The University of Hull

New materials preparation and miniaturised devices for solid phase extraction as sample preparation techniques for the analysis of transition metals

Being a thesis submitted in partial fulfilment for the degree of

Doctor of Philosophy

PhD

in the University of Hull

by

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Acknowledgements

I would like to take this opportunity to thank my supervisor, Dr. Tom McCreedy for his continued support and valuable suggestions throughout the development of this research.

I am grateful for the University of Taibah at Almadinah Almunawarah, for the generous sponsorship.

I would also like to gratefully acknowledge Mr. Bob Knight for his ideas and assistance in the practical work carried out in ICP laboratory. My appreciation must also be directed to the technical staff of the chemistry department, especially, Mr. Martin Cawley, Mr. Nigel Prakin, Mr. Terry Aspinall and Mr. Mike Bailey for their help on the fabrication of microdevices and lending many useful instruments and apparatus.

I wish to thank all the staff and research students of the analytical group at Hull, who have contributed time and knowledge.

My special thanks and final thanks are to my wife Saada, and my little daughters, Reem and Shaima for their continued support, motivation and patient during the years of my study.

A.O. AlSuhaimi (April 2006)

Acronyms and abbreviations

8-HQ-CPG: 8-hydroxyquinoline immobilised onto controlled pore glass

8-HQ-Si: 8-hydroxyquinoline immobilised onto silica

AE: auxiliary electrode or counter electrode

CAD: computer-aided design software

CCD: charge coupled devices

CE: capillary electrophoresis

CEC: capillary electrochromatography

CPG: controlled pore glass

DC: direct current

DCCAs: drying control chemical additives

DDDC: diethylammonium-N-Ndiethyldithiocarbamate

DDPA: ammonium diethyldithiophsphate

DNA: Deoxyribonucleic acid

DTT: DL-dithiothreitol

DVB: polystyrene divinyl benzene

EDTA: ethylenediaminetetraaceticacid

EPA: environmental protection agency

ETAAS: electrothermal atomic absorption spectroscopy

Ferrozine (FZ): 3-(2-pyridyl)-5,6-bis-(4-phenylsulfonate)-1,2,4-triazine)

FIA: flow injection analysis

FTIR: Fourier Transform Infrared

GC: Glassy Carbon

GFAAS: graphite furnace atomic absorption spectroscopy

HPLC: high pressure liquid chromatography

ICP: inductively coupled plasma

ICP-MS: inductively coupled plasma-mass spectroscopy

ICP-OES: inductively coupled plasma-optical emission spectroscopy

IDA: amino diacetic acid

L-Cys-Si : *L*-cysteine immobilised onto silica

LIF: laser-induced fluorescence

LIGA: from the German Lithographie, Galvanformung and Abformung, means, lithography electroplating and moulding

LLE: liquid–liquid extraction

LOD: limit of detection

LOQ: limit of quantification

LOV: lab on valve
MCN: micoconcentric nebuliser
MFS: microcolumn field sampling
*o*VTSC: *o*-Vanlinthio-semicarbazone
PAAm: and poly(acryl amide)
PAN: pyridylazo-naphthol
PAR: (pyridylazo) resorcinol
PDCA: pyridine-2, 6 dicarboxylic acid
PDMS: poly(dimethylsiloxane)
PEEK: poly(ether ether ketone)
PEG: polyethylene glycol)
PEO: poly(ethylene oxide)
PMMA: poly(methylmethacrylate)
PTFE : poly tetra fluro ethylene
PVC: poly vinyl chloride
PVP: poly(vinyl pyrrolidone)
R E: reference electrode
R&D: research and development
REEs: rare earth elements
RF: radio frequency
RSD: relative standard deviation
S/N: signal to noise ratio
SAM: self-assembled monolayer
SI: sequential injection systems
SNP: single nucleotide polymorphisms
SPE: solid phase extraction
TAS: totals analysis systems
TEOS: tetraethoxysilane
TLM: thermal lens microscope
TMOS: tetramethoxy-silane
UV-Vis: ultraviolet-visible
WE: working electrode
μ-TAS: micro totals analysis systems

Abstract

The subject of this thesis is the development of solid phase extraction (SPE) materials and miniaturised apparatus applicable for sample preparation in the analysis of transition elements from environmental matrices. It consists of five main chapters and a general conclusion.

