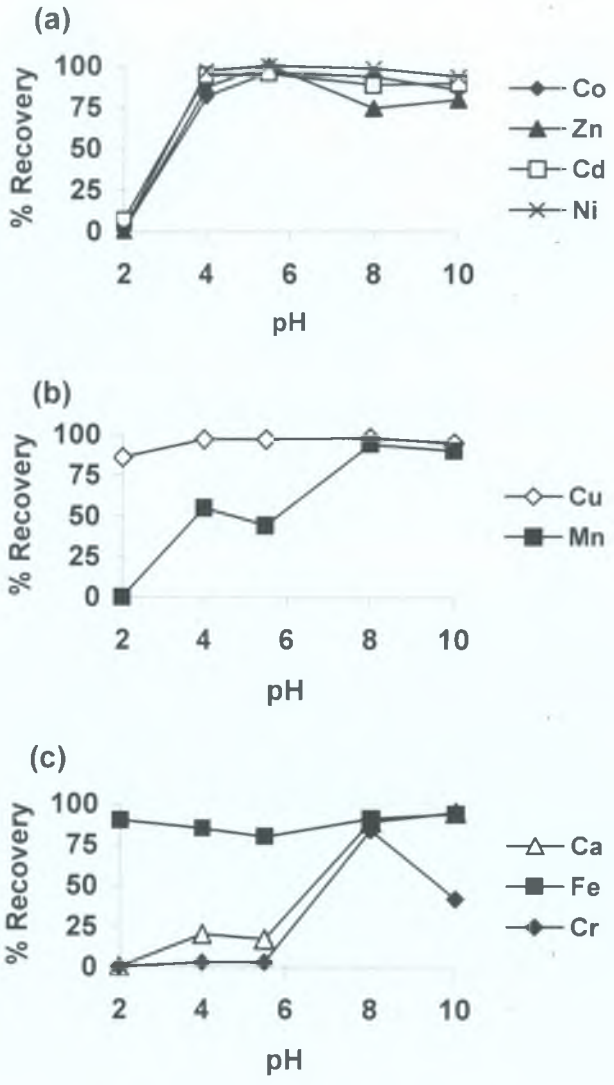


Fig. 3.2 Effect of ammonium acetate pH on Co, Zn, Cd, Ni, Cu, Mn, Ca, Fe and Cr.

The effect of pH on both Cr and Mn is similar and the recovery of both elements peaked at pH 8. It has been previously reported that with proper pH control Mn and Cr show an optimum recovery at this pH [105]. Although working at pH 8 may retain these elements however efficient matrix removal from the sample will not be achieved.

The high recovery of Cr at this pH is interesting in that there are few reports of this element having been successfully recovered using an iminodiacetate reagent using similar experimental procedures [115].

Athough Cr^{3+} has been shown to strongly complex with iminodiacetate in solution, its conversion to Cr^{6+} by the acetic acid present in the ammonium acetate buffer results in the low recovery of this element across the pH range.

A chromium (III) and total chromium speciation study was successfully developed using an iminodiacetate resin but ammonium acetate buffer was not used to control pH. The sample was adjusted to pH 3 using hydrochloric acid and passed through iminodiacetate chelating resin. Chromium (VI) in the sample was reduced to Cr (III) using hydroxylamine solution prior to being processed through the iminodiacetate column [115].

The recovery of the calcium was also studied during this experiment. Recoveries for calcium was <14% from pH 4-7 and almost quantitative recovery of 93% occurring at pH 8.5. Calcium behaves significantly different from most other elements and exhibits very poor recoveries at pH 6 and below.

An operational pH in the range of pH 4-6 would appear suitable for samples if the objective is to generate conditions promoting poor retention of calcium (usually a matrix interferent) while achieving high recoveries for the other elements. Overall the optimum compromise pH for high recovery of trace elements (Co, Cd, Zn, Ni Cu and Fe) and efficient matrix removal of calcium is generally pH 5.5.

The recovery for Mn and Cr was poor (<3%) at pH5.5. Mn recovery is low due the competition effect of calcium present in the multielement spike solution for the iminodiacetate reagent. The strength of the Mn iminodiacetate complex Ca iminodiacetate complex is at least 100 times weak across the pH range studied.

This indicates that this element will have the same pH behaviour as Ca on the iminodiacetate reagent. Increasing the concentration of calcium above the level of manganese will magnify this effect. The oxidation of Chromium (III) to Chromium (VI) by the acetate buffer ensures poor recovery of this element by the chelating reagent.

Overall these results compared well to the literature describing the use of iminodiacetate chelating reagents for matrix removal/preconcentration techniques.

The optimum pH for matrix removal/preconcentration systems has been shown to be pH 5.5-6 because of its high retention of trace elements while achieving efficient removal of the Ca and Na matrix elements from the sample.

At this pH range the high conditional formation constants of Cu, Zn and Cd relative to the weak formation constants of Ca and Na result in the efficient separation of these elements on the iminodiacetate chelating reagent (see **Appendix A**).

3.4.2 Effect of pH on the recovery of Tl, Al, Ti, Sn, Mo, V, Ce, Pb, Th and U.

The pH study results for Tl, Al, Ti, Sn, Mo, V, Ce, Pb, Th and U are given in **Table 3.7** and the data is graphed **Fig. 3.3**. Recoveries for thallium over the entire pH range are poor but do increase with increasing pH. It has been previously reported that thallium has not been quantitatively retained on the iminodiacetate reagent [100].

Speciation procedures for Tl using an iminodiacetate resin by a different experimental procedure have been reported [116, 117].

These procedures do not use ammonium acetate to control the pH of the sample. The presence of ammonium acetate may prevent the uptake of this element and this effect may be similar to one that causes the low recovery of chromium on this reagent.

Table 3.7 Effect of ammonium acetate pH on the recovery of Tl, Al, Ti, Sn, Mo, V, Ce, Pb, Th, U

	pH					
	4		5.5		10	
Element	**% Recovery**	**% RSD**	**% Recovery**	**% RSD**	**% Recovery**	**% RSD**
Tl	1	27	1	10.6	25	12
Al	39	4	85	3.7	80	12
Ti	86	4	79	7.4	83	12
Sn	43	22	79	n/a	64	16
Mo	65	13	2	2.5	0	13
V	92	4	89	0.7	12	28
Ce	111	5.9	98	3.7	59	1.0
Pb	104	12	105	5.6	49	12.7
Th	91	2.7	99	4.0	55	2.6
U	78	5.2	79	7.6	50	3.5

n=3

The recoveries for Al and Ti reach greater than 79% at pH 5.5 (**Fig. 3.3a**). The pH dependence of Sn, V and Mo is shown in **Fig. 3.3b**. The recoveries of Sn and V are greater than 79% at pH 5.5. The recovery for Mo reached 80% at pH 2 and reduced to 65% at pH 4.

As the pH increases > pH4 the iminodiacetate reagent loses its ability to chelate Mo and no longer retains the element. The optimum pH for molybdenum was determined to be less than pH 4.

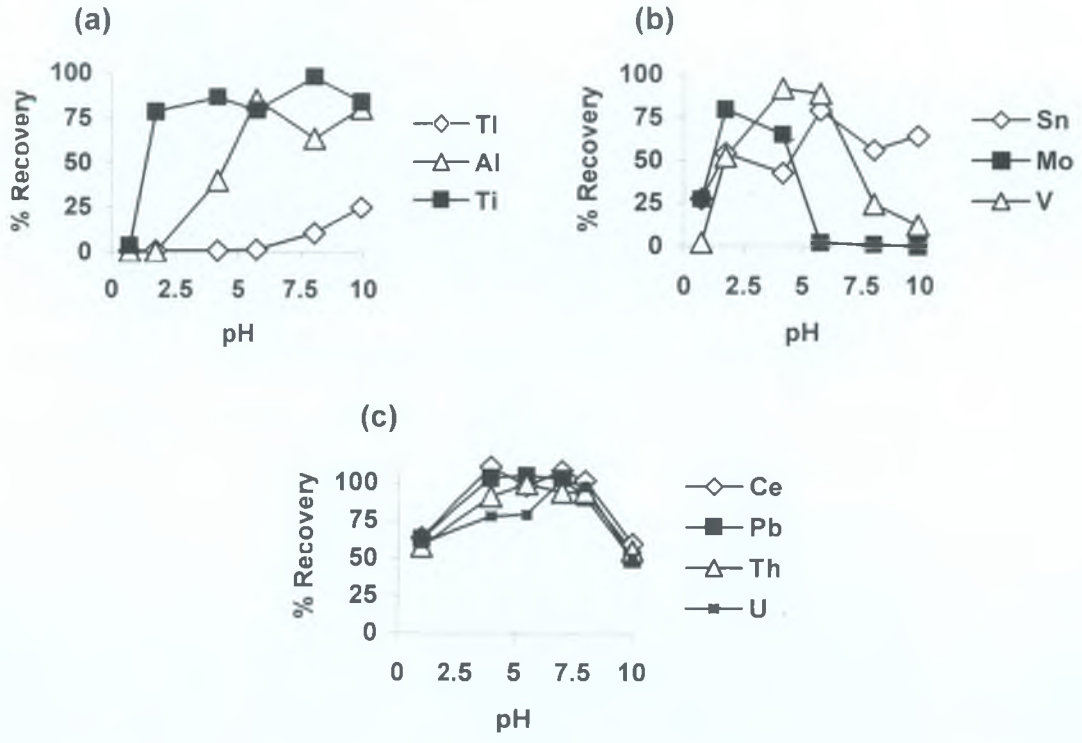


Fig. 3.3 Effect of ammonium acetate buffer pH on the recovery of trace elements

The pH behaviour of both Sn and Mo compare favourably with work carried out by other researchers [119, 120]. However conflicting results indicating low recovery for Sn and Mo across the pH range have been reported [34]. This may indicate that the chemistry of these elements in solution varies and influences their recovery from the iminodiacetate reagent.

Good recoveries of >91% for Ce, Pb, Th and U are observed from pH 4 and above. Each element has a characteristic optimum pH range for preconcentration on the iminodiacetate reagent. High recovery of Ce, Pb and Th is achieved above pH 4 while U is 79% recovered above pH 5.5.

3.4.3 Effect of ammonium acetate pH on the recovery of alkaline earth elements and Beryllium.

The optimum pH for the analysis of trace levels of these elements is greater than pH 8. The recoveries of these elements at this pH are summarised in **Table 3.8.**

Table 3.8 Recoveries of Alkaline earth elements and Beryllium at pH 8.

Element	% Recovery	% RSD
Ba	96	2.8
Ca	95	4.1
Mg	96	4.6
Sr	100	4.5
Be	83	6.8

n = 3

The pH dependence profile for the Ba, Ca, Mg, Sr and Be is also shown in **Fig 3.4**. Quantitative recovery of these elements (95-100%) is achieved at pH 8.

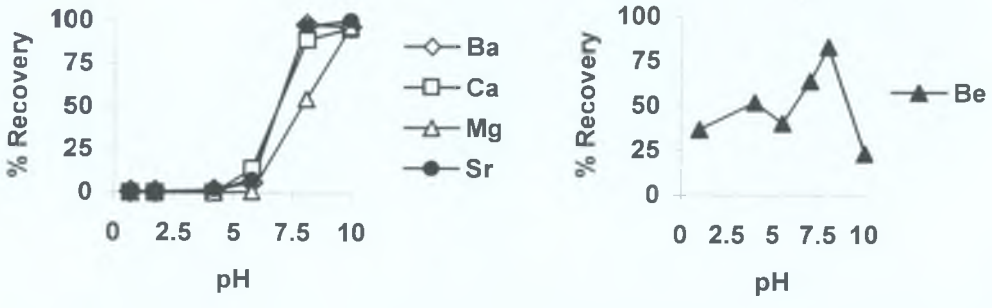


Fig. 3.4 Effect of ammonium acetate buffer pH on the recovery of the Alkaline earth elements and Beryllium.

The pH study results obtained here compare with previous work which noted that high recovery of alkaline earth and beryllium is obtained at a pH greater than 8 [121]. A number of publications have taken advantage of this pH behaviour to determine alkaline earth metals in brine solution [122-123]. The toxicity of beryllium in the environment [124] has required the use of iminodiacetate based preconcentration techniques and the pH 8 was used for high recovery of this element from rock [125] and water samples [126].

The matrix elements Ca and Mg are present at macros level when compared to trace elements in environmental samples. Therefore the efficient matrix removal of these elements from the iminodiacetate reagent at pH <6 or lower is required. The poor retention of these elements pH <6 is in agreement with a previous retention study of Ca and Mg on a similar iminodiacetate resin [127].

The best overall compromise pH for the high recovery of the majority of trace elements while achieving efficient matrix removal of alkaline earth elements (Ca and Mg) is about pH 5.5. In the case of some elements the use of a pH higher than pH 5.5 can lead to the formation of hydroxides resulting in lower trace element recoveries [114].

3.5 Reproducibility.

The next stage of the research was to evaluate the reproducibility of the Tygon tube based iminodiacetate reagent packed column and the associated analytical methodology. Preconcentrating a low level spike of 30 $\mu g\ l^{-1}$ copper tested the reproducibility of the system incorporating this reagent. Replicate spiked samples of 30 $\mu g\ l^{-1}$ copper in ultra-pure water were buffered to pH 5.5 using the ammonium acetate buffer as described previously. The sample was then pumped through the column system at a flow-rate of 4 ml min^{-1} using a peristaltic pump. The experimental conditions used are given in **Table 3.9**

Table 3.9 Experimental conditions using the column system.

Sample volume, ml	100
Eluent volume, ml	10
Preconcentration Factor	10
Acid Eluent, M	2
Sample Buffer, M	0.25
pH	5.5

The concentration of the spike in this sample was beyond the detection limit of the Flame Atomic Absorption Spectrometer. However, after preconcentration, Flame AAS can be used to determine the copper in the acid eluent. 100 ml of buffered sample was pumped through the column and a buffer wash step was used to remove any matrix elements.

A water rinse step was used to remove residual matrix and buffer from the column. The copper was eluted from the column using 10 ml of 2M nitric acid eluent. The copper analyte was then preconcentrated by a factor of approximately ten and was therefore easily measured by flame atomic absorption spectrometry. Eight replicate spikes were processed through the column system and the recovery of each spike is show in **Table 3.10.**

Table 3.10 Preliminary column recovery of copper from Prosep CPG.

Sample	% Recovery	%RSD
1	95.7	2.1
2	94.8	1.2
3	94.5	3.1
4	91.3	5
5	97.3	1.8
6	94.3	1.4
7	90.3	2.4
8	93.7	3.1

n=3

The average recovery of the spike from the water sample was 94% with average %RSD of 2.4%. This recovery is similar to that reported in the literature for copper under similar experimental conditions [105]. This demonstrated that the basic column design was capable of carrying out its required function in an accurate and reproducible manner. The reproducibility of the system is good and is superior to the burette-based system described previously, see **Table 3.4.**

3.6 Factors Influencing Matrix Removal.

3.6.1 The influence of the ammonium acetate buffering system.

The ammonium acetate buffer system buffers the sample to the optimum pH of 5.5 in order to achieve efficient analyte recovery and matrix removal. The ammonium ion of the buffer competes for iminodiacetate complexation sites with the matrix elements. It is also present at a high concentration when compared to concentration of matrix elements in a sample thereby impeding retention of these elements via equilibrium effects.

The ammonium ion has a number effect on the formation of iminodiacetate complexes with trace elements such as Cu, Cr, etc. This buffer therefore promotes efficient matrix removal, >99%, for calcium, magnesium and sodium while allowing trace elements to remain on the resin. In order to select the optimum buffer concentration, its effect on matrix removal must be considered.

3.6.2 The effect of varying sample buffer concentration on retention of Ca, Mg and Na.

The buffer concentration of a sample containing 10000ppm matrix of the common elements Ca, Mg and Na was varied from 0.25-2M. The pH of the sample was maintained at pH 5.5 ± 0.1. This pH was determined by experiment and through examination of literature to be the optimum pH for efficient matrix removal. No buffer wash step was carried out and only a water rinse step was used to remove any matrix elements and acetate buffer remaining in the column. This was done in order to measure only the effect of sample buffer concentration on matrix removal. The residual matrix, which was retained on the chelating reagent, was then eluted using 3% nitric acid eluent. The matrix elements in the acid eluent were determined using the techniques of Flame Atomic absorption Spectrometry (FAAS) and Flame Atomic Emission Spectrometry (FAES). The results indicate that varying the sample buffer concentration (0.25-2M) had no significant effect on the efficiency of matrix removal, **Table 3.11**.

Table 3.11 Effects of ammonium acetate buffer concentration on matrix removal.

Buffer, M	Na	% RSD	Mg	% RSD	Ca	% RSD
0.25	99.5	0.028	99.8	0.054	99.8	0.103
0.5	99.5	0.073	99.5	0.027	99.8	0.137
1	99.7	0.042	99.5	0.008	100	0.116
2	99.8	0.025	99.8	0.097	99.7	0.109

n=3

99.5-100% of the 10000ppm matrix (Na, Mg and Ca) was successfully removed from the original sample. The level of matrix elements retained in the acid eluent is <50ppm on average. This represents <0.5% of 10000ppm matrix remaining in the acid eluent after the matrix removal step was carried out, **Fig 3.5**.

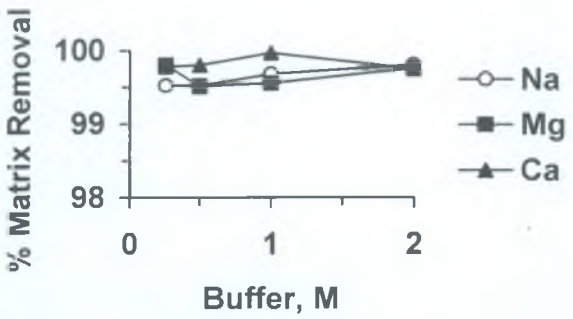


Fig 3.5 Effect of ammonium acetate buffer concentration on matrix removal.

Increasing the buffer concentration did not lead to more efficient matrix removal (<50ppm). The lowest concentration of buffer (0.25M) can therefore be chosen for subsequent experiments.

The main benefit of a lower concentration of sample buffer is its lower trace element blank levels. This is an important consideration as blank levels in the chemical reagents used in the method can affect the overall efficiency of the matrix removal/preconcentration system i.e. degraded method detection limits. In summary, these results indicate that a sample buffer concentration of 0.25M is suitable for efficient matrix removal of alkali/alkaline earth elements using this laboratory-designed system.

3.6.3 Effect of matrix concentration on matrix removal.

The original concentration of matrix elements (Ca, Mg and Na) in a sample may also have an effect on the efficiency of matrix removal. This possible effect was studied by varying the concentration of these elements from 5000-40000ppm while the ammonium acetate buffer concentration of the sample was kept constant at 0.25M.

No buffer wash step was carried out and only a water rinse step was used to remove any remaining matrix elements and acetate buffer remaining in the column prior to analyte elution. The matrix elements in the acid eluent were determined using the techniques of Flame Atomic absorption Spectrometry (FAAS) and Flame Atomic Emission Spectrometry (FAES).