The first chapter is a general introduction. Considerable attention is given first to underlining the role of SPE as an efficient sample preparation approach in trace analysis. Subsequently the materials and techniques most frequently exploited for the synthesis of SPE materials specific for metals are discussed with the aid of various demonstrative examples from recent literature. Then the current research trends towards downscaled analytical systems and the efforts to integrate SPE apparatus within miniaturised devices are pointed out. Particular attention is paid to the fabrication techniques predominantly exploited to construct microfluidic devices, i.e., from glass and polymers. Given that the developed SPE systems in this thesis were coupled with various analytical detectors/instruments including atomic spectroscopy (i.e., ICP-OES, ICP-MS), optical absorption spectroscopy (in UV-Vis range) and electrochemical (amperometry) monitoring, the essential fundamentals of these detection techniques are presented.

In the second chapter, the development of a rapid and an environmentally friendly chemical transformation, consisting of minimum steps in comparison with the traditional method, to immobilise oxins (i.e., 8-hydorxyquinoline (8-HQ)) on silica surfaces is reported. The produced chelating resin shows excellent performance as SPE materials for on-line sample preparation (preconcentration and matrix elimination) of some transition metals prior to their determination by ICP-OES. The applicability of this SPE material was tested by analysing Cu, Co, Zn, Ni and Pb in the range of 50-300 ng ml^{-1} from a synthesis matrices simulating sediment. The recovery values were ranged from 100% for Zn to 70% for Ni. The work in this chapter also presented a method to mask the environmentally abundant (major) transition metals (i.e., Fe, Al and Mn). The system used at its optimised parameters to

analyse the studied ions from different sediments reference materials. The results show good agreement with the certified values.

Chapter three describes methods to fabricate miniaturised SPE columns from monolithic materials. Short monolithic columns were fabricated from silica materials using a simple sol-gel method relaying on the hydrolysis of potassium silicate (21% SiO_2, 9% K_2O) using formamide and/or acetamide, then functionalised with 8-HQ and *L*-cystiene via two different *in situ* procedures. The functionalised monolithic microcolumns encapsulated inside a house made connector, and thus easily incorporated within a FI manifold coupled with UV/Vis spectrometer allowing the eluted metals to be derivatised, with chromogenic reagents (i.e., PAR and ferrozine), and monitored on-line. The system has been characterised for Co, Cu and Fe (II). The microcolumns functionalised with *L*-cystiene operating at flow rate of 0.3 ml min^{-1} for 4 minutes, the linear range for the Co, Cu and Fe (II) ions were 20-240, 10-200 and 5-180 ng ml^{-1} respectively. For those functionalised with 8-HQ, the linear range were 15-300, 10-250 and 5-250 ng ml^{-1} in the same order.

In chapter four, downscaled SPE apparatus applicable for sample preparation prior to ICP-MS monitoring, have been constructed making use of the lab on a chip concept. Standard photolithography and wet etching were used to fabricate glass microfluidic devices accommodating three microchannels, each of them incorporating a defined section that could be packed with SPE resin. The microdevice interfaced with the ICP-MS instrument throughout a low flow rate concentric nebuliser using a Teflon connector, and coupled with FI delivering sample and reagents via a splitting valve. The feasibility of this miniaturised system to perform SPE of trace metals was proved by analysing trace metals, Cd, Co, and Ni, in seawater reference materials.

Chapter five reports two designs to integrate microfluidic devices with electrochemical detection. In the first one, a microfabricated glass microfluidic device incorporating single microchannel with a packed section was coupled with a specially designed miniaturised electrochemical cell in a configuration that permits the working electrode to be mounted opposite to the channel outlet to facilitate end channel

amperometric detection. The miniaturised electrochemical cell was made from three pieces of glassy carbon, silver and platinum rods of 2 cm length as working, reference and auxiliary electrodes respectively. These rods were assembled in a miniaturised Perspex block, stabilised with insulating epoxy resin and their ends were polished to mirror like discs. In the other design, the microfluidic devices were fabricated from PDMS by a simple casting and moulding techniques permitting the construction of three dimensional (3D) microchannels. The elastic characteristics of PDMS offer a great degree of flexibility for the placement of the microelectrodes inside the microchannel; thus, the monitoring is performed in-channel. To minimise variation in background current due to the pH change, the SPE process was carried out in buffer media i.e., the metals were loaded in acetate buffer of pH 4.8 and eluted with buffered solution of 50 nM Pyridine-2,6-dicarboxylic acid (PDCA) containing 50 mM of KCl as a supporting electrolyte to maintain a constant conductivity. The system shows good performance in the SPE and monitor Cu ion from standards solution in the range 100-400 ng ml^{-1} with LOD at 52 ng ml^{-1}

In chapter six a general conclusion and a concise prospective for further work are presented.

Table of contents

Chapter 1

Introduction

Chapter 1–Introduction

1.0 Sample Preparation (General)

Over the last few decades, the increased demand for accurate determination of many transition and heavy metals at trace levels in a diverse range of matrices (e.g., natural and waste waters, geological, biological and industrial materials, substances of high purity) has stimulated interest in trace and ultra trace metals analysis. Despite the real advances in instrumentation technology, providing analytical science with highly selective separation and sensitive quantification for trace elements down to sub femtograms or below, in a wide range of matrices, the simple approach of "dilute and shoot"[1] is not usually applicable. In fact, as highly sensitive analytical systems became a common standard for analysts it was realized that the preparation of samples was an important limiting factor in the general progress in trace analysis. It became apparent that any mistake occurring in collecting and processing samples could lead to a substantial error in the final result, regardless of the excellent performance of the state-of-the-art analytical technique applied subsequently. Moreover, the result of a recent survey among analysts indicated that two-thirds of the analysis time was spent on sample collection and preparation.[2] This finding verified the magnitude of sample preparation in the analytical process.

A comprehensive discussion of the fundamentals of the generally applied preparation procedure is well documented in the text by Anderson, [3] and an extensive overview of the most encountered techniques is well presented in the recent monograph edited by Mitra.[4] The most recent development and new trends in this growing area have been reviewed recently.[1,5,6]

The basic concept of sample preparation method is to convert a real sample into a format suitable for analysis by separation or some other analytical technique. A central step frequently used in sample preparation processes involves extraction of the analyte from other interference species (matrix). Nonetheless, many techniques based on this principle have seen little change over the last 100 years.[5] They all have the following aims in common: [2] (1) analyte isolation and enrichment, (2) removal of matrix effects, (3) improving the detection limit, and (4) allowing the application of simpler instrumentation in the final measurement.

Although many traditional sample preparation methods are still in use, the trends in recent years have been towards: [4, 5]

- using smaller initial sample sizes even for trace analysis.
- greater specificity or selectivity in extraction.
- on-line methods thereby reducing manual operations and sample exposure to the ambient atmosphere
- environmentally friendly approaches with less waste production or the use of smaller amounts of organic solvents.
- minimise the number of operations and processes utilised at the sample preparation stage.

1.1 Solid phase extraction

To facilitate the above criteria, the classical extraction techniques, such as evaporation, liquid–liquid extraction (LLE), precipitation or co-precipitation have in recent years been largely replaced by those based on sorbent extraction (liquid-solid) which are usually referred to as solid phase extraction (SPE).

The principle of SPE is similar to that of LLE, involving a partitioning of solutes between two phases. However, instead of two immiscible liquid phases, as in LLE, SPE involves partitioning between a liquid (sample matrix) and a solid (sorbent) phase. Preconcentration and purification of analytes from matrix by sorption on a solid sorbent has been a remarkable advantage of this technique.

The first experimental applications of SPE was fifty years ago[7]. However, its growing development as an alternative approach to liquid–liquid extraction for sample preparation started only in the mid-1970s. It has been extensively used in the past fifteen years for the preconcentration of trace level of organic pollutants, especially pesticides, in water samples.[8] Correspondingly, ever increasing studies have demonstrated the great potential of this technique in the preconcentration and speciation of trace metals.[9]

1.2 SPE materials for trace metals

During the last twenty years, SPE has established itself as a replacement for the traditional technique to perform analyte preconcentration and matrix elimination in the analysis of trace metals from difficult matrices. A wide range of materials have been used effectively for this process. The mechanism of partition (retention) depends on the nature of the sorbent, and may include simple adsorption, chelation or ion-exchange. Sorbent materials are classified based on the chemical phenomena involved into two broad classes, namely, ion exchanges, and complexation reactions with an appropriate chelating reagent. The latter can be exploited either as organic collectors or immobilized chelating resins.