The results indicate that varying the original matrix concentration (5000-40000ppm) in the sample had no significant effect on the efficiency of matrix removal, **Table 3.12**.

Table 3.12 Effect of matrix concentration on % matrix removal efficiency.

Matrix, ppm	Mg*	% RSD	Na**	% RSD	Ca*	% RSD
5000	98.9	0.013	99.9	0.141	99.7	0.197
10000	99.8	0.019	99.8	0.09	99.8	0.131
20000	99.7	0.032	99.9	0.085	99.9	0.128
40000	99.9	0.015	99.9	0.021	n/a	n/a

n=3
Method of determination: *FAAS, **FAES

99.7-99.9% of the matrix elements (Na, Mg and Ca) were successfully removed from original sample across the studied matrix concentration range (5000-40000ppm). As the concentration of matrix elements increased, the level of matrix elements retained in the acid eluent is consistently <50ppm. These results are illustrated in **Fig 3.6**.

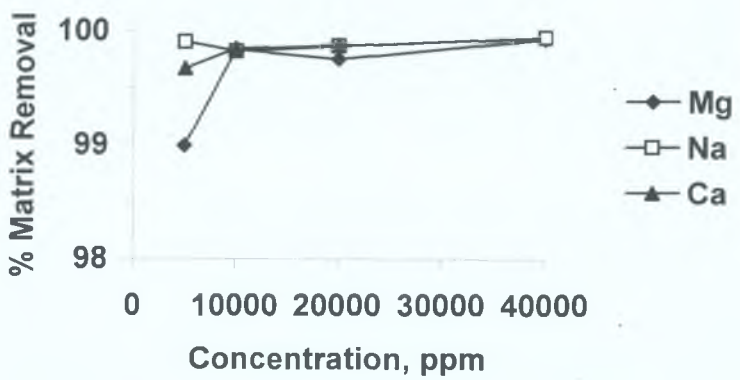


Fig. 3.6 Effect of Matrix concentration on % Matrix removal.

In summary, increasing the matrix concentration did not reduce the efficiency of the 0.25M ammonium acetate buffer in removing the matrix. This system is therefore effective in matrix removal over a wide range of matrix element concentrations.

3.6.4 Comparison of buffer wash and water rinse steps on matrix removal efficiency.

The use of an optimum sample buffer concentration (0.25M) has been shown to remove >99.5% of Ca, Mg and Na matrix elements over a wide concentration range (5000-40000ppm). After the use of a water rinse step the concentration of these matrix elements remaining in nitric acid eluent was <50ppm on average.

The following experiment was designed to determine if a buffer wash step improved matrix removal efficiency when compared to the use of a water rinse step. Solutions containing 50ppm and 10000ppm of matrix elements (Ca, Mg and Na) were buffered to pH 5.5 using 0.25M ammonium acetate and pumped through the chelating column followed by 5 ml of water rinse or 0.25M buffer. The remaining matrix elements after the water or buffer wash step were then eluted from the chelating column using the nitric acid eluent. Calcium and magnesium matrix elements in the acid eluent were then analysed by Flame Atomic Spectrometry and sodium by Flame Atomic Emission Spectrometry. The results of these studies are shown in **Table 3.13**.

Table 3.13 Comparison of a buffer wash and water wash step on matrix removal efficiency.

	Water Rinse		Buffer Wash		Buffer Enhancement Factor, (Water Rinse/ Buffer Wash)
	ppm in Eluent	% RSD	ppm in Eluent	% RSD	
Element	**50ppm Matrix**				
Mg	7	41	<0.5	n/a	14
Ca	13	23	<0.5	n/a	27
Element	**10000ppm Matrix**				
Na	31	37	<5	n/a	6
Mg	45	13	<5	n/a	9
Ca	22	19	<5	n/a	4

n=3
Method of determination: Mg and Ca by FAAS, Na by FAES

The residual concentrations of calcium and magnesium from the 50ppm sample in the acid eluent were 13ppm and 7ppm respectively when only water was used to wash column. This represents >63% removal of the matrix elements from the original 50ppm in the sample.

The concentration of Ca and Mg in the acid eluent was <0.5ppm when a buffer wash step was used and therefore achieved >99% removal of the matrix elements from the sample.

The residual concentration of calcium, magnesium and sodium in the acid eluent from the 10000ppm sample was 31ppm 45ppm and 22ppm respectively when only water was used to wash the column. This represents >99.5% removal of the matrix elements from the original 10000ppm matrix in the sample. The concentration of Na, Mg and Ca in the acid eluent was <5ppm when a buffer wash step was used and therefore achieved >99.95% removal of the matrix elements from the sample. The use of a buffer enhancement factor illustrates more clearly how the use of a buffer wash step is more efficient at matrix removal when compared to a water rinse step, **Table 3.13**.

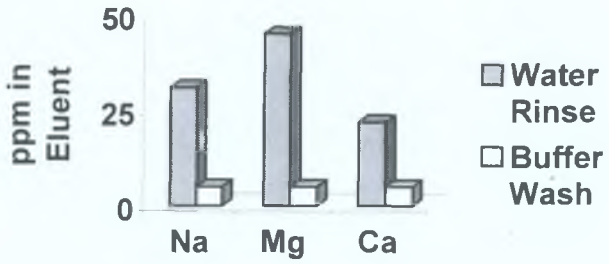


Fig 3.7 Comparison of buffer wash versus water rinse steps on matrix removal efficiency.

The use of a buffer wash step ensures that Na, Mg and Ca removal is 4-27 times more effective when compared to the use of a water rinse step alone. The effectiveness of a buffer wash step over a water rinse is represented graphically in **Fig 3.7**.

3.6.5 Effect of buffer wash and water rinse volume on matrix removal.

The volume of buffer and water used to wash the column after sample loading is an important experimental parameter. This parameter must be optimised in order to determine the minimum volume required for efficient matrix removal.

The use of a 5ml water rinse step reduced 10000ppm concentration of Ca, Mg and Na matrix elements to <50ppm on average. Although this represents 99.9% matrix removal there is a possibility that these levels of matrix elements may still interfere with the determination of some ultra trace elements in solution. The results of varying the water and buffer wash volume are shown in **Table 3.14**.

Table 3.14 Effect of water and buffer volume on matrix removal.

		Volume, ml				
		1	2	3	4	5
		ppm in Eluent				
Calcium	Water Rinse	29	23	34	28	20
	Buffer Wash	<5	<5	<5	<5	<5
Magnesium	Water Rinse	59	52	49	45	44
	Buffer Wash	<5	<5	<5	<5	<5
Sodium	Water Rinse	44	48	56	43	37
	Buffer Wash	<5	<5	<5	<5	<5

n=3

The volume of water used to rinse the column does not appear to significantly increase the efficiency of matrix removal. This demonstrated that in most cases a 1-2 ml water-rinse step may achieve sufficient removal for certain analytical applications (e.g. quantitative retention of weakly complexed manganese on the iminodiacetate reagent, which would be prematurely eluted if a buffer wash step is used).

The use of a buffer wash step is at the discretion of the analyst. If approximately <50ppm matrix elements are acceptable in the acid eluent then a buffer wash step can be avoided. The buffer wash step reduced the concentrations of Ca, Mg and Na in the acid eluent to <5ppm on average. Here again, the volume of buffer used in the wash step alone did not increase the efficiency of matrix removal.

However, a water rinse step is required in the method to remove residual ammonium acetate buffer from the column. The concentrated buffer would be otherwise co-eluted into the acid eluent thus introducing sample based matrix interferences.

This would have led to the deposition of carbon onto the sampling interface of the ICP-MS instrument. The results show that use of 2 ml buffer wash step prior to nitric acid analyte elution was appropriate for efficient matrix removal of Ca, Mg and Na.

3.6.6 Comparison of matrix removal efficiency with other designs.

The design of the preconcentration column is one of the factors influencing the efficiency of matrix removal. The column allows a continuous flow of buffered sample through the iminodiacetate reagent while simultaneously removing any matrix [106].

The recommendations by Fang [69] on column size and tubing inner diameter have improved the efficiency of matrix removal when compared to other systems. However this Prosep reagent based system only used a matrix removal based procedure and did not take advantage of the preconcentration capabilities of this chelating reagent [105]. It used a sample loop where a small volume of sample (3 ml) was mixed on-line with a high concentration of ammonium acetate buffer (1.5M) prior to loading onto the chelating column.

The dimensions of the column (2.5cm x 3mm i.d.) were half the length of the column (4.5cm x 2.29mm i.d.) used in this present work. This reduced length leads to a shorter time for the sample matrix and buffer to mix. Poor buffering caused by inadequate mixing of the sample and buffer could lead to the higher retention of matrix elements. The sample also has a shorter residence time while exposed to the chelating reagent and with the sample buffer flowing through the column.

The current system uses a larger column and also a larger volume of sample (25 ml). The sample is buffered to the correct pH before it is pumped through the column. The buffer is being continuously exposed to chelating reagent and can remove any matrix elements that are weakly retained on reagent. An optimum sample buffer concentration (0.25M) achieves efficient matrix removal while also reducing the trace element blanks levels associated with using high concentrations of ammonium acetate buffer. This group did not investigate the use of a buffer wash step.

The Dionex Metpac CC-1 column (5cm x 4mm) although larger in size when compared to our column (4.5cm x 2.29mm) was also successfully used for matrix removal/preconcentration in estuarine and seawater samples [114]. Metpac CC-1 is a polystyrene divinylbenzene substrate based iminodiacetate polymer resin and as described previously, suffers from swelling problems [69].

An application of this system requires 2M ammonium acetate buffer in order to remove the matrix elements. The higher sample buffer concentration is used for a number of reasons. Firstly the swelling behaviour of the polystyrene divinylbenzene resin substrate requires a higher concentration of buffer to condition the resin prior to sample loading. This leads to increased analysis time due to the use of buffer conditioning steps between each elution cycle.

Secondly, diffusion of ions through the pores of the polymer resin requires a higher concentration of buffer in both the sample and the buffer wash solution to remove the matrix elements from the resin. Finally the use of high buffer concentration leads to coelution of residual buffer with the nitric acid eluent. This high carbon content buffer in the eluent causes both physical and spectral based interferences in ICP-MS analysis.

In conclusion, the results of these experiments demonstrate that the matrix removal efficiency of this laboratory-based system is equivalent to or exceeds the capability of other commercially available matrix removal/preconcentration systems.

3.7 Factors Influencing Analyte Recovery.

3.7.1 Effect of ammonium acetate buffer concentration on analyte preconcentration.

The concentration of the ammonium buffer in a sample is an important parameter for efficient matrix removal but can also have an impact on the preconcentration of trace elements by the iminodiacetate reagent. The concentration of buffer was varied from 0.25-2M and a 50 $\mu g\ l^{-1}$ multielement spike was added to each sample. The optimum pH of the buffer was maintained at pH 5.5. The recoveries obtained for the eleven analyte elements are reported in **Table 3.15**.

Table 3.15 Effect of ammonium acetate buffer on analyte recovery

	Ammonium Acetate Buffer, M							
	0.25		0.5		1		2	
Elements	% Recovery	% RSD	% Recovery	% RSD	% Recovery	% RSD	% Recovery	% RSD
Cu	104	0.6	88	3.7	89	3	79	2.6
Cd	95	2.4	84	1.1	94	2.1	83	1.2
Ce	90	2.0	86	2.3	90	6.7	79	1.8
Th	80	2.8	84	3.3	75	4.6	74	3.4
Co	92	0.3	89	1.8	91	4.3	82	4.3
Ni	93	1.7	86	2.8	79	9.4	79	3.8
Zn	103	2.7	102	3.2	112	3.1	88	4
Pb	103	2.9	98	2.2	91	7.5	58	2.4
U	88	2.2	78	1.7	50	4.5	26	2.4
Al	108	4.8	82	1	87	6.2	72	6
Mn	86	4.1	71	1.3	52	5.9	31	1.1

n=3

The effect of the ammonium acetate buffer concentration versus recovery is plotted in **Fig. 3.8**.

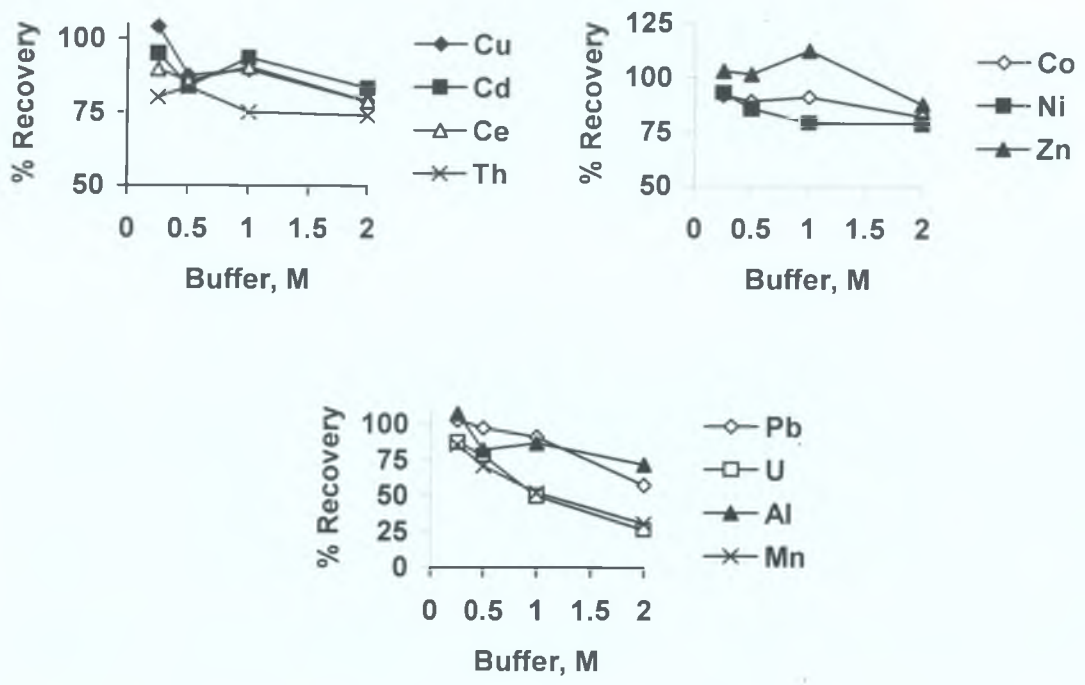


Fig. 3.8 Effect of ammonium acetate concentration on analyte recovery.

Recovery of Cu, Cd, Ce, Th, Co, Ni and Zn decreased as the concentration of buffer was increased from 0.25-2M. The %RSD is less than 9% indicating that the trend is reproducible. The recoveries of Al, Pb, Mn and U decreased rapidly as the concentration of buffer increased.

The behaviour of Mn is not surprising as it has similar weak complexing ability as the matrix element Ca for iminodiacetate (**Appendix A**). The increasing concentration of buffer in the sample results in greater competition between manganese and ammonium ions in the buffer for free sites on the iminodiacetate reagent.

Uranium in the form of the uranyl ion (UO_2^{2+}) has much stronger complexing ability for the iminodiacetate regent when compared to manganese (27). However this does not seem to explain the trend of reduced recovery with increasing concentration of the ammonium acetate buffer.

In the work carried out by Nelms et al [105], a high concentration of buffer was used because this was deemed necessary to achieve efficient removal of Ca from the iminodiacetate reagent. However this also reduced Mn and U recovery. The problem with this observation is that the same level of spike, 10 $\mu g\ l^{-1}$ U, Mn and Ca was used in this study. This low level spike of calcium may distort the trend observed here.

The calcium matrix is present in real samples at concentrations of mg l^{-1} compared to the $\mu g\ l^{-1}$ levels of most trace elements. The results of matrix removal studies using this laboratory system, discussed in section 3.6, has highlighted that the efficiency of calcium removal was not improved by an increasing buffer concentration.

The type of matrix removal system used may dictate the efficiency of U, Mn uptake and also the concentration of buffer to remove matrix elements. Jimenez et al [128] described a matrix removal/preconcentration system using Chelex 100 resin.

They highlighted the advantages of a low sample buffer concentration as higher recovery of analyte elements and low blank contamination levels. They also reported that a higher buffer concentration led to a higher recovery of most elements (Pb, Mn, Co and Al). These results appear to in conflict with the results of other publications and also with this work.

3.7.2 Effect of ammonium acetate buffer concentration on analyte recovery in a sodium matrix.

The effect of buffer concentration on analyte recovery in the presence of 10000ppm sodium matrix was studied. Sodium has extremely low complexing ability with iminodiacetate and only an ion exchange mechanism is dominant with this element at the pH chosen for this analysis.

The high concentration of this element was added to the sample to determine if the recoveries of the analytes are affected by its presence. The experimental results for analyte recovery with the variation of buffer concentration in 10000ppm sodium matrix are shown in **Table 3.16**.

Table 3.16. Effect of ammonium acetate buffer on analyte recovery in 10000ppm sodium.

	Ammonium Acetate Buffer, M							
	0.25		0.5		1		2	
Element	% Recovery	% RSD	% Recovery	% RSD	% Recovery	% RSD	% Recovery	% RSD
Al	55	10	73	8	73	11	58	11
Mn	63	2	43	1	33	8	28	2
Fe	45	6	56	3	80	16	66	10
Y	89	1	91	2	99	9	88	1
Cd	101	1	100	3	109	8	100	3
Ce	94	1	97	1	106	8	94	1
Co	94	1	86	2	90	9	83	1
Ni	95	2	88	3	88	9	80	3
Zn	95	3	93	1	105	8	92	3
Cu	89	3	84	3	85	10	79	4
Pb	103	1	101	4	105	8	72	3
U	69	1	60	8	30	6	10	8

n=3

Ammonium acetate buffer concentration versus recoveries in a 10000ppm sodium matrix on the retention of selected elements is illustrated in **Fig. 3.9**. The recovery of Y, Cd, Ce, Co, Ni, Zn and Cu is high (>80%) across the buffer range.

Iron recovery increases to 80% in the buffer however, poor precision indicates erratic behaviour on the iminodiacetate reagent. Mn recovery was depressed with 10000ppm Na when compared to the results without any matrix present.

The high concentration of the Na competes with Mn through an ion exchange mechanism of ion exchange for free sites on the iminodiacetate. Recovery of aluminum was low across the buffer range and may be due in part to the hydroxide forming ability of this element.

The recovery of Pb and U followed a similar trend to that observed in the absence of a Na matrix.