1.2.1 Ion exchange resins

Observations of ion exchange phenomenon date back to ancient times, when people used some soils, sands, natural zeolites and plants as tools for improving the quality of drinking water by desalinating or softening it, but the mechanism of ion exchange reaction, however, was first established by Thompson and Way in 1850.[10]

Ion exchangers as they are known today are insoluble polyelectrolyte structural matrices having free ions that can reversibly interchange with ions in the surrounding area. In a true ion exchange process the exchange of ions takes place stoichiometrically between the matrix and the solution. In general, cation exchanger has negative anionic sites (R^-) with cations A^+ electrostatically bound but free to undergo exchange with cation B^+ according to equation (1.1)

$$R^-A^+_{(solid)} + B^+_{(solution)} \rightleftharpoons R^-B^+_{(solid)} + A^+_{(solution)} \quad (1.1)$$

In turn, anion exchanger R^+ has positive cationic sites with anions A^- again electrostatically bound, but free to be exchanged with anions B^- as represented by equation (1.2)

$$R^+A^-_{(solid)} + B^-_{(solution)} \rightleftharpoons R^+B^-_{(solid)} + A^-_{(solution)} \quad (1.2)$$

The ion exchange process is reversible, i.e. it can be reversed by suitably changing the concentration of the ions in the solution. The process is, in many respects, analogous to the adsorption process but is not exactly the same. The most characteristic difference between the two processes is that the ion exchange takes place stoichiometrically, really by the effective exchange of ions, while in an adsorption process the adsorbent takes up the dissolved substances from the solution without releasing anything into the solution

(molecular adsorption). However, the two processes may occur simultaneously in practice, particularly in inorganic ion exchangers.

Based on structural matrix, ion exchangers can be grouped into two main categories; inorganic and organic exchangers. Both groups include synthetic and natural materials.

Natural inorganic ion exchanger materials can be grouped into three main categories: zeolites, clay minerals (crystalline aluminosilicates) and oxides. Synthetic inorganic materials on the other hand encompass many classes, including zeolites, hadrous oxides, acidic salts of polyvalent metals, salts of heteroply acid and other ionic compounds. [10]

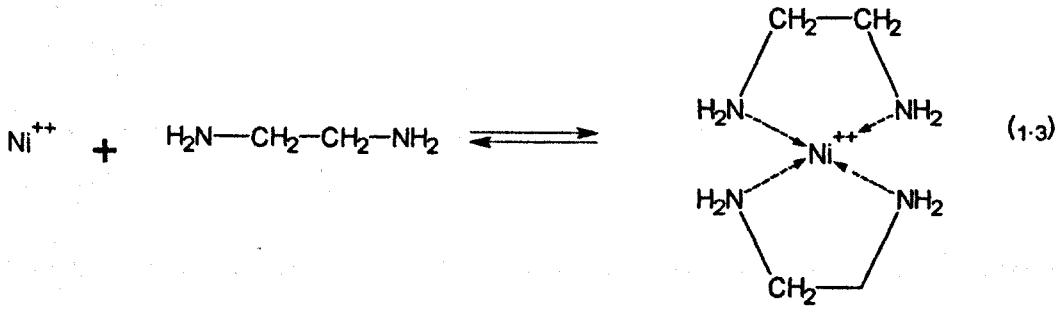
Nearly all the synthetic organic ion exchange resins are based on styrene-divinely-benzene, phenolic or methcrylic acid structures functionalised with certain ionogenic groups. According to the nature of these groups, the resins are classified into two main classes, i.e., cation and anion exchangers. For example the exchangers with sulfonic acid or caboxylic acid groups attached are cationic, while exchangers functionalised with primary, secondary, tertiary, or quaternary ammonium groups are anionic exchangers.[11]

Although ion exchangers are used overwhelmingly in industrial applications including water softening, soil remediation, hazardous waste treatment involving reactive wastes, and metallurgical industry,[7] they are also convenient for trace analysis applications especially in ion exchange chromatography.[12,13,14] Due to the relatively high exchange capacity, ion exchangers are frequently used to preconcentrate and/or separate ionic analytes from species of opposite charge. However, the use of these as materials for analytical applications (i.e., SPE of trace metals) is limited by their relatively poor selectivity. A strong cation exchanger, for example, will retain metal ions effectively; but,

it will accumulate innocuous cations such as sodium, calcium and magnesium. This poses a definite problem when the concentration of these 'matrix' ions is large compared to the heavy metals content. There are, in addition some examples where anion-exchangers can be used to preconcentrate metals, particularly those that form basic halide (e.g., floro-chloro-) or oxo-complexes, but again the applications are limited.[15]

1.2.2 Complexation reactions

The term complexation process is applied to the reaction, in which ligand species react with a metal ion to form a complex, usually by coordination through one or more atoms capable of donating a pair of electrons to the bond. The most useful analytical complex, however, is that in which the legand is attached to the metal by two or more atoms (multidentate) to form a ring structure, wherein coordinating atoms and the metal ion all form part of the ring. [16] This kind of complex is usually referred to as a metal chelate. The optimum size of the ring that could form a stable metal chelate is 5 or 6 atoms.[17] The reaction of the chelating reagent, 1,2-diaminoethane with Ni (II) to form two 5-membered rings, is a clear example (equation 1.3). The arrows represent the coordination bonds.



(1·3)

The number of types of group involved in chelated ring formation is strictly limited by the requirement that the atoms bonded to the metal in the complex must be able to donate a pair of electrons to the union. In practice this almost restricts the choice to ligands bearing N, O, S and to the less common P atoms. As a general guideline, metals are classified into three groups depending on the donating atoms (groups) on the ligands that they are attracted to;[16,18] *Oxygen bearing ligands* may coordinate as phenoliates or carboxylates onto alcoholic OH, or ether-oxygen, or –C=O of ketones, aldehydes, and carboxylic groups. These groups react preferentially with the ions having electronic distributions similar to those of the inert gas atoms. Examples include Na^{+}, Ca^{2+}, and Al^{3+}. These ions form rather weak outer-sphere-complexes with only oxygen ligands. Nitrogen may be present in the ligand as amines, nitro, nitroso, azo, or diazo groups; or as a nitrile or acid amide. These ligands, particularly the polarisable portions such as amino and heterocyclic nitrogen, attract the first two rows of transition metals with slight overlap with mercury. Sulphur bonding may occur because of ionized thiol and thiocarboxylate anions, thioethers and thioketone, and through disulfide groups. These highly polarisable ligands are attracted by metals with filled d orbitals such as Ag, Zn, and Ga since some d electrons can be back-bonded in the vacant orbitals on S atoms.[16] The concept of soft and hard doners class metals also a useful classification guide.

To summarise, the ligand selectivity toward certain ion or group of ions depends primarily on the relative values of the overall formation constant of the complex formed which in turn are affected by the electronic configuration of the metal ion and by the Lewis base character, stereochemistry and polarisability of the ligand.[19] Formation

constants can vary over a wide range. For example, the overall formation constants of 8-quinolinol-metal complexes vary from about 10^2 for Ba^{2+} to about 10^{38} for Fe^{3+}.[20] Therefore this variation in relative values of formation constants can be taken as a significant selectivity parameter.

1.2.2.1 Adsorption and sorption

The general term sorption includes adsorption, the process by which a solute clings to a solid surface, and absorption, the process by which the solute diffuses into a porous solid and clings to the interior surface. The SPE of metal ions by the sorption process involves three mean steps. Firstly, a suitable chelating reagent is added to the sample solution, either in batch or on-line, to form a metal-complex with the targeted metal. Secondly, the complexes produced are accumulated on an inert solid phase (sorbent). Finally, the metal-complex or metal ion is desorbed off the sorbent into a small amount of a suitable solvent. The process, therefore, can be considered as a liquid-liquid extraction process in which the organic solvent is replaced with a suitable solid sorbent. Although, the principle had been in use for a long time for multielement determination by liquid chromatography,[21, 22] the technique gained much popularity in SPE of trace metals after the work of Ruzicka and Arndal in 1989 [23], particularly with flow injection systems. The extensive selection of chelating agents and sorbent materials to be used off-shelf as required, and the simplicity of the technique, are among the reasons for its popularity. The sorbent materials on which the metal-complex is retained could be any chromatographic solid phase (i.e., polar, non-polar, or graphitized carbon) and the selection of a particular sorbent often based on the polar characteristics of the complex. The interactions between the complex and sorbent surface are typically weak electrical

forces including dipole-dipole interactions, π-π interactions and induced and dipole-dipole interactions (Van der Walls force), and may be hydrogen force.[3,4] Because the bonds are physical rather than chemical, the attachment can occur over multiple layers onto the adsorbent surface. This could explain the relatively high values of the preconcentation factor and the capacity exchanges that have been reported.[24]

Theoretically, a wide range of reagents (such as those used comprehensively in liquid extraction) can be employed to collect trace metals, though it is preferable to use ligands that can form water soluble complexes with metals and desorbed easily with water miscible organic solvents. This made the use of hydroxyquinoline and dithiocarbamates and their derivatives very popular as collectors for the SPE of metals prior to atomic spectroscopy, especially flame atomic absorption and electrothermal atomic absorption. Usually the metal complexes of these chelating agents are adsorbed onto reverse phase packing materials containing C18 hydrocarbon chain immobilised on a silica substrate, or macro-reticular e.g., Amberlite XAD-4. There are however, an enormous number of chelating reagents that exhibit some degree of selectivity which can be used as collectors for metals. To name but few examples: dithiophsphate derivatives[25], 2-(5-Bromo-2-pyridylazo)-5-*N*-propylamino)phenol[26], cyclohexane-ondioxime[27], dimethylgly-oxime[28], nitoro-R-salts and cetyltrimethyl- ammonium bromide, benzophenone[29] and thiocynate[30] have been used regularly by many investigators. Besides the extensive use of the reverse phase solid supports such as C18, there are, many other materials including zeolites[31], C60-70 fullerenes,[32,33] and polyurethane foam, [30] that have proven to be ideal solid phase materials for the accumulation of many metal-complexes in various applications.