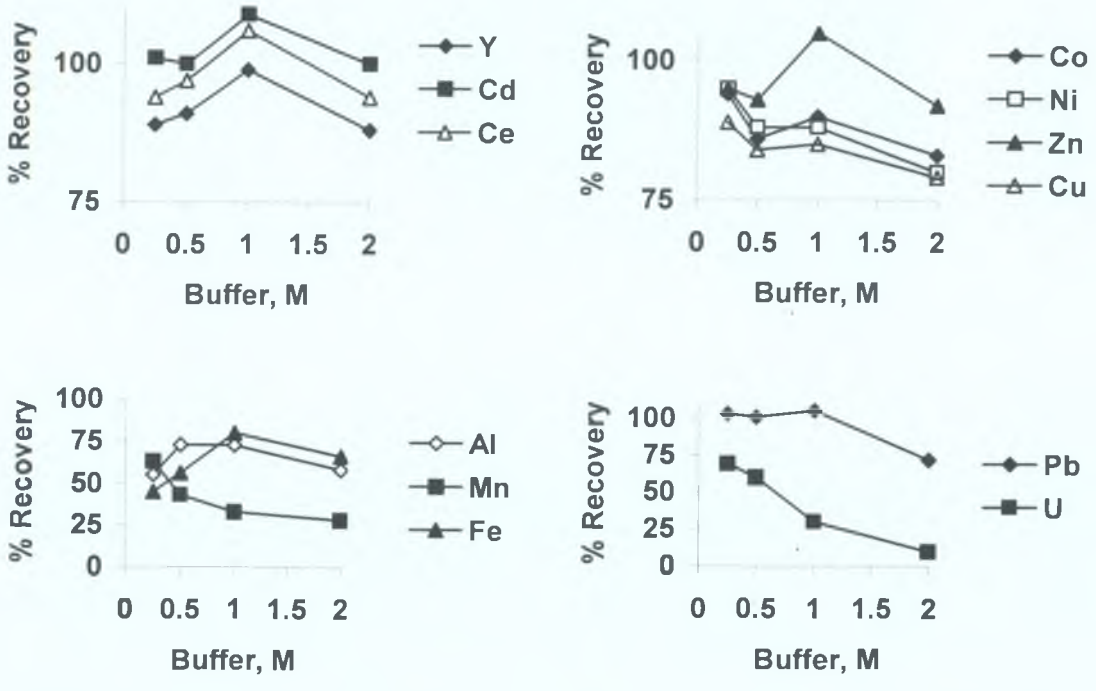


Fig. 3.9 Effect of ammonium acetate buffer on analyte recovery in 10000ppm sodium.

3.7.3 Effect of ammonium acetate buffer concentration on analyte recovery in magnesium matrix.

The effect of buffer concentration on analyte in the presence of 10000ppm magnesium matrix was studied. High concentrations of this alkaline earth element can compete for free iminodiacetate sites on the reagent and may reduce the recovery of trace analytes. Experimental results of analyte recovery with the variation of buffer concentration in 10000ppm magnesium matrix are given in **Table 3.17**.

Table 3.17. Effect of ammonium acetate buffer on analyte recovery in 10000ppm magnesium.

	Ammonium Acetate, M							
	0.25		0.5		1		2	
Element	% Recovery	% RSD	% Recovery	% RSD	% Recovery	% RSD	% Recovery	% RSD
Cu	81	6	75	3	79	7	77	2
Y	87	1	83	3	91	3	93	3
Cd	102	3	96	3	97	4	91	3
Ce	95	1	94	3	102	4	105	4
Mn	34	10	23	2	21	1	23	9
Fe	51	12	56	14	75	8	106	11
Al	80	6	67	6	87	5	82	3
Co	81	1	80	0	84	7	82	3
Ni	109	1	107	2	109	8	102	2
Zn	92	8	87	3	93	7	90	4
Pb	107	1	98	1	91	8	57	3
U	77	3	56	4	24	3	8	7

n=3

Ammonium acetate buffer concentration versus recovery in a 10000ppm magnesium matrix for selected elements is plotted in **Fig. 3.10**. Recoveries for Cu, Y, Cd, Ce, Co, Ni, Al and Zn are high (>80%) across the buffer range studied. Fe recovery increased as the buffer concentration was increased to 2M.

Manganese recovery was low across the buffer range due to the high concentration of the competing magnesium matrix in sample solution. The recovery of Pb and U in the presence of magnesium matrix followed a similar trend as the previous study carried out in the absence of matrix.

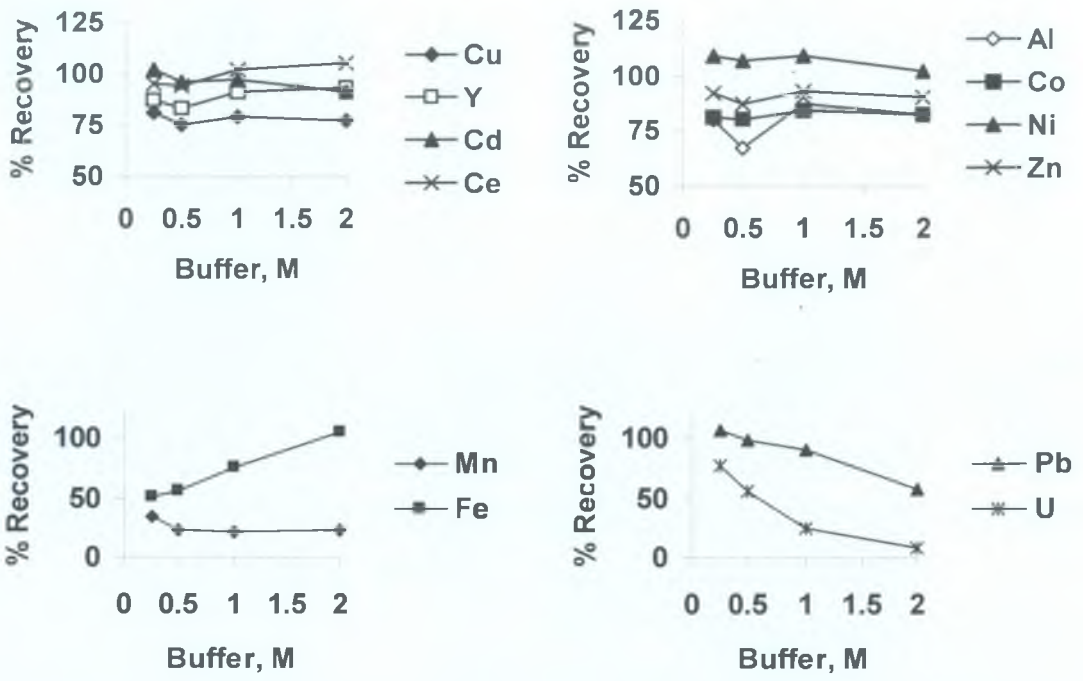


Fig. 3.10 Effect of ammonium acetate buffer on analyte recovery in 10000ppm magnesium.

A combination of buffer concentration versus recovery in different matrix types are shown for Mn, U, Pb and Cu in **Fig. 3.11** and a number of interesting observations can be made. The recovery of Mn and U is reduced as the buffer concentration is increased. The presence of high concentrations of Na and Mg reduces the recovery of both elements even further as the buffer concentration increases and the reduction levels off in 1-2M buffer. The presence of magnesium reduces Mn recovery significantly when compared to Na matrix and in the absence of matrix element.

The recovery of lead is high (>90%) in 0.25-1M ammonium acetate buffer in the different matrix types examined. However, the use of 2M buffer concentration however caused a significant decrease in lead recovery. The possibility of a lead acetate complex competing with iminodiacetate at this concentration of buffer cannot be ignored.

Ammonium ion concentration may also lower the recovery of this element at high buffer concentrations. The presence of large concentrations of Mg or Na matrix elements as buffer concentration was varied also reduced the recovery of lead.

However the main effect of lower lead recovery is due to the higher buffer concentration.

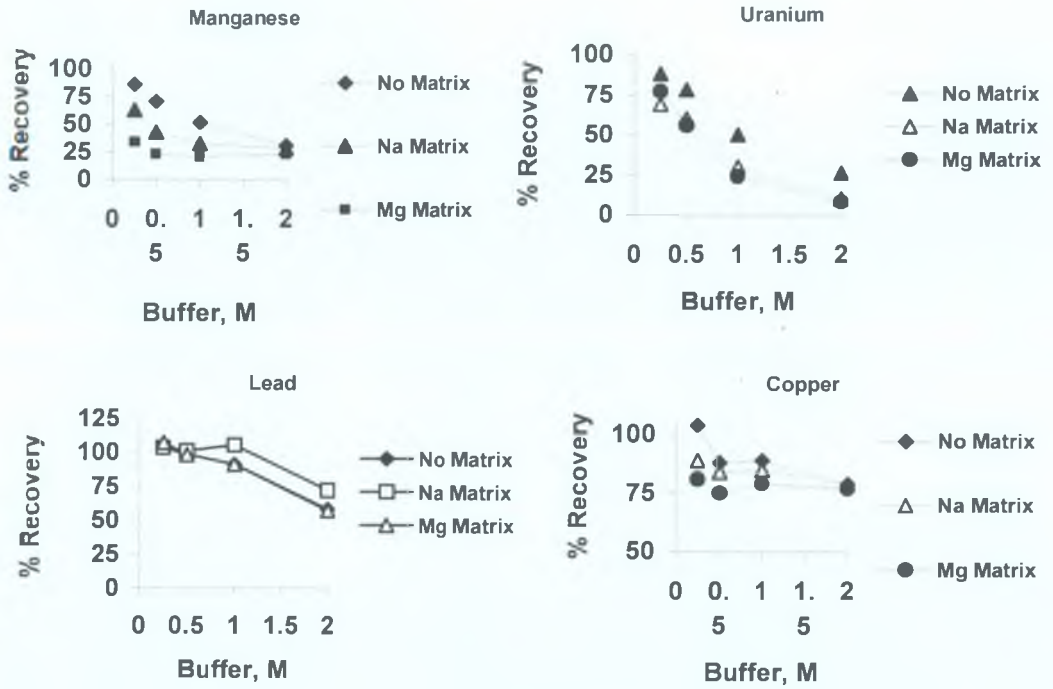


Fig 3.11 Effect of buffer concentration in different matrix types on Mn, U, Pb and Cu recovery.

Recovery of copper across the buffer range studied was greater than >75% and was not significantly effected by the presence of Na and Mg in the sample. The highest recovery for most elements is at the lowest buffer concentration (0.25M) in the absence of the matrix.

Recovery of analytes in the magnesium matrix is lower when compared to the Na matrix and in the absence of the matrix elements. This is not unexpected as this element has a higher affinity for iminodiacetate than sodium.

The combination of matrix removal efficiency results and this buffer concentration study have shown that the optimum buffer concentration of 0.25M was suitable for this matrix removal/preconcentration system.

3.7.4 Effect of matrix element concentration on analyte recovery.

The effect of Ca, Mg and Na matrix element concentration on analyte recovery was examined. The concentration of these elements in solution was varied from 0-40000ppm. The buffer concentration and pH of the solution was kept constant. A buffer wash step was not used in this experiment.

3.7.4.1 Effect of calcium concentration on analyte recovery

The results of varying the calcium concentration are given in **Table 3.18**. The recovery of Co, Ni, Cu, Zn, Cd and Pb decreased slightly (10-20%) when calcium was present in higher concentrations.

Table 3.18 Effect of calcium concentration on analyte recovery.

	Calcium Concentration, ppm							
	0		5000		10000		20000	
Element	% Recovery	% RSD	% Recovery	% RSD	% Recovery	% RSD	% Recovery	% RSD
Co	92	0.3	84	1.2	79	0.2	78	0.4
Ni	93	1.7	85	4.8	77	1.1	72	5.2
Cu	104	0.6	79	3.2	76	0.8	80	3.8
Zn	103	2.7	87	6.7	93	1.2	91	1.1
Cd	95	2.4	84	2.9	83	0.4	80	0.7
Ce	90	2	94	0.9	85	0.3	90	1.8
Pb	103	2.9	85	4.7	87	2.9	82	0.9
Al	108	4.8	66	4.8	61	2.6	72	6.3
Mn	86	4.1	37	2.6	36	1.9	49	3.2
U	88	2.2	84	2.4	74	1.1	87	2.1

n=3

Recovery of U was did not vary significantly with increasing calcium concentration. The recovery of Al and Mn decreased to less than <72% and <50% respectively as the calcium concentration was increased. The competitive uptake of Al and Mn in high concentration of Ca for iminodiacetate leads to a lower recovery.

The recovery of these and several other trace elements is illustrated in **Fig. 3.12**

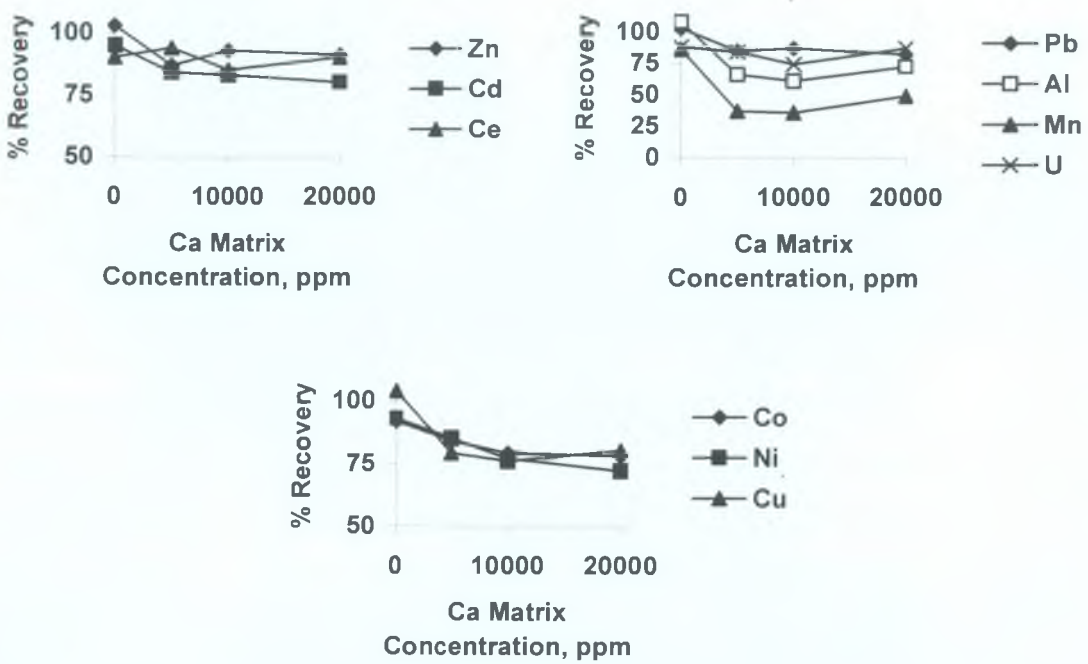


Fig. 3.12 Effect of calcium concentration on analyte recovery.

3.7.4.2 Effect of magnesium concentration on analyte recovery.

Varying the magnesium concentration (0-40000ppm) provides a similar trend to that found in the previous calcium matrix concentration study, **Table 3.19**. This is expected since magnesium is in the same alkaline earth element group as calcium and therefore its effect on trace element recovery would be similar.

Table 3.19 Effect of magnesium concentration on analyte recovery.

	Magnesium Concentration, ppm							
	0		10000		20000		40000	
Element	% Recovery	% RSD	% Recovery	% RSD	% Recovery	% RSD	% Recovery	% RSD
Co	92	0.3	89	1	91	5	103	6
Ni	93	1.7	98	1	104	7	97	5
Zn	103	2.7	100	2	107	5	103	9
Cu	104	0.6	78	4	85	4	99	6
Cd	95	2.4	102	2	105	9	106	5
Ce	90	2	94	0	93	7	109	6
Pb	103	2.9	104	2	112	6	113	7
Al	108	4.8	40	10	47	11	49	1
Mn	86	4.1	28	2	32	4	35	3
U	88	2.2	79	2	87	6	95	6

n=3

The recovery of trace elements in varying concentration of magnesium are illustrated in **Fig. 3.13**

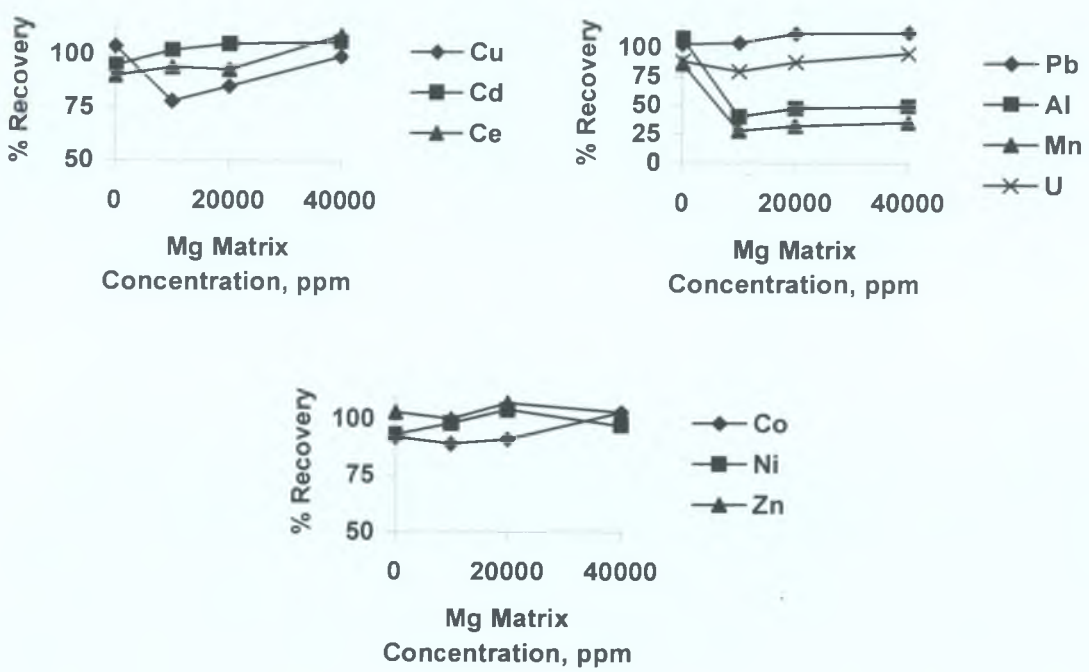


Fig. 3.13 Effect of magnesium concentration on analyte recovery.

3.7.4.3 Effect of sodium concentration on analyte recovery.

The sodium concentration was varied from 0-40000ppm and its effect on trace element recovery was determined. The recovery of the majority of elements (Co, Ni, Zn, Cd, Ce, Pb and U) did not decrease significantly as the concentration of the sodium matrix was increased, **Table 3.20**.

Table 3.20 Effect of sodium concentration on analyte recovery.

	Sodium Concentration, ppm							
	0		10000		20000		40000	
Element	% Recovery	% RSD	% Recovery	% RSD	% Recovery	% RSD	% Recovery	% RSD
Co	92	0.3	90	1	78	3	89	5
Ni	93	1.7	83	3	81	3	82	4
Zn	103	2.7	94	1	89	4	91	6
Cu	104	0.6	73	1	75	3	72	3
Cd	95	2.4	103	5	100	5	111	2
Ce	90	2	99	3	93	7	98	4
Pb	103	2.9	107	2	101	2	105	4
Al	108	4.8	46	3	42	23	47	22
Mn	86	4.1	68	3	70	3	66	3
U	88	2.2	78	5	70	8	79	6

n=3

The recovery of Al and Mn from the sodium matrix followed the same trend as Ca and Mg matrices. Reproducibility of Al retention decreased dramatically as the sodium concentration was increased.

The decrease in Mn recovery appears to be lower than Ca and Mg matrix concentration studies. The result of the Na matrix concentration study is shown in **Fig. 3.14**.

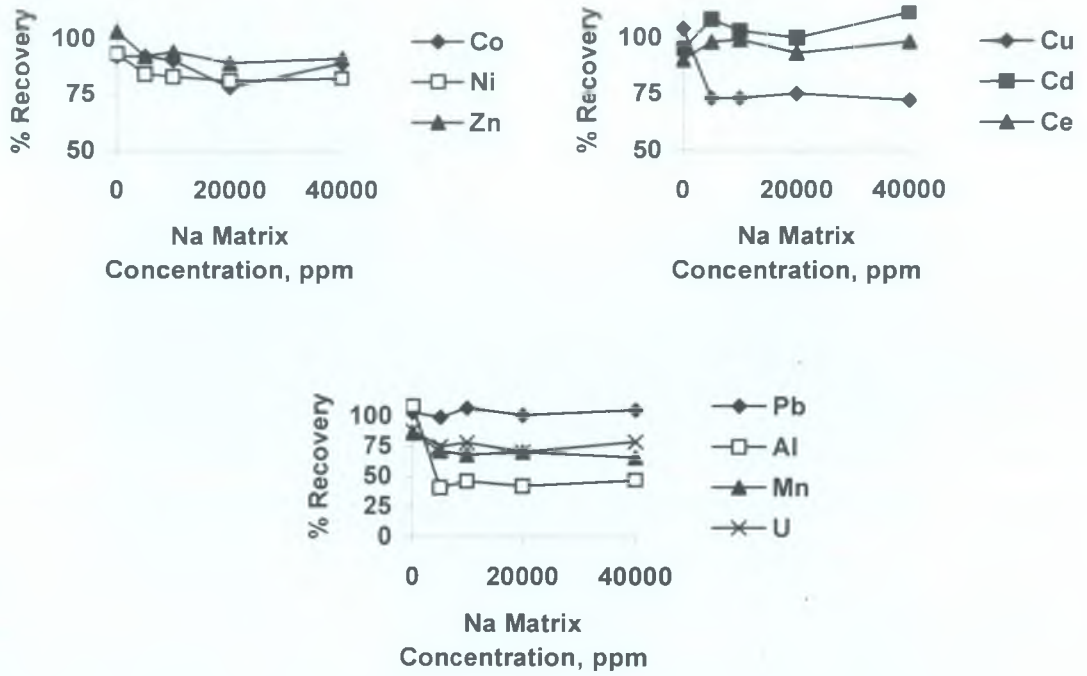


Fig. 3.14 Effect of sodium concentration on analyte recovery.

3.7.4.4 Comparison of the effect of Mg, Ca and Na matrix concentration on Mn, U, Al and Co recovery.

Comparison of the effects of Na, Ca and Mg matrix concentrations on recovery are shown for Mn, U, Pb and Co in **Fig 3.15**. Manganese recovery is less than 50% in the presence of Ca and Mg while the recovery is >66% in presence of Na. These results confirm again that the similar complexing ability of Mn, Ca and Mg with iminodiacetate is the primary reason why Mn recovery is poor.

The behaviour of Co in Mg, Ca and Na matrices represents the majority of the elements studied. The recoveries for these elements are reduced slightly, but reproducibility is not affected in the presence of higher concentrations of the Mg, Ca and Na.

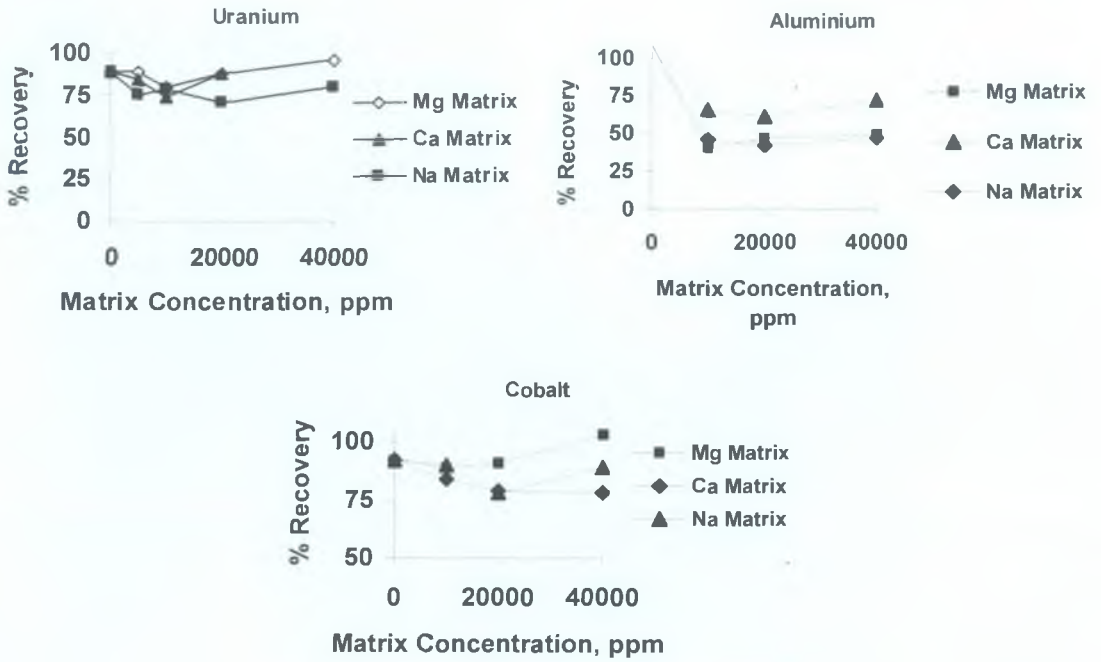


Fig. 3.15 Effect of Mg, Ca and Na matrix concentration on Mn, U, Al and Co recovery.

3.7.5 Effect of buffer wash volume on analyte recovery.

The volume of buffer wash is an important parameter influencing matrix removal and also the effect on trace analyte recovery. In this experiment the volume of the buffer wash was varied from 0-5 ml for samples having 10000ppm Ca, Mg and Na matrix.

3.7.5.1 Effect of buffer wash volume on analyte recovery in calcium matrix.

The results of the buffer wash volume study in the presence of 10000ppm Ca matrix are shown in **Table 3.21**.

The use of a buffer wash step reduced the recovery of Co, Ni, Cu, Cd, Ce and Pb by 25% while the recovery of Zn remained constant at >85%.

Table 3.21 Analyte recovery versus buffer wash volume on analyte recovery from 10000ppm calcium matrix.

	Volume of Buffer Wash, ml					
	0		1		2	
Element	% Recovery	% RSD	% Recovery	% RSD	% Recovery	% RSD
Al	108	5	68	3	62	2
Mn	86	6	33	1	31	2
Co	92	4	86	1	77	1
Ni	93	9	86	1	78	1
Cu	104	3	85	1	76	2
Zn	85	3	92	1	85	1
Cd	99	2	89	1	81	3
Ce	90	7	88	2	80	1
Pb	103	1	91	1	83	1
U	88	5	57	2	50	4

n=3

Al and Mn recoveries decreased to <62% and 31% respectively due to the buffer wash. The recovery of U decreases to 50% when a buffer wash step was used. The volume of buffer wash did not decrease the recovery significantly (>75%) for the majority of elements while it did not reduce the recoveries of Al, Mn and U further. The results of this study are illustrated in **Fig. 3.16**.

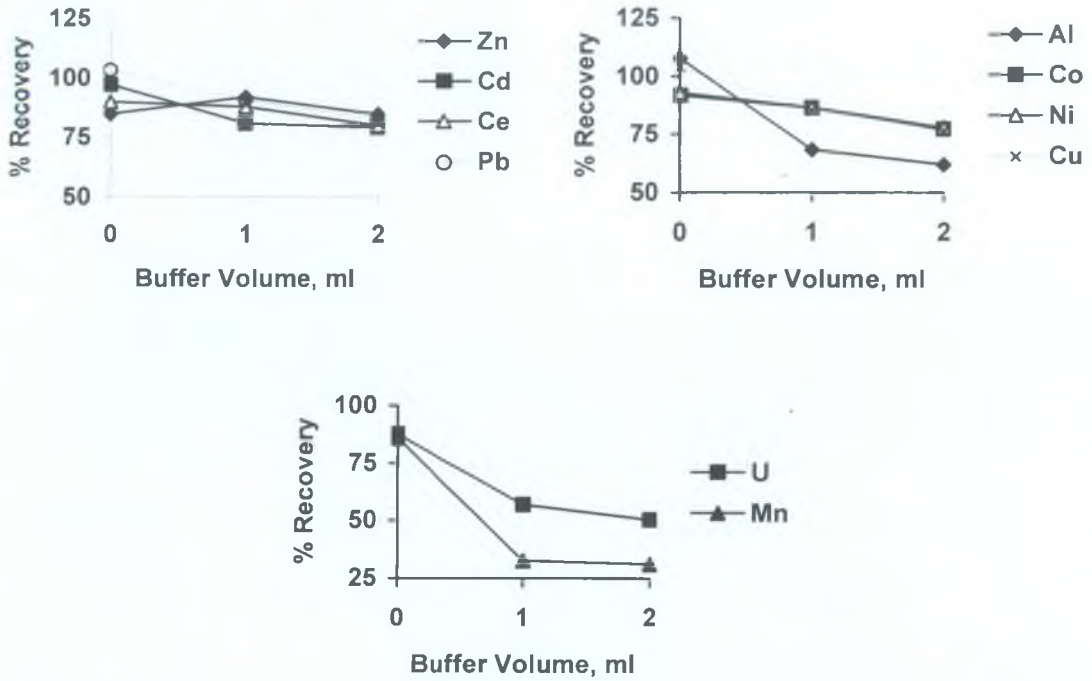


Fig. 3.16 Effect of ammonium acetate wash volume on analyte recovery in 10000ppm calcium matrix.

3.7.5.2 Effect of buffer wash volume on analyte recovery in magnesium matrix.

The results of the buffer wash volume study in the presence of 10000ppm Mg matrix are shown in **Table 3.22**. The recovery did not decrease for Co, Ni, Cu, Zn, Cd, Ce, Pb and U (>81%). The recovery of Al was erratic (53-86%) and the precision of the analysis varied from 6-35% RSD. Mn recovery decreased in the presence of 10000pppm Mg and the use of a buffer wash step. Increasing the buffer volume reduced recovery across the volume range studied

Table 3.22 Effect of buffer wash volume on analyte recovery from a 10000ppm magnesium matrix.

	Volume of Ammonium Acetate Buffer, ml							
	0		1		3		5	
Element	% Recovery	% RSD	% Recovery	% RSD	% Recovery	% RSD	% Recovery	% RSD
Al	108	4.8	53	6	86	34.7	78	18.8
Mn	86	4.1	27	2.1	30	5.7	26	6.3
Co	92	0.3	93	2.6	92	4.6	91	2.7
Ni	93	1.7	101	1.8	105	8.5	101	3.8
Cu	104	0.6	101	3.3	109	14.4	105	4.4
Zn	103	2.7	101	3.2	113	12.1	106	10.2
Cd	95	2.4	91	2	94	6	92	3.1
Ce	90	2	95	2.5	101	6	97	3.5
Pb	103	2.9	91	1.3	97	7.4	92	2.4
U	88	2.2	80	1	85	7.4	81	1.5

n=3

The result of varying buffer volumes in a 10000ppm magnesium matrix is shown in **Fig. 3.17.**

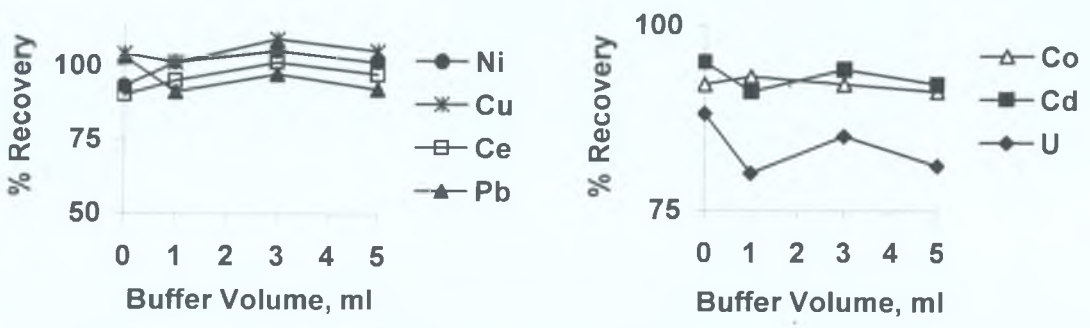


Fig 3.17 Effect of ammonium acetate wash volume on analyte recovery in 10000ppm magnesium matrix.

3.7.5.3 Effect of buffer wash volume on analyte recovery in sodium matrix.

The results of the buffer wash volume study in the presence of 10000ppm Na matrix are shown in **Table 3.23**. Results for Co, Ni, Cu, Zn, Cd, Ce, Pb and U show relatively constant recovery and are not dependant on the volume of buffer used. The recovery of Al appears erratic (48-69%) but the precision is acceptable at less than 11%. Mn recovery was reduced due to the buffer wash.

Table 3.23 Buffer Wash volume in 10000ppm sodium matrix.

	Volume of Ammonium Acetate Buffer, ml							
	0		1		2		5	
Element	% Recovery	% RSD	% Recovery	% RSD	% Recovery	% RSD	% Recovery	% RSD
Al	108	4.8	59	6	48	9	69	11
Mn	86	4.1	64	5	65	5	44	1
Co	92	0.3	95	3	94	1	94	4
Ni	93	1.7	95	5	93	2	93	4
Cu	104	0.6	102	5	98	1	101	2
Zn	103	2.7	99	4	100	0.2	100	1
Cd	95	2.4	95	3	91	2	94	4
Ce	90	2	93	5	92	0.4	97	3
Pb	103	2.9	97	4	93	0.5	96	1
U	88	2.2	89	5	85	3	87	3

n=3

The result of varying buffer volumes for a sample with a 10000ppm sodium matrix is shown is **Fig. 3.18.**

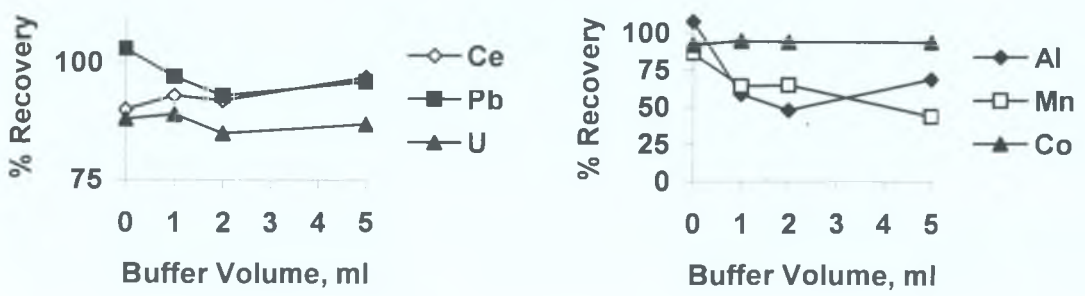


Fig. 3.18 Effect of buffer wash volume on analyte recovery for samples in 10000ppm sodium matrix.

3.7.5.4 Effect of buffer volume for samples Ca, Mg and Na matrices on Mn, U, Al and Co recovery.

The comparison of buffer wash volume on samples with different matrix types is shown for Mn, U, Pb and Ce in **Fig 3.19**. Mn follows similar behaviour to Al in that both exhibit lower recovery in the presence of high concentrations of Ca, Mg, and Na. The use of different volumes of buffer wash does not appear to greatly affect the recovery profile of these elements. The recovery of uranium appears to be lowered (<60%) when buffer wash is used for a Ca matrix but not for Mg or Na matrices. The recovery behaviour of lead and cerium represents the recovery profile of the other elements and is not affected by the volume of buffer wash used.

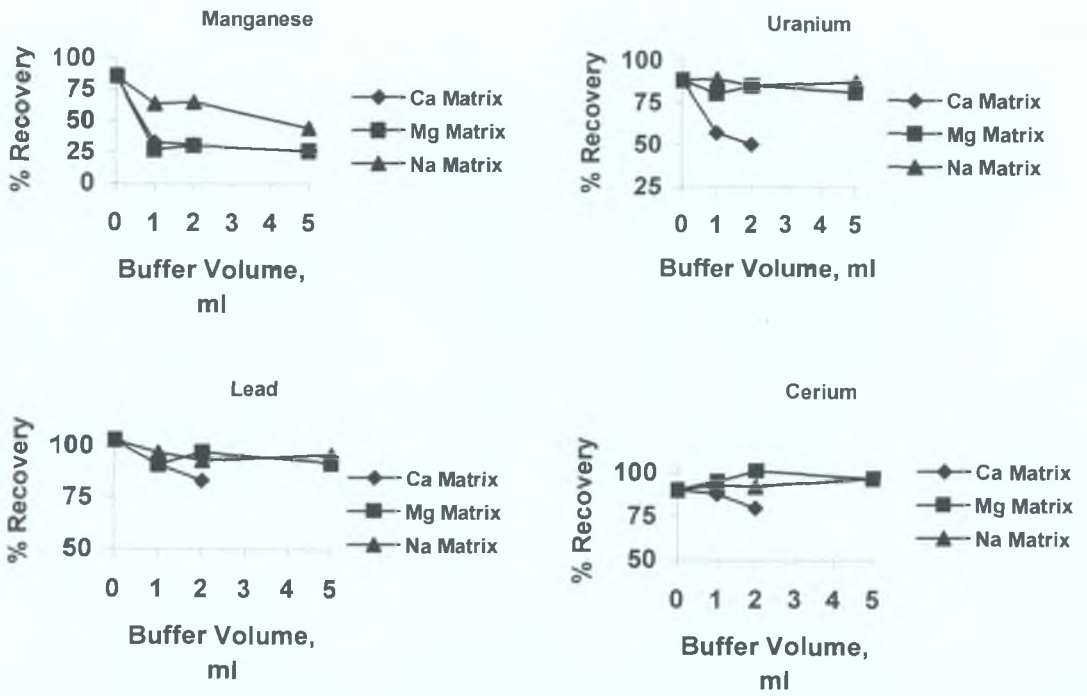


Fig. 3.19 Effect of buffer wash volume on Mn, U, Pb and Ce recovery.

Overall, the volume of buffer wash does not significantly lower the recovery of trace analytes. The use of a buffer wash has been shown to be effective for the removal of high concentrations of matrix elements

3.7.6 Effect of Flow-rate on analyte recovery.

The results indicate that there are no significant changes in recoveries over the range of flow-rates from 1-4 ml min^{-1} is given in **Table 3.24**, and **Fig 3.20**. The ability to use flow-rates as high as 4 ml min^{-1}, without sacrificing recovery, is important in order to maximise sample throughput. This indicates that the current system design is suitable for efficient element preconcentration.