1.2.2.2 Immobilised chelating reagents and their supports

Chelating resins are groups of materials having ligands, with suitable donor atoms, attached to their surface. A popular example of synthetic resin is Chelex 100. It is based on the chemical modification of a matrix of a styrene-divinylbenzene polymer with iminodiacetic acid group (see figure 1.1). The attached groups incorporated within the chelating resins interact with metal to form stable metal-complex through a coordination bond, as in the free chelating ligand. The most obvious benefit however, is the reusability of the resin for many cycles.

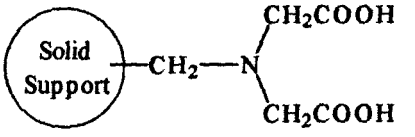


Figure 1.1 The chemical structure of Chelex 100

The synthesis of chelating resin involves the attachment or immobilisation of particular chelating ligands or functional groups to or within a solid support. These are usually inert (preferably porous) polymeric or inorganic matrices. More frequently the synthesis is accomplished using one of the following techniques: [34,35]

a) *Impregnation Techniques* in which the reagent is bonded onto the support by physical adsorption, chemical sorption or electrostatic bonding. Practically, impregnation can be achieved by exposing the porous support (e.g., silica gel) to a concentrated solution of reagent to be immobilised followed by solvent evaporation.

b) *Chemical doping* by which the reagent to be immobilised is added as an organic modifier (usually organic polymer) during support production throughout polymerisation or the moulding processes.

c) *Copolymerisation of modified reagents* is accomplished using a two-step synthesis. Firstly, a certain monomer such as alkoxysilane, containing covalently bound chelating reagent is prepared, and subsequently used as a precursor in the synthesis of support polymers. This approach is commonly applied in the synthesis of resins based on macrocyclic molecules. [35]

d) *Chemically immobilisation by covalent binding to pre-prepared matrices.* This process is an extensively researched technique and by far the most commonly utilised for the attachment of chelating ligands, because of the strength of the binding, and the availability of numerous choices of ideal supports.

The synthesis of chelating resins has been a fruitful research area for many years. Studies on organic/inorganic ligands have led to a real development in the synthesis of resins possessing various chelating properties. This is encouraged by the increased demand, in analytical as well as in preparative inorganic chemistry, for chelating resins which combine the ease of operation of the conventional ion exchangers together with specificity and selectivity of the ligands.[36,37] Most of the recent research activities in the field and the new trends have been reviewed by many authors. The following reviews represent good examples: Chow and co-workers[38] summarized various synthetic processes utilised in the production of different chelating resins and emphasized their applications in the extraction/preconcentration of trace metals from water samples. These authors paid particular attention to the increased use of polymers and related materials as

supports for the immobilisation, and also cited several examples illustrating the performance of commercial resins. More recent review papers by Bilba *et.al.*[42] and Garg,

et al.[39] described in depth most of the existing chelating resins, essentially, the most common chemical transformations for their preparations and their usefulness in various applications. The new trends in the chelating resins based on silica materials were critically reviewed by Sharam *et al.*[40] Pasekova and Morosanova dedicated an excellent work to the synthesis and applications of specific chelating resins based on macrocyclic compound.[35] In addition to chelating resin based on complexing agents, other specific materials that have attracted increasing interest as chelating reagents are biological substrates. The advance in their application in the SPE of trace metals, and the most employed immobilisation process to realise their attachment onto solid supports have been reviewed by Madrid and Camara[41]

Solid support materials most extensively exploited for the immobilisation of chelating reagents can be classified as in the scheme in Figure 1.2. [42] In the following sections, some of the characteristic properties and the widely employed chemical transformations to attach chelating reagent onto these solid supports will be summarised briefly.