Table 3.24 Effect of Flow-rate on analyte recovery.

	Flow Rate, ml min^{-1}							
	1		2		3		4	
Element	% Recovery	% RSD	% Recovery	% RSD	% Recovery	% RSD	% Recovery	% RSD
Al	95	13	91	5	87	23	92	11
Co	94	4	92	2	91	9	95	12
Ni	98	6	96	2	91	5	95	7
Zn	94	12	93	2	82	7	101	15
Cu	104	3	101	3	94	n/a	98	13
Cd	99	7	92	3	90	2	80	4
Ce	107	6	89	2	109	5	110	3
Ti	69	1	65	2	66	7	60	12
Fe	76	2	66	6	65	10	64	5
Pb	110	4	88	10	110	1	97	4
U	101	9	76	2	123	2	96	5
V	97	5	91	2	94	4	93	6

n=3

The column design, tubing diameter, and the type of peristaltic pump used can all have an influence on the flow-rate of the system.

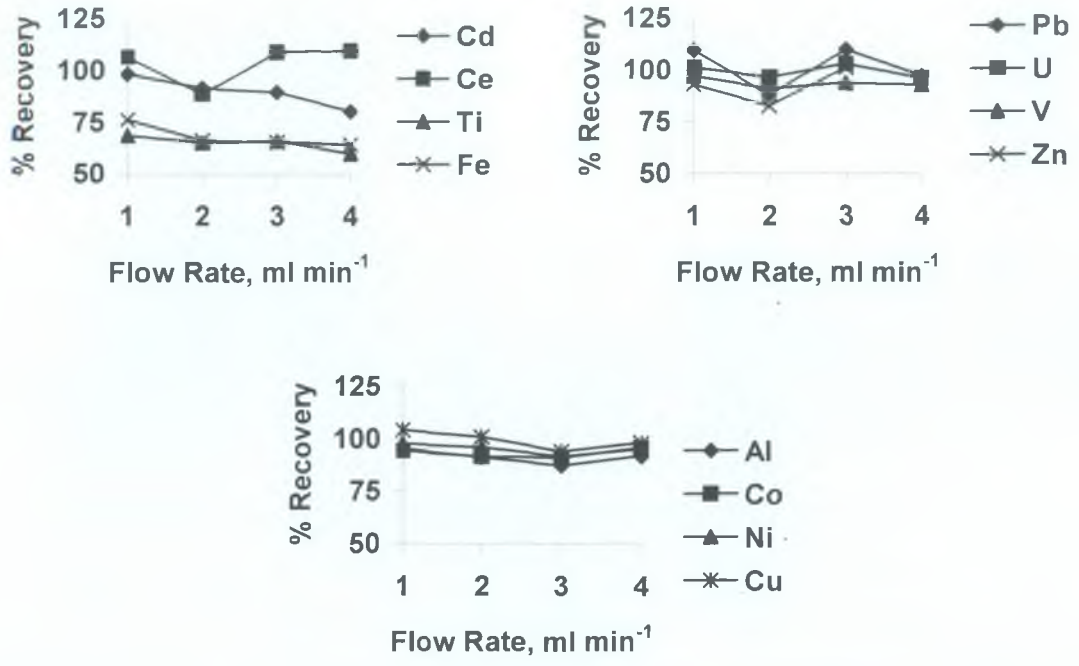


Fig. 3.20 Effect of Flow rate on the recovery of trace elements, Relative Standard Deviation, (RSD) <5%.

At flow-rate of >4 ml min^{-1} is not possible for this type of sample pretreatment system due to the build up of back pressure in the column itself and the fundamental limitations of peristaltic pumps.

The recoveries of Ti and Fe show the greatest decrease with increasing flow-rate, **Table 3.25**. A default flow-rate of 4 ml min^{-1} was chosen for most experiments to ensure maximum sample throughput, and also quantitative analyte recovery. Variation of peristaltic pump flow-rate by ±0.5 ml min^{-1} did not cause a significant decrease in recoveries of trace analytes for these experiments as the volume of the sample loaded onto the columns was measured volumetrically and was therefore not dependant on the control of the pump flow-rate.

The independence of analyte recovery from flow-rate is the most important observation of these results. The stability of the peristaltic pump flow as discussed in **section 2.7.3** means the system can be automated without significant problems.

3.7.7 Effect of nitric acid eluent concentration on analyte recovery.

The complexes formed between the analytes and the iminodiacetate reagent is broken down in the presence of high concentration nitric acid eluent. Determining the optimum concentration for efficient elution is important if maximum trace element recoveries are to be obtained. The effect of increasing nitric acid concentration on analyte recovery is given in **Table. 3.25**.

Table 3.25 Effect of eluent concentration on Al, Mn, Ni, Cu, Cd, Ce, Pb, Th and U recovery.

	Acid Eluent Concentration, %					
	2		6		12	
Element	% Recovery	% RSD	% Recovery	% RSD	% Recovery	% RSD
Al	90	3.4	77	2.3	85	2.0
Mn	81	1	74	3.9	77	5.8
Ni	75	1	80	1.3	82	4.9
Cu	79	0.4	77	1.7	73	5.2
Cd	82	1.2	80	1.5	71	5.6
Ce	85	1.3	93	2	90	5.0
Pb	93	0.8	86	1.9	81	3.5
Th	71	0.5	92	2.2	102	2.8
U	85	1.3	94	2.4	98	4.2

n=3

The behaviour of nine elements was investigated at an acid concentration of 2-12%, **Fig 3.21.** Acid concentrations greater than 12% were not investigated as this could degrade the column over prolonged use.

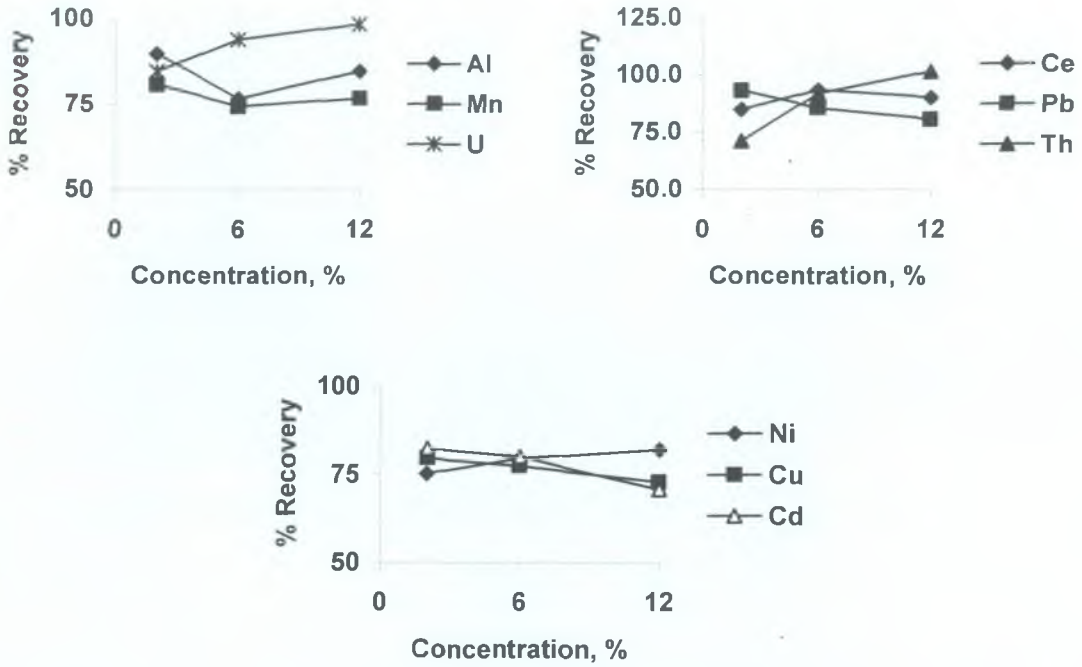


Fig. 3.21 Effect of acid eluent concentration on analyte recovery.

The recoveries of Ni, Th and U increases with nitric acid concentration and reached a plateau above 6% Nitric acid. The higher concentrations of nitric acid in the samples (6-12%) could affect nebulisation efficiency and this could explain the small decreases in element recovery.

3.7.8 Effect of nitric acid eluent volume on analyte recovery.

Acid eluent volume is also an important system parameter to optimise. The main purpose of the elution step is to ensure quantitative removal of the analytes from the iminodiacetate reagent.

The correct eluent volume eliminates potential memory effects from high levels of analytes and also regenerates the iminodiacetate reagent prior to introduction of the next sample. The complexes formed between the analytes and iminodaicetate reagent are broken down using the acid eluent.

The nitric acid eluent concentration was maintained at 3% and its volume was varied from 1-10 ml. The experimental results obtained for ten analyte elements are presented in **Table 3.26** and graphed in **Fig. 3.22**.

Table 3.26 Effect of nitric acid eluent volume on analyte recovery.

	Acid Eluent Volume, ml							
	1		2		5		10	
Element	% Recovery	% RSD	% Recovery	% RSD	% Recovery	% RSD	% Recovery	% RSD
Al	59	4.9	72	13.4	109	23.1	69	22.3
Co	78	2.3	106	4.1	97	9.0	85	12.8
Ni	77	1.5	102	5.6	82	4.8	90	14.9
Zn	77	2.4	90	12.3	104	7.3	94	12.8
Cu	93	3.0	111	3.0	97	n/a	100	7.5
Cd	78	2.7	106	6.7	93	1.8	89	2.8
Ce	79	2.0	107	5.9	109	4.8	110	2.6
Pb	88	9.5	110	3.7	110	1.3	97	4.0
Th	48	39.3	64	3.7	108	3.3	94	5.5
U	76	1.8	101	8.7	103	2.3	96	5.5

n=3

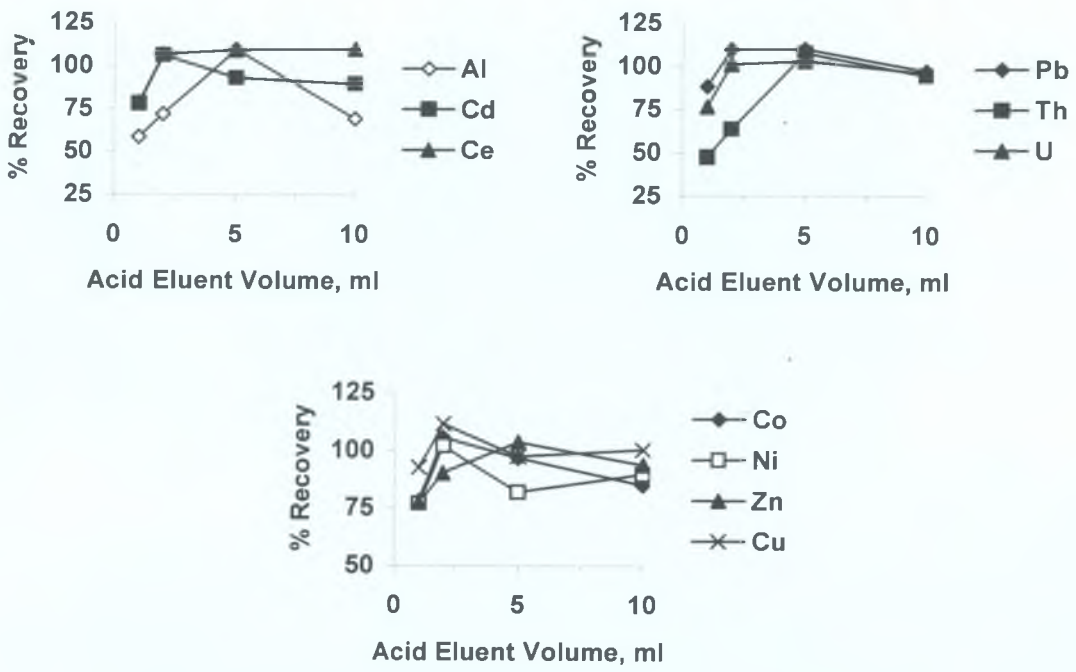


Fig. 3.22 Effect of acid eluent volume on analyte recovery.

The use of a smaller elution volume increases the efficiency of the system by increasing the preconcentration factor, PF, achieved for the analysis. All elements with the exception of Al, Th and Zn tend to provide maximum recoveries with 2ml eluent volume and either plateau or decrease slightly thereafter.

Aluminium reached a maximum at 5 ml eluent volume however the reproducibility achieved is poor. Thorium shows a similar behaviour to aluminium and the 1ml eluent volume results are not reproducible. In general, thorium and aluminium appear to have a different optimum for eluent concentration and volume compared to other elements. Some elements also have a low recovery but this is not improved by increasing the eluent concentration or volume (≥5mls eluent for 3% acid eluent).

Overall, optimisation of the system for the largest number of environmental elements and efficient preconcentration with matrix removal is the aim of the project. The concentration of the eluent has an impact on the detection limits, DL, of the method. A higher concentration of acid results in a higher overall signal for the method blank. The lower the volume of eluent used to remove the analytes from the reagent, the greater will be the preconcentration efficiency of the system. This feature can be further improved if a low flow microconcentric nebuliser is used in the ICP-MS analysis.

In conclusion this study has found that an eluent volume of 2 ml of 3% nitric acid is acceptable for most elements. Care however should be taken to avoid memory effects for some elements (e.g. Al, Zn and Th) when these conditions are used. One approach would be to accept lower recoveries for these latter elements but use an acid wash step following elution in order reduce memory effects.

3.7.9 Linearity.

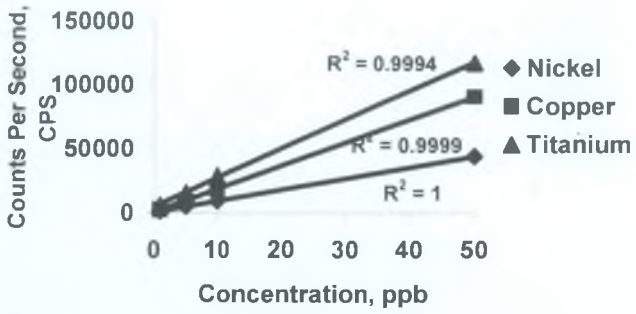
Validation of the linearity was carried out using the elements (Ni, Cu, Ti, Mo, Co and V). Replicate measurements of multi-element standard solutions were used to check system linearity. Calibrations were made with four standards in the range 1–50 μg l^{-1} and were preconcentrated by a factor of 5. In general, linearity was obtained for all the elements measured despite the low concentrations used, **Table 3.27**. The calibration graphs are plotted in **Fig. 3.23.**

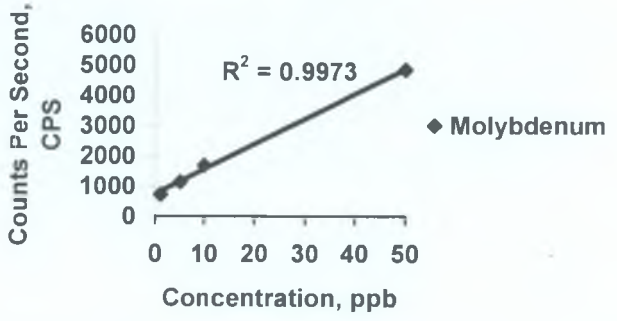
Table 3.27 Linearity results for Ni, Cu, Ti, Mo, Co and V.

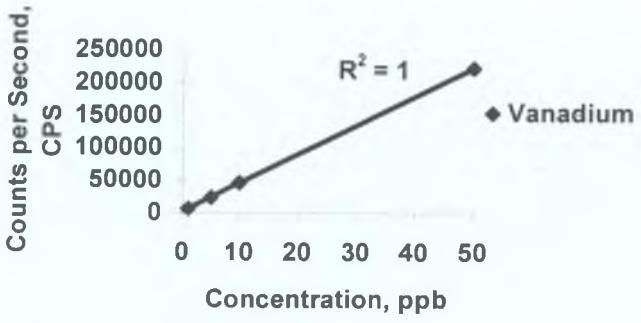
	Concentration, μg l^{-1}							
	1		5		10		50	
Element	% Recovery	% RSD	% Recovery	% RSD	% Recovery	% RSD	% Recovery	% RSD
Ni	98	13.5	96	2.8	94	n/a	95	3.4
Cu	104	6.4	91	4.4	94	n/a	98	2.3
Ti	90	13.0	69	6.6	69	n/a	59	7.4
Mo	18	4.1	20	1.3	21	n/a	16	2.5
Co	104	8.8	99	1.6	95	n/a	96	2.7
V	105	7.8	97	2.1	95	n/a	93	0.8

n = 3

Correlation coefficients of between 0.990 and 0.998 were obtained for Co, V, Ni, Cu, Ti and Mo. However, high blank concentrations for some elements such as Al, Fe and Mn meant that the 1-10 μg l^{-1} standards were indistinguishable from the blank. The recoveries for Ni, Cu, Co and V were >90% over the entire linear range while the recoveries of Ti and Mo were lower but reproducible over the entire linear range.







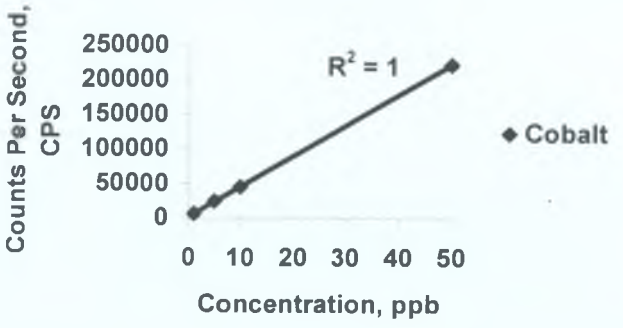


Fig. 3.23 Calibration curves (1–50 μg l^{-1}) for Ni, Cu, Ti, Mo, V and Co obtained using the preconcentration system.

The recovery of 1 μg l^{-1} Ti is not reproducible due to blank levels rather than successfully preconcentrating the element. The poorest precision in general was obtained for the 1μg l^{-1} spike since the spike level was very close to the limit of detection of the instrument.

This is due in part to impurities in the reagents used and instrument variability at these low concentrations. An evaluation of the capacity of the iminodiacetate reagent may require further study if samples with high concentrations of analytes are used.

3.6.10 Detection Limits

An important feature of the matrix removal/preconcentration system is the large improvement in detection limits achievable as the preconcentration factor is increased. However trace element impurities in the ammonium acetate buffer does limit these detection limit improvements for certain elements such as Ni, Cu and Cd.

The results given in **Table 3.28** compare matrix removal preconcentration system detection limits to ICP-MS instrument detection limits. The limits of detection (LOD) are based on 3σ of six replicate measurements of the blank. A combination of a twenty fold preconcentration factor and low levels of impurities in the reagents leads to a significant improvement in detection limits.

Table 3.28 Matrix removal/preconcentration versus ICP-MS instrument detection limits.

Element	LOD ($\mu g\ l^{-1}$)		Instrument/System Improvement Factor
	System	ICP-MS	
Co	0.013	0.37	28
Ni	0.381	0.023	0
Zn	0.163	0.649	4
Cu	0.488	0.515	1
Cd	0.456	0.49	1
Ce	0.013	0.012	1
Pb	0.044	1.15	26
Th	0.044	3.74	85
U	0.025	0.617	25

n=6, PF=20

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These results prove the effectiveness of using a matrix removal/preconcentration system for obtaining improved detection limits for Co, Pb, Th and U. No improvement in detection limits is obtained for Ni, Zn, Cu, Cd and Ce due to the presence of these elements as contaminants in the ammonium acetate buffer.

3.7.11 Reagent Capacity

Another important consideration when using this system is the capacity of the reagent. Thus far experiments have been conducted with solutions having relatively low concentrations. If larger concentrations are expected then care must be taken not to overload the column.

The capacity of the reagent for specific analytes is listed in **Table 3.29**. For multielement Preconcentration, the total analyte load must be estimated and the system validated for each specific sample type

Table 3.29 Capacity of Prosep from a list of elements using a batch technique [105].

Element, E	Capacity, mmol/g of Prosep	Molecular Weight, g	mg of E/1g of Prosep	mg of E/100mg of Prosep
V	0.22	50.94	11.2	1.12
Cr	0.1	52	5.2	0.52
Mn	0.14	54.94	7.7	0.77
Fe	0.29	55.85	16.2	1.62
Co	0.13	58.93	7.7	0.77
Ni	0.11	58.7	6.5	0.65
Cu	0.11	63.55	7.0	0.70
Zn	0.13	65.38	8.5	0.85
Cd	0.1	112.41	11.2	1.12
Ce	0.1	140.12	14.0	1.40
Pb	0.13	207.2	26.9	2.69
U	0.04	238.03	9.5	0.95

Larger quantities of the Prosep reagent leads to greater retention of matrix elements, longer buffer steps, poorer detection limits caused by higher blank levels and also backpressure problems which cause erratic flow-rates and possible column blockage. The quantity of reagent used in the current columns (i.e. 100mg) represents an optimum for the conditions under which the current experiments were conducted.

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Chapter Four

4.0 Elemental Fingerprinting of the Irish Marine Environment using ICP-MS with Matrix Removal/Preconcentration System.

4.1 Introduction.

In November 2002 the oil tanker Prestige sank several hundred kilometres off the coast of northern Spain. Before the tanker sank, up to two million gallons of oil leaked from the vessel affecting 1000 km of coastlines of Northern Portugal, Spain and South West France. The impact of the Prestige oil spill has highlighted how the large areas of European coastline can be affected by one large scale pollution incident. The consequences of this spill have both short term problems due to the cleanup process and longer term problems due to accumulation of pollutants in the marine environment.

Monitoring of pollution in the European marine environment is based on "The Protection of the Marine Environment of the North-East Atlantic Convention" (OSPAR Convention 1992) [129]. The convention coordinates marine environmental policies and related scientific research within the area of latitude 36° north, and between longitudes 42° and 51° west, excluding the Baltic and Mediterranean seas. The convention entered into force on 25 March 1998. Ireland has signed and ratified the OSPAR convention and is obliged to produce a report on the current state of the marine environment. This report provides a comprehensive overview of the current state of the marine environment and includes the analysis of various inorganic and organic based pollutants present in shellfish.

Participating countries
I – Arctic Waters, Norway, Denmark (including Faroe Islands) and Iceland
II – Greater North Sea, Netherlands Belgium, Denmark, France, Germany, Norway, Sweden and UK
III – Celtic Seas, Ireland and UK
IV – Bay of Biscay and Iberian Coast, France, Spain and Portugal
V – Wider Atlantic, Iceland, Portugal, Belgium, Denmark, France, Germany, Ireland, Netherlands, Norway, Spain, Sweden and UK

Fig. 4.1 The maritime area covered by the OSPAR Convention [129]

4.1.1 Bivalve tissue as indicator of marine pollution.

The ease with which trace elements bioaccumulate in the food chain means that shellfish often serve as bioindicators in areas contaminated with these pollutants.

Trace metal analysis of shellfish tissue has been used to assess the background levels of Pb, Cu, Ni, Zn, Hg, and Cr around the Irish and U.K. coastline [130]. The report "Measurement of stress effects (scope for growth) and contaminant levels in mussels (Mytilus edulis) collected from the Irish Sea" [131] provides more detailed information on this area of research.

The measurement of trace element pollutants in bivalve shellfish has been used for monitoring the marine environment since the 1970's. Shellfish are good bioindicators in that their pollutant content is a measure of its exposure to the surrounding environment [132-134].

Some of the features of shellfish as bioindicators of trace element pollution are:

1. The concentration of trace elements in the shellfish tissue is higher than in the surrounding water or sediment.
2. Only the fraction of the pollutant that is biologically available is measured.
3. An index of pollution can be obtained which provides the rate of uptake and excretion by the shellfish.
4. Bivalve shellfish are commonplace in the marine environment, easily collected in large numbers, and are sedentary.
5. Because bivalves are filter feeders, they pass large volumes of water over their body surfaces, accumulate pollutants almost continuously and act as indicators of trace element exposure over long time periods.

One primary reason given for the measurement of trace elements in shellfish is that the direct analysis of seawater and sediments is difficult and expensive. Usually, trace element concentrations are much lower in water and sediment samples than in the shellfish and levels are often below analytical detection limits of the instrumentation used. The water currents may disperse the pollutants over a wide area, even though the shellfish remaining at a locality have been contaminated. This makes predicting the accumulation of trace elements in shellfish particularly difficult if these predictions are based on the results of water or sediment analysis.

The biological nature of shellfish has a number of disadvantages which limits the ability to accumulate trace elements over a long period of time, and, therefore, few species of shellfish meet all of the criteria for an ideal bioindicator species. Different species appear to be able to regulate the retention and excretion of trace elements from its tissue structure. The accumulation of a certain level of trace elements may affect the growth of shellfish and limit the uptake of more trace elements. The toxicity of the accumulated trace elements may ultimately cause the death of the bioindicator organism. Decomposition of the organism destroys the record of trace element pollution accumulated over the lifetime of the shellfish.

4.1.2 Bivalve Shell as indicator of marine pollution.

The trace element content of bivalve shells when used as an indicator of marine pollution has a number of advantages when compared to bivalve tissue analysis. The shells which constitute a rigid calcium carbonate structure are easy to store, and appear to accumulate trace elements in the shell structure over a long period of time. Since shell growth occurs incrementally they can provide trace element data related to the surrounding marine environement for a discrete time period. Tissues conversely are strong accumulators of metals and integrate trace elements over the life of the organism. Such information has been used to assess the effects of anthropogenic inputs into coastal waters. This approach has considerable potential for determining the historical changes in the trace element chemistry of the marine environment [135].

4.1.3 Trace Element Analysis of Bivalve Shells.

The direct trace element analysis of the calcium carbonate based bivalve shell by atomic spectrometry encounters a number of analytical problems. The calcium matrix causes physical, chemical, ionisation and spectral interferences for FAAS and ICP-MS based analytical techniques. The dissolved solids content of samples must also be limited to less than 0.2% w/v due to nebulisation problems and blockage of sampling orifices in ICP-MS.

A number of methods can be used to overcome the interference problems associated with this sample type. Firstly, by simple dilution of the sample, the level of calcium matrix in solution is lowered but at the expense of degraded detection limits for many trace elements. The use of flow injection instrumentation prior to sample introduction reduces the contact time of the high concentration matrix with the sample introduction system.

Currently, the most recent instrumentation innovation is the use of reaction cell technology for interference reduction in ICP-MS instruments. Using this technology various gases (He, H_2, NH_3 and CH_4) selectively react with the ionised matrix elements in the plasma prior to ion separation by the quadrupole based ICP-MS [136].

The small number of elements whose detection limits can be improved using this method, instrumentation complexity and extra costs mean that this technique is limited to specialised applications.

Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS) has been used for the determination of Cu, Zn and Pb in bivalve shells [137]. This is a very powerful technique but once again the instrumentation is very expensive. The levels of trace elements were determined for shells in two sites in the North Sea, a known dump-site and a control site which was distant from known sources of pollution. The results of the study appeared to confirm elevated levels of these elements in the dump site compared to the control site.

This work formed the basis the current study on trace element composition in bivalve shellfish from around Ireland's coastline and one site in the Irish Sea using the matrix removal/preconcentration system.

A baseline survey of the trace element composition of shellfish was carried using the laboratory developed matrix removal/preconcentration system. The system was applied to the removal of the calcium matrix from the shellfish and therefore solves a number of analytical problems outlined above [138]. The results reported in these publications and additional results from further work in this application area are included in the following sections.

4.2 Analytical Methodology.

4.2.1 Bivalve sampling and preparation.

The shellfish sampling sites chosen encompasses most of the Republic of Ireland's coastline. Sites ranging from Mulroy Bay, Co. Donegal to Carlingford Lough, Co. Louth were sampled. The majority of shell samples were collected by the Marine Institute of Ireland from commercial shellfish grounds. Dr. Bret Danilowicz (Department of Zoology, University College Dublin) also provided bivalve shell samples from several east coast sites. A list and description of the shellfish sampling sites is shown in **Table 4.1** and indicated on the map of Ireland in **Fig 4.2**.

Table 4.1 Shellfish sampling sites around Ireland's coastline.

Site No.	Site Name	No. of Shells/Samples
1	Mulroy Bay, Donegal	10
2	Sligo Harbour, Sligo	10
3	Drumcliff Bay, Sligo	10
4	Clew Bay, Mayo	10
5	Shannon Estuary Oysters	10
6	Shannon Estuary Mussels	10
7	Cromane, Kerry	10
8	Spanish Island, Kerry	3
9	Castletownbere, Cork	3
10	Bantry Bay, Cork	3
11	Mizen Head, Cork	4*
12	Cork Harbour, Cork	10
13	Wexford Harbour, Wexford	17
14	Carnsore point, Wexford	4*
15	Sandycove, Dublin	3*
16	Rush, Dublin	2*
17	Seapoint, Louth	3*
18	Dundalk, Louth	3*
19	Carlingford Lough, Louth	10
20	Irish Sea	8*
21	Lough Derg	1*
22	Lough Key	2*
23	Belgium	1
24	Holland	1

* composite shell samples

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Fig. 4.2 Location of shellfish sampling sites around Ireland's coastline.

Sampling bivalves from commercial shell fishing grounds guaranteed that the shells were grown at the same site for a controlled duration (i.e. within the previous two years). A minimum of twenty shells were randomly selected from each site. The bivalve tissue was separated from the shell and the outer and inner surfaces were cleaned using acid washed plastic implements. The cleaned shells were rinsed three times in ultrapure water to remove any remaining debris. Finally, the shells were air dried in an oven at 90°C for 24 hours prior to sample digestion.

4.2.2 Sample digestion procedure.

The digestion method used is based on the US Environmental Protection Agency (USEPA) method 3050B [139]. The shell was broken into small fragments and a sample size of 2-10 grams (depending on the size of the shell) was accurately weighed. 10 ml of ultrapure water was added, followed by the slow addition of 4 ml of concentrated nitric acid (Romil-SpA, UK).

The beaker was covered with a watch glass and the sample was digested at 90°C for 1 hour on a hotplate. The digest was then allowed to cool followed by the slow addition of 5 ml of ultrapure water and 1 ml of 30% hydrogen peroxide (Romil SpA, UK). The beaker was again covered with a watch glass and the digestion was continued at 90°C for a further hour.

This sample digest was allowed to cool, filtered into a 50 ml volumetric flask and made up to volume with ultrapure water. In order to reduce the excess acidity and buffer the sample prior to matrix removal, an appropriate aliquot of the above solution was adjusted to a pH of approximately 5.5 using concentrated ammonia (Romil SpA, UK) followed by the addition of 6 ml of the 4M ammonium acetate buffer. The final volume was made up to 100 ml with ultrapure water.

Three replicate 25 ml aliquots of this solution were each preconcentrated using the matrix removal/preconcentration system and eluted with 2 ml of 0.5M nitric acid eluent into polyethylene sample containers. These samples were subsequently spiked with 0.5 ml of 500 µg l^{-1} internal standard solution (Be, Sc, In, Rh and Bi) immediately prior to ICP-MS analysis. Therefore, the concentration of the internal standard in the final sample was 100 µg l^{-1}.

4.2.3 Off-line Matrix Removal System.

The procedure used for matrix removal and analyte preconcentration using the matrix removal system was previously described in **Section 3.2.7**. The optimised conditions used are shown in **Table 4.2.**

Table 4.2 Optimised matrix removal system parameters.

Chelating Reagent	Prosep
pH	5.5 ±0.1
Ammonium Acetate Sample Buffer	0.25M
Ammonium Acetate Buffer Rinse	0.25M
Nitric Acid Eluent	0.5M
Flow Rate	4 ml min^{-1}

4.3 Results and Discussion.

4.3.1 The coastal geography of Ireland.

Mulroy Bay, Drumcliff Bay, Sligo Harbour and Clew Bay.

The coast from Mulroy Bay in the north to Clew Bay in the south is predominantly rocky but has many large bays with fine sandy beaches. It is also heavily indented and has a number of large promontories exposed to strong westerly winds and some of the largest waves around the Irish coast. The North West is one of the more remote and undeveloped regions of the country and is a popular destination for tourists from home and abroad. Commercial fishing and fish processing industries also play a major role in the local economy and there are a number of important fishing ports in the region. The largest coastal town in the region is Sligo with a population of 17,786.

Shannon Estuary, Cromane, Spanish Island, and Bantry Bay.

The coastline is very rocky and divided into a series of large bays and inlets that provide a degree of shelter from the prevailing south-westerly winds. Large Atlantic waves are the prominent features of the west coast environment. To the south, there is a series of long narrow inlets separated by mountainous peninsulas. The inlets support important commercial shellfish cultivation grounds and tourism is an important industry in this region. On the Shannon estuary, industrial estates at Limerick and Shannon are important centres of manufacturing industry.

The largest industry in this region is Aughinish Alumina Ltd, an alumina refinery situated on Aughinish Island on the south side of the Shannon estuary between Askeaton and Foynes, 20 miles downstream from Limerick City, Ireland. Aughinish Alumina Ltd, produces alumina (Al_2O_3) by treating bauxite ore, a reddish brown earth, using the Bayer process. Alumina is a fine white granular powder, which is exported to aluminium smelters for processing into aluminium metal. In addition to the commercial ports at Foynes and Limerick, the only other port handling general cargoes is at Galway. The most important fishery harbours in terms of landed catches are Castletownbere, Dingle, Valentia and Fenit in Co. Kerry and Rossaveal on the Northern side of Galway Bay.

Mizen Head, Cork Harbour, Carnsore Point, and Wexford Harbour

The southern coastline of Ireland, from Carnsore Point in the east to Mizen Head in the west, is rockier and more indented than the east coast and is approximately 1,300 km in length. It has large estuaries at Cork Harbour and Waterford Harbour. The south coast is moderately sheltered from the prevailing west south-west winds. To the east, the Wexford coast has sandy beaches for more than half its length with rocky and muddy substrates comprising about 16% and 13% respectively.

Cork is the largest city on the south coast and an important centre of manufacturing for the chemical and pharmaceutical sector. Cork Harbour is a large natural inlet with a narrow entrance from sea, providing port facilities for cargo vessels up to 100,000 tonnes. A ferry terminal provides links with the UK and continental Europe. There are also a number of important fishery ports in the region.

Sandycove, Rush, Seapoint, Dundalk, and Carlingford Lough

From Carlingford Lough in the north to Camsore Point in the south, Ireland's east coast is approximately 450 km in length. It is less indented than other Irish coastal areas and constitutes less than 7% of the national coastline. From Carlingford Lough to Howth Head, the shoreline is characterised by softer forms of intertidal substrates and includes extensive linear sandy beaches.

With the notable exceptions of Dublin Bay and Wexford Harbour, the coast from Dublin Bay to Camsore Point is distinguished by an absence of bays and inlets. Dublin and Rosslare (Wexford) are the two major ports on the east coast and have developed around the estuaries of the Liffey and Slaney.

Another significant inlet is Carlingford Lough at the border with Northern Ireland which has relatively narrow entrances and large expanses of intertidal substrate. Dublin is Ireland's capital city and occupies a central position on the east coast. The city and surrounding areas support a large population and a wide range of commercial activities. The proximity of the east coast to the UK and Europe strongly influenced the development of Dublin and other east coast ports as centres of trade and commerce. The east coast is used intensively for residential, recreational, agricultural and commercial purposes but there are also extensive and important habitats for flora and fauna. The coast between Howth Head, Dublin and Bray, Wicklow is this most heavily populated coast in Ireland.

The population density is the lowest in northwest and west and increases rapidly from South to east coast. Coastal cities with large populations include Galway, Limerick, Cork, Wexford and Dublin. This would indicate that higher population density leads to higher inputs of marine pollution into the marine environment. Information obtained from the Irish Department of Marine "Dumping at Sea" permits indicate that 4.25-million tonnes of sewage sludge and dredge spoil have been dumped off Dublin's coastline in the period 1996-2000.

Irish Sea

The Irish Sea is generally defined as the area north of a line from St. Anne's Head (Wales) across to Hook Head (Ireland), and south of a line from Rathlin Island (Northern Ireland) to Mull of Kintyre (Scotland). It is a relatively small, enclosed sea area of 52,000 km^2, with shallow coastal waters of <50 m depth representing 60% of the area.

The Irish Sea is subject to a wide range of human uses and activities including shipping, oil terminals, direct sewage dumping, industrial waste, cooling water for nuclear and non-nuclear power stations, gravel extraction and fishing [130]. Studies carried out by the International Atomic Energy Agency (IAEA) have shown the Irish sea is one of the most contaminated waters from man made radioactivity in the European Union. A number of monitoring programs have been set up to measure the effects of pollution in the Irish sea.

4.3.2 Analytical Figures of Merit.

The performance characteristics of the matrix removal/preconcentration system for the determination of trace elements in bivalve shells (i.e. spike recovery, precision, background equivalent concentration and method detection limits) is discussed in this section. The results for the shell analysis are shown in the subsequent section. Shells or shell composites were analysed from each site. Each sample was analysed in triplicate using the matrix removal/preconcentration system and ICP-MS.

The recovery of a 50 µg l^{-1} spike of multielement solution (Co, Ni, Cu, Y, Cd, Ce, Pb and U) from a 5% w/v mollusc shell solution is given in **Table 4.3**. The results indicate that the recovery of elements from the shell solution is consistently within acceptable limits. The extensive results represent the analysis of shells from 12 separate sites, spiked and analysed in triplicate by ICP-MS (i.e. thirty six separate analyses).