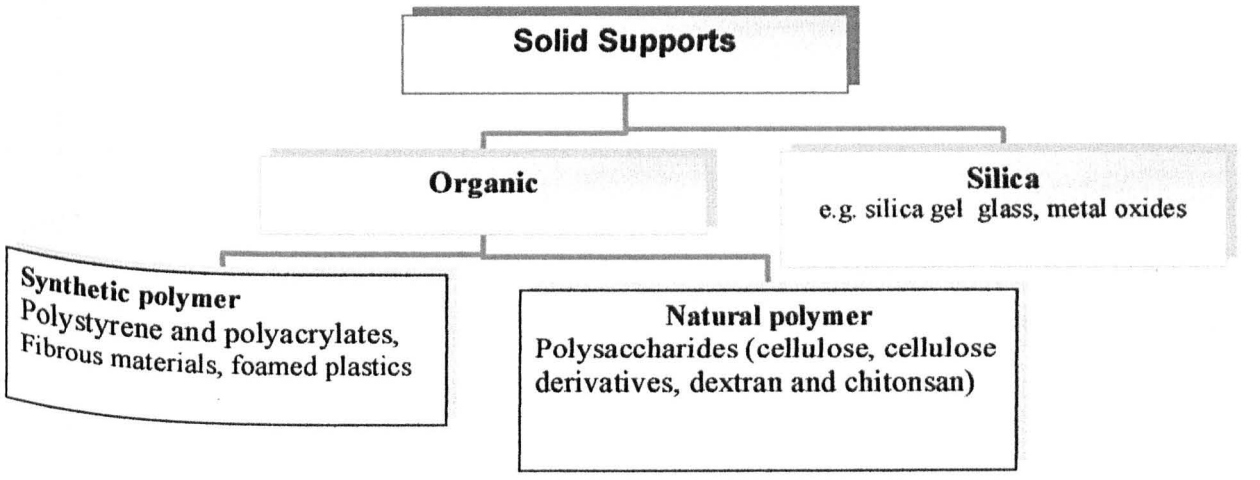


Figure 1.2 Classification of solid support materials for the immobilisation of chelating reagents.

1.2.2.3 Chelating resins based on organic polymers

The incorporation of chelating groups into polymeric chains can be effected using one of the following three methods:[43]

1. direct polymerisation and copolymerisation of monomers containing the desired functional groups,
2. chemical modification of preformed polymer,
3. a combination of 1 and 2 .

Direct polymerisation has been extensively employed in the early applications to prepare various resins, mostly throughout condensation reaction. In this method, chelating molecules such as oxins, aromatic amines and phenols are condensed with formaldehyde in the presence of a suitable cross-linking agent.[44] It has been realised, however, that the chelating resins prepared using condensation methods have irregular beads and some exhibit a very low capacity exchange.[45] In the recent years, however, most of reported immobilisation methods rely on the incorporation of chelating ligands into preformed polymers (matrices). This is because the synthetic polymers, particularly chain growth polymers such as polystyrene, have the advantages of a high surface area, uniform pore distribution and rigid structure, and are available at low cost.[46] Generally, the polymer is activated by incorporating a reactive functional group into its matrix by chemical reaction or photo radiations. Subsequently, these reactive groups (such as chlormethyl, epoxy, amino etc.) interact with complexing reagents in the coupling process. For example, for the attachment of aminodiacetic acid (IDA) on a polyvinyl styrene matrix, the polymer is activated first through a transformation to a chloromethylated derivative by means of its treatment with chloromethylether under Friedel-Crafts reaction conditions.[47] After that,

IDA is allowed to react with the chloromethylated intermediate in triethyl amine or any other amines. Chelex 100, which is based on this modification, is perhaps the oldest chelating resin to be developed and commercialised.[48] Although it has been used successfully for isolation of trace metals from many matrices, (e. g., seawater), it suffers from instability caused by swelling and shrinking. This problem can be minimised when a polymer of high crosslinking is utilised. For example, Muromace A-1 which has the same aminodiaceticacid functional group as Chelex 100 does not experience substantial swelling problems because of its high crosslinking.[49]

Another easy process, by which polymers such as divinylbenzene copolymers and Amberlite XAD are activated, is to use nitration (by treatment with nitric acid) to generate reactive nitro phenyl groups which can then be easily utilised in diazotisation coupling. This transformation method has been used to functionalise polystyrene polymer with many chelating reagents including; azobenzyphosphonic acid,[50] Alizarin Red-S,[51] Salicylic Acid,[52] 2-(2-Thiazolylazo)-5-dimthylaminophenol,[53] and *o*-Vanlinthio-semicarbazone (*o*VTSC).[54] The resulting resins were proved to be very useful for preconcentration/matrices elimination during the trace analysis of a wide range of transition and heavy metals, either in batch or column modes. Likewise, numerous chelating resins based on the attachment of wide spectra of chelating reagents, mainly those extensively applied in liquid extraction, such as hydroxyquinoline,[55] dithiocarbamate[56] and its derivatives, *L*-cysteine,[57] Mercapto group,[58] *N*-phenylhydroxyamine,[59] pyridylazo-naphthol (PAN),[60] have been synthesised using the above transformations or others.