Table 4.3 Average recovery of a 50 µg l^{-1} spike of Co, Ni, Cu, Y, Cd, Ce, Pb and U from a 5% w/v mollusc shell solution using iminodiacetate matrix removal at pH 5.5 (n=3)

Element	Spike (µg l^{-1})	Spike Recovery (%)
Co	50	87 ± 2.8
Ni	50	81 ± 2.8
Cu	50	76 ± 1.9
Y	50	85 ± 1.5
Cd	50	85 ± 2.3
Ce	50	87 ± 1.9
Pb	50	94 ± 2.3
U	50	73 ± 4.5

Precision (as % RSD), background equivalent concentration (BEC) and method detection limits are reported in **Table 4.4**. The precision is determined from six replicate measurements of a 50 µg l^{-1} multielement spike solution.

The background equivalent concentration of Co, Y and Ce are very low, reflecting the absence of spectral and other interferences at these masses. However the background levels of Ni, Cu, Cd, Pb and U are relatively high due mainly to trace element impurities in the reagents used. The primary source of contamination is from the acetic acid used in the preparation of the ammonium acetate buffer. Although high purity reagents were used in this work, however further purification of these reagents would seem to be required.

Table 4.4 Limits of detection, precision, background equivalent concentrations and method detection limits for Co, Ni, Cu, Y, Cd, Ce, Pb and U. (n=6, The 3σ MDL's are normalised for the analysis of 1 g shell sample preconcentrated into 2 ml of 2% nitric acid eluent).

Element	RSD (%)	BEC (µg l^{-1})	MDL(µg kg^{-1})
Co	3.2	0.75	0.6
Ni	3.5	0.93	4.3
Cu	2.5	3.21	4.4
Y	1.8	0.02	0.1
Cd	2.7	0.94	1.2
Ce	2.2	0.71	0.3
Pb	2.4	2.11	1.3
U	6.4	0.49	1.1

The method detection limits are based on 10 times the standard deviation of the blank level and expressed as µg kg^{-1} in the sample, **Table 4.5**. The MDL level is set at this relatively high level in order to minimise the probability of reporting spurious results. The levels of aluminium and zinc in shells exceeded 1 mg kg^{-1} but the variation in the blank levels made it impossible to report reliable results.

Table 4.5 Method detection limits. (n=6, The 3σ MDL's are normalised for the analysis of 1 g shell sample preconcentrated into 2 ml of 2% nitric acid eluent.)

Element	Batch 1 MDL, µg kg^{-1}	Batch 2 MDL, µg kg^{-1}
Ni	23	94
Cu	192	102
Y	0.3	n/a
Cd	16	34
Ce	3	16
Pb	124	98
U	2	2

The results for the trace element analysis of each bivalve shell site are shown in **Tables 4.6-4.29**. Each result represents the average concentration determined for the analysis of a bivalve sample carried out in triplicate. In the following section, observations are made regarding the trace element profiles determined for these sites.

4.3.3 Yttrium and Cerium.

Both yttrium and cerium are rare earth elements and their pollution impact is limited due to the low concentrations of these elements in the marine environment. Their presence in a particular site may be due to the natural geology of the site. The method detection limits, MDL's, for these elements is <0.3 µg kg^{-1} for yttrium and 3-16 µg kg^{-1} for cerium.

Interesting observations can be made by comparing levels in Sligo harbour to those observed for the Drumcliff, Co. Sligo. Although the shells were grown within a relatively short distance of each other, the level of yttrium in Drumcliff is below the MDL, while levels are elevated in Sligo Harbour for a large number of separate shell analyses. The opposite trend is observed for cerium with lower levels in Sligo harbour relative to the Drumcliff site. Regional geographical differences can therefore play a part in the trace element composition over relatively short distances.

The results for the Shannon Estuary, Askeaton, Co. Limerick also highlighted elevated levels of yttrium and cerium for all the samples analysed. Analyses of shells from the Irish sea have the highest concentration of cerium relative to all the other sites studied, however a subsequent analysis of the same batch of shells had cerium levels close to the detection limit of the technique.

The variability of the results for this batch of shells from the Irish sea is probably due to the random sampling of each shell. Samples from commercial fishing grounds had a definite place of origin and the duration which the shells were exposed to the marine environment is known. A larger sampling plan over a wider geographical area and the use of a control site would have helped to reduce the uncertainty for samples from the Irish sea.

4.3.4 Cadmium.

Cadmium is an element that occurs naturally in low concentrations in environment. It has many uses in industrial and consumer products such as batteries, pigments, metal coatings and plastics. It is usually present in the environment as various types of minerals combined with other elements such as oxygen or sulphur. It also forms solid compounds that may dissolve in water and can often be found in, or attached to, small particles present in the air. The level of cadmium in the environment is increasing due to human activity such as agriculture, industrial and urban activities.

The levels of cadmium in shells across the sites studied are below the method detection limit. These findings are similar to a shellfish tissue study of the same sites carried out by the Irish Marine Institute [140-141].

4.3.5 Uranium.

The levels of uranium for the majority of sites studied were also below the method detection limit. The levels of uranium in shells from the Irish sea are significantly higher than any levels observed around the entire coastline.

Three batches of shells were analysed on three separate occasions from the Irish sea site. Levels of uranium in two batches of shells analysed in September 2002 were relatively high when compared to the third batch analysed in May 2003 whose levels were below the MDL.

The relatively high uranium levels in the Irish sea may be related to historical dumping of nuclear waste in the sea. All sample from sites at Bantry Bay, Spanish Island and Dundalk registered uranium levels above the detection limit. The reasons for these elevated levels may be geological in origin. A more comprehensive study is required to confirm the results of this limited study.

4.3.6 Nickel and Copper.

The MDL's for copper and nickel were adversely affected by the presence of relatively large quantities of these element in the chemicals used for the analysis. The levels of nickel and copper are generally low and do not appear to vary significantly (Ni <100 μg kg^{-1} and Cu <500 μg kg^{-1}) across the range of sites. Elevated levels for these elements were noted in Sligo harbour and the Shannon Estuary. The levels of copper and nickel were above the MDL for the majority of sites. The high level of copper from shellfish tissue in the Shannon Estuary reported by the Irish Marine Institute appears to correlate with the high levels of this element in the shells from this site [140].

4.3.7 Lead.

The levels of lead in shells are low from Mulroy bay in the northwest to Carnsore Point in the southeast, with a background level of less than 500 μg kg^{-1}. The level of lead can be observed to be higher at the following sites: Wexford Harbour, Rush and the Irish sea. These higher levels of lead occur close to large industrial centres with a large population density (i.e. Wexford, Dundalk, Sandycove), which may indicate anthropogenic influences of this element in the environment. The highest lead results in shells are from the Irish sea site. A more detailed study of the Irish sea would require more sampling sites over a larger area and the selection of appropriate control sites. Again, the application of a comphrensive statistical analysis of the data would yield more definitive information.

Table 4.6 Analysis of shells from Mulroy Bay, Donegal (n=3).

	Sample No. (µg kg^{-1})									
Element	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Yttrium	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3	4	<0.3	<0.3
Cadmium	<16	<16	<16	<16	<16	<16	<16	<16	<16	<16
Cerium	<3	<3	<3	<3	<3	<3	<3	11	<3	<3
Uranium	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2
Nickel	59	53	90	75	65	62	85	74	44	53
Copper	<192	<192	<192	243	<192	<192	<192	<192	<192	<192
Lead	<124	<124	<124	<124	<124	<124	<124	<124	<124	<124

Table 4.7 Analysis of shells from Sligo Harbour, Sligo (n=3).

	Sample No. (µg kg^{-1})									
Element	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Yttrium	<0.3	29	12	5	17	21	<0.3	13	2	<0.3
Cadmium	<16	<16	<16	<16	<16	<16	<16	<16	<16	<16
Cerium	<3	<3	74	5	18	24	9	<3	6	4
Uranium	<2	<2	<2	4	35	<2	<2	<2	35	8
Nickel	934	208	133	74	49	107	97	<23	118	118
Copper	403	890	1459	274	1157	576	<192	<192	213	<192
Lead	201	184	182	177	<124	146	233	<124	157	139

Table 4.8 Analysis of shells from Drumcliff Bay, Sligo (n=3).

	Sample No. (µg kg^{-1})									
Element	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Yttrium	<0.3	<0.3	<0.3	<0.3	<0.3	14	<0.3	<0.3	<0.3	<0.3
Cadmium	<16	<16	24	30	<16	18	<16	<16	<16	<16
Cerium	7	24	3	18	14	15	8	41	19	11
Uranium	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2
Nickel	54	59	61	62	55	90	69	110	51	58
Copper	<192	391	200	<192	237	287	707	<192	208	<192
Lead	312	477	658	230	293	403	288	126	<124	202

Table 4.9 Analysis of shells from Clew Bay, Mayo (n=3).

	Sample No. (µg kg^{-1})									
Element	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Yttrium	7	<0.3	<0.3	0	<0.3	5	14	7	20	<0.3
Cadmium	<16	<16	<16	<16	<16	<16	<16	<16	17	<16
Cerium	<3	<3	<3	3	<3	10	39	13	60	<3
Uranium	<2	<2	<2	<2	<2	37	<2	46	<2	<2
Nickel	83	97	90	97	62	145	133	88	94	26
Copper	<192	<192	381	<192	307	513	388	772	<192	<192
Lead	<124	<124	<124	<124	<124	158	305	<124	<124	<124

Table 4.10 Analysis of shells from Shannon Estuary Oysters (n=3).

	Sample No. (µg kg^{-1})									
Element	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Yttrium	<0.3	17	12	60	26	33	5	40	45	<0.3
Cadmium	<16	<16	<16	<16	<16	<16	<16	<16	<16	<16
Cerium	<3	23	21	40	14	27	15	30	46	<3
Uranium	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2
Nickel	91	90	155	67	129	107	185	111	175	147
Copper	537	813	<192	1252	505	783	435	843	1004	<192
Lead	142	191	284	179	284	230	324	159	260	224

Table 4.11 Analysis of shells from Shannon Estuary Mussels (n=3).

	Sample No. (µg kg^{-1})									
Element	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Yttrium	44	60	6	25	<0.3	2	106	<0.3	13	<0.3
Cadmium	<16	<16	<16	<16	<16	<16	<16	<16	<16	<16
Cerium	42	53	8	41	<3	4	139	13	20	<3
Uranium	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2
Nickel	291	278	158	285	80	193	462	178	166	137
Copper	750	316	593	<192	492	1692	620	264	439	<192
Lead	270	287	159	238	<124	182	498	181	169	142

Table 4.12 Analysis of shells from Cromane, Kerry (n=3).

	Sample No. (µg kg^{-1})									
Element	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Yttrium	<0.3	<0.3	3	22	<0.3	<0.3	8	18	6	1
Cadmium	<16	<16	<16	<16	<16	<16	<16	<16	<16	<16
Cerium	<3	6	10	21	<3	4	15	10	12	<3
Uranium	32	<2	<2	6	<2	<2	55	26	<2	<2
Nickel	36	46	43	44	34	30	64	88	71	44
Copper	<192	428	620	<192	<192	<192	290	<192	<192	<192
Lead	<124	<124	<124	<124	<124	<124	<124	<124	<124	<124

Table 4.13 Analysis of shells from Spanish Island, Kerry (n=3).

	Sample No. (µg kg^{-1})		
Element	**1**	**2**	**3**
U	45	69	28
Ce	4	13	6
Pb	<120	<120	<120

Table 4.14 Analysis of shells from Castletownbere, Cork (n=3).

	Sample No. (µg kg^{-1})		
Element	**1**	**2**	**3**
U	41	21	<7
Ce	12	16	<4
Pb	214	<120	<120

Table 4.15 Analysis of shells from Bantry Bay, Cork (n=3).

	Sample No. (µg kg^{-1})		
Element	**1**	**2**	**3**
U	51	32	92
Ce	16	12	23
Pb	154	<120	<120

Table 4.16 Analysis of shells from Mizen Head, Cork (n=3).

	Sample No. (µg kg^{-1})			
Element	**1**	**2**	**3**	**4**
Co	<9	<9	<9	<9
Ni	<23	<23	<23	<23
Cu	<192	185	269	114
Y	0.4	10	7	0.8
Cd	<16	<16	<16	<16
Ce	<3	13.4	12.3	4.9
Pb	<124	320	286	185
U	<2	<2	<2	<2

Table 4.17 Analysis of shells from Cork Harbour, Cork (n=3).

	Sample No. (µg kg^{-1})									
Element	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Yttrium	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3
Cadmium	<16	<16	<16	<16	29	<16	<16	<16	<16	<16
Cerium	<3	<3	<3	6	<3	<3	<3	<3	<3	<3
Uranium	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2
Nickel	29	117	26	53	92	55	47	64	50	39
Copper	<192	<192	<192	<192	<192	<192	<192	<192	<192	<192
Lead	125	394	152	280	193	188	166	170	138	155

Table 4.18 Analysis of shells from Wexford Harbour (n=3).

	Sample No. (µg kg^{-1})									
Element	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Yttrium	<0.3	<0.3	<0.3	<0.3	4	<0.3	1	<0.3	<0.3	<0.3
Cadmium	<16	<16	<16	<16	<16	<16	<16	<16	<16	<16
Cerium	5	<3	<3	<3	13	10	16	<3	<3	<3
Uranium	<2	<2	<2	<2	8	<2	44	<2	<2	<2
Nickel	54	48	51	24	35	105	63	48	37	66
Copper	<192	<192	<192	<192	799	207	<192	<192	260	<192
Lead	192	<124	159	<124	263	305	290	162	<124	<124

Table 4.19 Analysis of shells from Wexford Harbour (n=3).

	Sample No. (µg kg^{-1})		
Element	**1**	**2**	**3**
U	9	<7	<7
Ce	44	72	37
Pb	1140	837	576

Table 4.20 Analysis of shells from Wexford Harbour (n=3).

	Sample No. (µg kg^{-1})			
Element	**1**	**2**	**3**	**4**
Co	<9	<9	<9	<9
Ni	<23	26.2	42.8	24.7
Cu	<192	442	212	<192
Y	0.8	0.8	0.4	0.6
Cd	<16	<16	18.8	<16
Ce	<3	<3	<3	<3
Pb	<124	<124	<124	265
U	<2	<2	<2	<2

Table 4.21 Analysis of shells from Carnsore Point, Wexford (n=3).

	Sample No. (µg kg^{-1})			
Element	**1**	**2**	**3**	**4**
Co	<9	<9	<9	<9
Ni	35	28	<23	26
Cu	<192	<192	<192	<192
Y	0.6	1	0.4	0.6
Cd	<16	<16	<16	<16
Ce	6.7	<3	<3	<3
Pb	<124	127	<124	<124
U	<2	<2	<2	<2

Table 4.22 Analysis of shells from Sandycove, Dublin (n=3).

	Sample No. (µg kg^{-1})		
Element	**1**	**2**	**3**
U	43	39	48
Ce	48	67	51
Pb	416	258	388

Table 4.23 Analysis of shells from Rush, Dublin (n=3).

Element	Sample No. (µg kg^{-1})	
Ni	<94	<94
Co	<15	<15
Cu	323.9	<102
Cd	<34	<34
Ce	<16	<16
Pb	513.7	292.0
U	<2	<2

Table 4.24 Analysis of shells from Seapoint, Dublin (n=3).

Element	Sample No. (µg kg^{-1})		
Ni	<94	<94	<94
Co	<15	<15	<15
Cu	<102	<102	<102
Cd	<34	<34	<34
Ce	<16	<16	<16
Pb	136.4	<98	273.7
U	<2	<2	<2

Table 4.25 Analysis of shells from Dundalk, Louth (n=3).

	Sample No. (µg kg^{-1})		
Element	**1**	**2**	**3**
U	73	91	41
Ce	95	118	76
Pb	134	362	369

Table 4.26 Analysis of shells from Carlingford Lough, Louth (n=3).

	Sample No. (µg kg^{-1})									
Element	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Yttrium	<0.3	2	<0.3	2	<0.3	<0.3	8	2	1	<0.3
Cadmium	<16	<16	<16	<16	<16	<16	<16	<16	<16	<16
Cerium	<3	3	<3	3	<3	6	16	5	5	<3
Uranium	<2	<2	<2	<2	<2	18	<2	<2	8	<2
Nickel	65	75	44	58	98	104	76	55	43	82
Copper	228	<192	299	<192	<192	<192	215	<192	281	<192
Lead	155	174	<124	137	<124	<124	134	127	<124	141

Table 4.27 Batch one analysis of shells from the Irish sea in September 2003 (n=3).

	Sample No. (µg kg^{-1})		
Element	**1**	**2**	**3**
U	315	278	248
Ce	119	114	147
Pb	1074	1038	338

Table 4.28 Batch two analysis of shells from the Irish sea in September (n=3).

	Sample No. (µg kg^{-1})		
Element	**1**	**2**	**3**
U	362	227	107
Ce	115	92	113
Pb	1893	1818	2429

Table 4.29 Batch three analysis of shells from the Irish sea in May 2003 (n=3).

	Sample No. (µg kg^{-1})	
Element	**1**	**2**
Co	<9	<9
Ni	89	66
Cu	199	<192
Y	4	3
Cd	<16	<4.3
Ce	5	9
Pb	1034	1685
U	<2	<2

4.3.8 Results for Freshwater Zebra mussels.

Considering the importance of monitoring the spread of zebra mussels and their impact on the environment, the trace element composition of shells analysed form Lough Key and Lough Derg was obtained, **Table 4.30**. However, only a limited number of shell samples from each Lough were analysed and a more detailed study of these areas is required.

The trace levels of cobalt and nickel in Lough Key and Derg are high relative to the levels observed for the coastal shell study. The use of a trace element fingerprint could aid research in the control of this non-native shellfish species in Irish waters.

Table 4.30 Analysis of Zebra mussel shells from Lough Key and Derg (n=3).

Element	Lough Key (µg kg^{-1})	3σ	Lough Derg (µg kg^{-1})	3σ
Ni	377	18	905	13
Co	309	101	38	2
Cu	431	53	203	47
Cd	<34	n/a	91	2
Ce	37	4	<16	n/a
Pb	<98	n/a	248	21
U	<2	n/a	<2	n/a

4.3.9 Belgium and Holland Mussel Samples.

The data for shell samples obtained from Belgium and Holland was analysed to determine if there was any large differences in the trace elements profile relative to the sites studied Ireland. Elevated levels of cobalt and nickel are noted for Belgium and Holland samples while the levels of cadmium, cerium, lead and uranium are below the MDL's. The level of copper in the samples is not significantly different from the Irish coastal study.

Table 4.31 Analysis of shells from Belgium and Holland (n=3).

Element	Belgium (µg kg^{-1})	3σ	Holland (µg kg^{-1})	3σ
Ni	266	84	433	43
Co	100	46	245	18
Cu	132	43	360	26
Cd	<34	n/a	<34	n/a
Ce	<16	n/a	<16	n/a
Pb	<98	n/a	<98	n/a
U	<2	n/a	<2	n/a

4.3.10 Intra-Shell Variation.

The variation of trace elements in the environment over the period of growth of a shell can affect the levels observed. A number of complex parameters can play a part in the growth of a shell, local geology, temperature, pH, salinity, and tidal forces. It was decided to measure the intra shell variability of trace elements by analysing portions of a single shell.

The results are shown for two separate shells in **Table 4.32**. The shells were obtained from the Shannon Estuary site, (see **Table 4.10**). Five samples of each shell were analysed in triplicate. The average results of the intra shell analysis for Pb, Cu and Ni levels are similar to those obtained for the previous inter-shell study whether sampling one shell a number of times or ten separate shells at once.

The results of these studies provide a baseline survey of the levels of trace elements in shells in the marine environment. The increasing concentration of lead and uranium from west to east along the Irish coast is the most obvious trend observed. This trend appears to correlate with increasing population density and probably the historical impacts of dumping in the Irish sea.

Table 4.32 Intra-shell variation of elements in Shannion Estuary oysters, Shell (n=3).

	Sample No. (µg kg^{-1})				
Element	**1**	**2**	**3**	**4**	**5**
Co	70	72	64	71	45
Ni	121	125	72	109	110
Cu	626	912	638	393	783
Y	27	61	15	8	9
Cd	<16	<16	67	<16	<16
Ce	13	43	23	<3	<3
Pb	<124	187	178	<124	<124
U	34	32	12	30	65

Table 4.33 Intra-shell variation of elements in Shannion Estuary oysters, Shell 2 (n=3).

	Sample No. (µg kg^{-1})				
Element	**1**	**2**	**3**	**4**	**5**
Co	192	73	3	38	22
Ni	451	194	72	108	92
Cu	1247	649	200	308	129
Y	126	60	1	0.33	1
Cd	<16	<16	<16	<16	<16
Ce	181	71	<3	<3	<3
Pb	581	272	<124	<124	<124
U	15	17	4	<2	8

4.4 Conclusions.

In several ways, the characteristics of this iminodiacetate based matrix removal technique make it ideal for the ICP-MS analysis of bivalve shells. Under carefully controlled conditions, the technique facilitates the efficient removal of the main matrix interferent present (i.e. >99% calcium) following acid digestion of samples. Consequently, larger bivalve shell sample sizes can be used resulting in significantly improved detection limits for these samples. It should be remembered that the ICP-MS analysis of solution samples without matrix removal are generally limited to the 0.2% w/v dissolved solids content.

The concentration of the acetate buffer used with the matrix removal technique also influences both the detection limits and recoveries achieved for all the analytes. In general, a lower concentration of buffer will give better detection limits. This is particularly true for elements such as lead and copper, which are common contaminants in the reagents from which the original buffer was prepared. For best results it is recommended that the buffer is purified prior to use by passing it through a column of the iminodiacetate reagent.

A balance must be achieved between efficient matrix removal and quantitative recovery of the trace elements from the iminodiacetate reagent. Increasing matrix removal efficiency can, under some conditions, have a detrimental effect on trace analyte recovery. Compromise experimental conditions must be used to achieve efficient matrix removal, quantitative recovery of trace elements and low method detection limits.

The ICP-MS results of bivalve trace element levels for sites presented here clearly indicate the potential of this methodology for the provision of distinct elemental profiles or elemental fingerprints linked to the sampling location. The results for lead and uranium appear to indicate some anthropogenic impacts from the dumping of waste at sea but also indicate to some extent the geological marine conditions pertaining at the particular site.

In addition the results indicate that the multielement sensitivity of ICP-MS, used in association with the metal bioaccumulation features of bivalve shells, provide an effective biomonitoring tool for the marine environment.

The current method is not designed to determine the trace element levels at each stage of the shell's incremental growth. The growth layers of the shell can however be used to provide important historical information on environmental conditions etc in the bivalve's lifecycle (e.g. seasonal variations may be inferred from the elemental signatures). The current methodology could be adapted for this task but would require the physical sampling of each growth layer within the shell.

Chapter Five

5.0 Analysis of Certified Reference Material.

5.1 Introduction.

The analytical characteristics of the laboratory developed iminodiacetate matrix removal/preconcentration system have been successfully validated previously in chapter three. The final stage of the research involved the analysis of certified reference material water samples and mineral water using this system.

The NIST-SRM 1643D Water (NIST, Maryland, USA) and The National Water Research Institute TM28.2 certified reference material water (NWRI, Ontario, Canada) were analysed directly using the ICP-MS instrument and also by using the iminodiacetate matrix removal system.

5.2 Analytical Methodology.

All glassware and containers were soaked overnight with 10% nitric acid and rinsed several times with ultra pure water prior to any analysis. Optimisation and calibration of the ICP-MS instrument was carried out as described in **Section 3.2.6**. Multielement calibration standards of 1-500 µg l^{-1} Ni, Co, Cu, Zn, Cd, Ce, Pb and U were prepared in 2% nitric acid. The standards, samples and blanks are spiked to 100 µg l^{-1} using an internal standard solution (Be, Sc, Rh, In and Bi).

Instrument recovery was determined by spiking samples to 25 µg l^{-1} with a 10 mg l^{-} multi-element solution. Samples were analysed using the matrix removal/preconcentration system as described previously in **Section 3.2.10**

5.3 Results and Discussion.

The results of two separate analyses of the NIST-SRM 1643D certified water reference material over a period of six months illustrate for Co, Cu, Cd and Pb that instrument optimisation, calibration and the use of quality control samples have provided reliable analytical measurements. The results for Ni and Zn were lower than the specification but were within 15% of the certified values, **Table 5.1**.

Table 5.1 The direct ICP-MS analysis of NIST-SRM 1643D certified water reference material (n=3).

(a) Analysed in January 2003

Element	Determined (µg l^{-1})	3σ	Certified (µg l^{-1})	2σ
Ni	53.6	1.72	58.1	2.91
Co	24	0.77	25.0	0.50
Cu	19.5	0.62	20.5	0.39
Zn	69.9	2.24	72.5	0.72
Cd	7.41	0.24	6.5	0.39
Pb	17.3	0.55	18.2	0.73

(b) Analysed in July 2003

Element	Determined (µg l^{-1})	3σ	Certified (µg l^{-1})	2σ
Ni	51.45	1.64	58.1	2.91
Co	23.15	1.16	25	0.5
Cu	19.35	2.25	20.5	0.39
Zn	62	1.25	72.48	0.72
Cd	6.16	0.47	6.47	0.39
Pb	17.7	0.77	18.15	0.73

The direct ICP-MS analysis of NRWI TM28.2 reference water provides additional confirmation that the methodology can provide accurate and precise analytical measurements, **Table 5.2.**

Table 5.2 The direct ICP-MS analysis of NWRI TM28.2 certified water reference material (n=3).

Element	Determined (µg l^{-1})	3σ	Certified (µg l^{-1})	2σ
Ni	11.1	1.05	11.2	1.69
Co	3.8	0.57	3.6	0.78
Cu	6.3	2.19	6.3	1.7
Cd	2	1.2	1.3	0.44
Pb	4.2	0.96	4.1	1
U	7.5	0.99	5.7	0.72

The analysis of certified reference material waters using the iminodiacetate matrix removal/preconcentration system was carried out. The NIST-SRM 1643D water reference material was diluted by a factor of twenty. This dilutes the levels of elements in the reference material below the detection limit of the ICP-MS instrument. The matrix removal/preconcentration system was then used to preconcentrate the sample twenty times. The results of this study are shown in **Table 5.3**. The higher level of uncertainty is due to the original lower concentration of trace elements in the diluted solution.

Table 5.3 Determination of Co, Ni, Cu, Cd and Pb in NIST-SRM 1643D water using the preconcentration system. (n=6, 3σ MDL's, preconcentration factor 20).

Element	Determined (μg l^{-1})	3σ	Certified (μg l^{-1})	2σ
Co	23.8	1.6	25.0	0.5
Ni	44.1	2.2	58.1	2.9
Cu	22.3	1.6	20.5	0.39
Cd	8.3	1.6	6.5	0.39
Pb	18.4	1.9	18.2	0.73

The NWRI-TM28.2 water reference material was analysed using the matrix removal/preconcentration system and preconcentrated by a factor of sixteen. The results of the study are given in **Table 5.4** and compare favourably with the certified values in terms of both accuracy and precision.

Table 5.4 Determination of Ni, Co, Cu, Cd and Pb and U in NWRI certified reference material water, TM28.2, using the preconcentration system. (n=6, 3σ MDL's, preconcentration factor 16)

Element	Determined (μg l^{-1})	3σ	Certified (μg l^{-1})	2σ
Ni	10.1	1.3	11.2	1.69
Co	3	0.5	3.6	0.78
Cu	6.7	1.3	6.3	1.7
Cd	2	0.6	1.3	0.44
Pb	4.9	1.6	4.1	1
U	7	2.4	5.7	0.72

Overall, the analysis of NIST-SRM 1643D and NRWI TM28.2 certified reference waters using the matrix removal/preconcentration system confirm that the system can provide accurate and precise analytical measurements.

5.4 Analysis of Mineral Waters.

Finally, the research work focused on the analysis of mineral water sample using the matrix removal/preconcentration system. Ballygowan mineral water was analysed directly by ICP-MS and by the matrix removal/preconcentration system and the results of this analysis are shown in **Table 5.5**.

Table 5.5 Comparsion of direct solution nebulisation with preconcetration for the ICP-MS determination of trace elements in Ballygowan mineral water.(n=6, 3σ MDL's, preconcentration factor 16).

Element	Direct (µg l^{-1})	Preconcentration system (µg l^{-1})
Co	<0.23	<0.014
Ni	<0.8	0.44
Cu	<6.3	2.54
Zn	<3.28	0.64
Cd	<0.29	<0.459
Ce	<0.03	<0.013
Pb	<0.11	0.17
Th	<0.01	<0.043
U	1.4	0.88

The levels of most trace element in the Ballygowan sample are below the detection capability of both direct ICP-MS and the matrix removal/preconcentration system.

Other mineral waters (Tipperary and Riverrock) were analysed using the matrix removal/preconcentration system and the results are shown in **Table 5.6**.

Table 5.6 The analysis of mineral water using the matrix removal/preconcentration system (n=6, 3σ MDL's, preconcentration factor 20).

Element	Ballygowan (µg l^{-1})	Tipperary (µg l^{-1})	Riverrock (µg l^{-1})
Co	<0.013	<0.013	<0.013
Ni	<0.381	<0.381	<0.381
Zn	<0.163	<0.163	<0.163
Cu	<0.488	<0.488	<0.488
Cd	<0.456	<0.456	<0.456
Ce	<0.013	<0.013	<0.013
Pb	<0.044	<0.044	<0.044
Th	3.1	<0.044	5.4
U	0.62	0.45	<0.025

The levels for the majority of elements in these mineral water samples are also below the detection limit of this technique.

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Chapter Six

6.0 Conclusions.

The construction of a laboratory-based matrix removal/preconcentration system using an iminodiacetate chelating ion exchange reagent was successfully designed and operated. This work has highlighted how system design can have an important impact on the operational chemistry of the chelating reagent (i.e. buffer concentration and buffer wash volume). The performance of the push-fit connections used in the preconcentration column provided leak-free operation over long periods of use. However, the use of a uniform bore column made from plexiglass with threaded fittings is recommended for routine use.

Validation and optimisation of the essential experimental parameters (pH, buffer concentration, flow rate etc), which affect the performance of the system were studied and optimised. The optimised system was then successfully applied to the elemental fingerprinting of bivalve shells collected from sites around Ireland's coast.

Purification of the ammonium acetate buffer by pumping the solution through a column of chelating reagent can lead to improved detection limits (DL) for elements such as copper, nickel, lead, zinc and aluminium. However, if this does not obtain satisfactory results, the use of quartz sub-boiling distillation system would be required to obtain a higher purity buffer solution.

Further processing and particularly statistical analysis of the shell data in chapter 4 is required. The application of a chemometic based approach to the results would determine if correlations existed between sites of high and low population. Information about the geological source of trace elements in the marine environment would also assist in this analysis.

The data from the Irish sea samples indicate that a larger study and the selection of an appropriate control site would strengthen these preliminary results. The elevated levels of lead and uranium observed in the Irish sea samples highlight the potential of this technique for obtaining useful information about the current state of the marine environment.

6.1 Further Work.

The matrix removal/preconcentration system can be applied to a wide variety of applications including:

1. Trace elements in electronic grade inorganic acids and bases (used in the manufacture of computer chips).
2. Trace levels of alkaline earth metals in high purity chemicals or brine solutions.
3. Trace levels of Beryllium in environmental samples.
4. The analysis of trace metals in medical snd biological samples.
5. Ultra-trace levels of elements in Ireland's rivers and coastal seawater.

All of these samples are difficult to analyse due to the low levels of analyte in the sample and the physical/chemical based interferences caused by the samples matrix. Further validation of the system would also be required due to the different problems associated with each sample type.

The matrix removal/preconcentration system could be easily adapted for the analysis of arsenic and selenium by using a column packed with activated alumina. Arsenic and selenium would be retained on the column through an anion exchange mechanism.

The determination of arsenic in bivalve shells would provide an interesting extension to the trace element analysis of bivalve shells. Speciation analysis of chromium in the environment is also possible using this packing material.

The analysis of the fifteen rare earth elements (Y, La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, and Lu) in bivalve shells using the matrix removal/preconcentration system would provide a comprehensive trace element fingerprint of the sites studied.

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Chapter Seven

7.0 References

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Chapter Eight

8.0 Appendix A

Conditional Formation Constant for Iminodiacetate Chelates.

The use of conditional stability constants to describe the theoretical behaviour of iminodiacetate with trace elements as a function of pH is developed in this Appendix. This theory is based on similar textbook treatments by other authors [1]. The pH of a sample is an important analytical parameter to optimise when using a chelating ion exchange reagent. It affects the ability of iminodiacetic acid to chelate trace elements because the compounds carboxylic (-COOH) and amino ($-NH_2$) functional groups also form stable bonds with protons. Therefore the trace elements and protons in solution compete for iminodiacetic acid complexing sites. The use of conditional formation constants have been used to take into account the competition between metal ions (M) and protons (H) for the unprotonated iminodiacetic acid ligand (L^{2-}). If protonation of L^{2-} to form HL^- and H_2L occurs, the concentration of L^{2-} in solution must be calculated before the conditional constant can be used.

$$L^{2-} + M^{2+} \rightleftharpoons ML \qquad K_{ML} = \frac{[ML]}{[M^{2+}]\,[L^{2-}]} \qquad 1$$

Where K_{ML} is the critical stability constant for the metal-iminodiacetate complex (1). The concentration of L^{2-} in solution is determined more easily if the fraction of L^{2-} is known. The fraction of L^{2-} is calculated using equation (2).

$$\alpha_{L^{2-}} = \frac{[L^{2-}]}{[HL^-] + [H_2L] + [L^{2-}]} = \frac{K_1 K_2}{[H^+]^2 + K_1[H^+] + K_1 K_2} \qquad 2$$

K_1 Disscociation constant

K_2 Disscociation constant

$\alpha_{L^{2-}}$ Fraction of unprontonated iminodiacetic acid in solution

Where the acid dissociation constants of iminodiacetic acid are K_1 2.98 and K_2 9.98 respectively and these constants define the amount of ionisation of the compound in solution.

The expression for the conditional formation constant, K', is given by.

$$K'_{ML} = \frac{[ML]}{[M^{2+}]C_L} \qquad 3$$

where C_L is concentration of the iminodiacetic ligand in all forms other than ML. This constant only applies to specified experimental conditions. $\alpha_{L^{2-}}$ is related to C_L through

$$\alpha_{L^{2-}} = \frac{[L^{2-}]}{C_L} \qquad 4$$

K'_{ML} is related to K_{ML} by

$$K'_{ML} = \alpha_{L^{2-}} K_{ML} \qquad 5$$

Since the fraction, α, of L^{2-} varies with pH the conditional formation constant, K'_{ML}, also changes with pH. Therefore the strength of the iminodiacetic metal chelates formed varies with changes in pH and a larger formation constant leads to a more stable complex. The conditional formation constants for iminodiacetic acid with Cu, Zn, Cd. Mn, Ca and Na are given in **Table 8.1**, and were calculated using equation 5.

Table 8.1 The effect of pH on conditional formation constants for Cu, Zn, Cd, Mn, Ca and Na Iminodiacetic acid complexes

	Iminodiacetate Conditional Stabilty Constants					
pH	**Cu**	**Zn**	**Cd**	**Mn**	**Ca**	**Na**
0	4.6E-03	1.8E-06	6.5E-08	6.6E-09	5.0E-11	2.9E-13
2	4.2E+01	1.6E-02	5.9E-04	6.0E-05	4.6E-07	2.6E-09
4	4.2E+04	1.6E+01	5.9E-01	6.0E-02	4.6E-04	2.6E-06
5.5	1.4E+06	5.6E+02	2.0E+01	2.1E+00	1.6E-02	9.1E-05
6	4.6E+06	1.8E+03	6.4E+01	6.6E+00	5.0E-02	2.9E-04
8	4.5E+08	1.8E+05	6.4E+03	6.5E+02	4.9E+00	2.8E-02
10	2.0E+10	7.9E+06	2.9E+05	2.9E+04	2.2E+02	1.3E+00
12	3.6E+10	1.4E+07	5.1E+05	5.2E+04	3.9E+02	2.3E+00

The strength of the Cu, Zn, Cd and Mn iminodiacetate complexes increases exponentially as the pH increases from 1-10 and this trend is illustrated in **Fig 8.1**. At high pH these elements are not in a form that will easily complex with the iminodiaetate reagent and this is due to the formation of stronger hydroxide based complexes at greater than pH 8. The strength of the Ca and Na iminodiacetate complexes does not increase significantly until greater than pH 7.5 and pH 10 respectively.

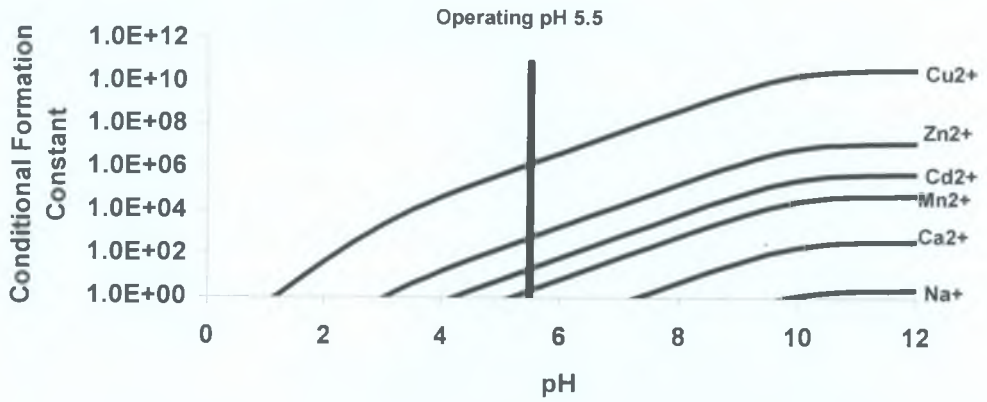

Fig. 8.1 Iminodiacetic acid chelate conditional formation constants for Cu, Zn, Cd, Mn, Ca and Na

Reference

[1] W.E. Harris, B. Kratochvil, An introduction to chemical analysis, Holt-sanders international editions, 1981.