1.2.2.3 Chelating resins based on natural organic polymers

Cellulose is one of the fascinating solid supports that have been used for the immobilisation of various ligands. This is because of its many useful charactesitics, mainly the high surface area and the established chemical transformations by which it can be functionlised. The recent review by Pyrznska and Tojanowicz illustrates the favourable properties of cellulose based resins for SPE of metals.[61] Gennaro *et al.* described a procedure for the covalent attachment of iminodiacetic acid groups to the commercial cellulose filters and demonstrated its feasibility by using it to preconcentrate some trace metals. [62] Gurnai *et al.*[63] developed a method to couple oxine (i.e., 8-hydroxyquinone) to cellulose surface making use of an innovative linker, $-NH-CH_2-CH_2-NH-SO_2-C_6H_4-N=N-$, and studied its sorption behaviour for metal ions. It proved to provide a promising stable matrix for the enrichment of several transition metals (e.g., Cu, Zn, Fe, Ni, Co, Cd and Pb) in comparison with those based on other linkers. Cellulose functionalised with 8-HQ (Ostsorb Oxin)[64], phosphoric acid (Cellex P) [65] and other groups [62] are available on commercial scale from many suppliers.

1.2.2.4 Chelating resins based on silica and related materials

Silica and metal oxide materials have been the most widely studied solid supports for the immobilisation of chelating reagents. They exhibit many properties as convenient supports to effect characteristic immobilisation. For instance, they do not swell excessively, have good mechanical strength and can undergo heat treatment. In addition, chelating agents can be easily bound chemically to the support, affording a higher stability. [66] Probably, the most useful characteristic is the presence of high concentration of silanol groups, (≡Si-OH), which is essential for the initiation of chemical reaction

involved in the immobilisation of functional groups. The increased applications of silica materials as efficient solid supports for the immobilisation of various reagents for analytical or industrial applications together with the chemistry commonly involved in the transformation have been reviewed by many authors. [67,37,39]

Unlike polymers, the silica surface is highly reactive due to the presence of silanol (≡Si-OH) or siloxane (Si-O-Si) groups. It can be functionalised using one of three main methods:[37] (i) through reaction between organosilanes or organic molecule and silica surface silanols (silylation), (ii) chlorination of silica surface followed by reaction of the Si-Cl with a suitable reactive reagents, (iii) incorporation of functional group via sol-gel methodology followed by postmodification if necessary. However, for attachment of chelating reagents to silica surface, a silylation using appropriate organofunctional silanes, most frequently aminoalkylsilane (e.g., 3-aminopropyl- triethoxysilane), is the most preferred step to activate the surface. The reaction taking place in this process involves four steps (see figure1.3).[68] Initially, hydrolysis of the alkoxy (i.e., methoxy) groups occurs. Following the hydrolysis of the first and second alkoxy groups, the condensation to oligomers takes place. The tendency toward self condensation can be controlled by using fresh solutions, alcoholic solvents, dilution, and by careful selection of pH ranges. Silanetriols are most stable at pH 3-6, but condense rapidly at pH 7-9.3. The third methoxy group upon hydrolysis is oriented towards, and hydrogen bonds with, hydroxyl sites on the substrate. Finally, during drying or curing, a covalent bond is formed with the substrate and water is liberated. This results in the formation of covalent bonding to silica via the siloxane bond, (Si-O-Si), which is known to exhibit much

greater hydrolytic stability compared to other linking such as Si-C or Si-N. At the interface, there is usually only one bond from each Si atom of the organosilane to the substrate surface.[69]

Silylation can be used to functionalise silica with reactive chelating groups through various processes; perhaps the simplest one, is to use a silylation agent on which the reactive functional group (e.g., amino or other reactive group), that is involved in the chelating process, is part of the silane. The most common type of chelating regents attached in this manner is ethylendiamine. Yet other reagents can be attached using this simple approach. For example, Howard and co-worker attached mercapto groups on silica gel in a single step via its silylation using (3-mercaptopropyl)-trimethoxysilane. [70,71] Similarly dithiocarbamate and xanthate functional groups have been anchored.[72] Iminosalicyl-modified silica gel was prepared via direct coupling of salcylaldehyde to siylanised silica gel in anhydrous diethyl ether.[73]

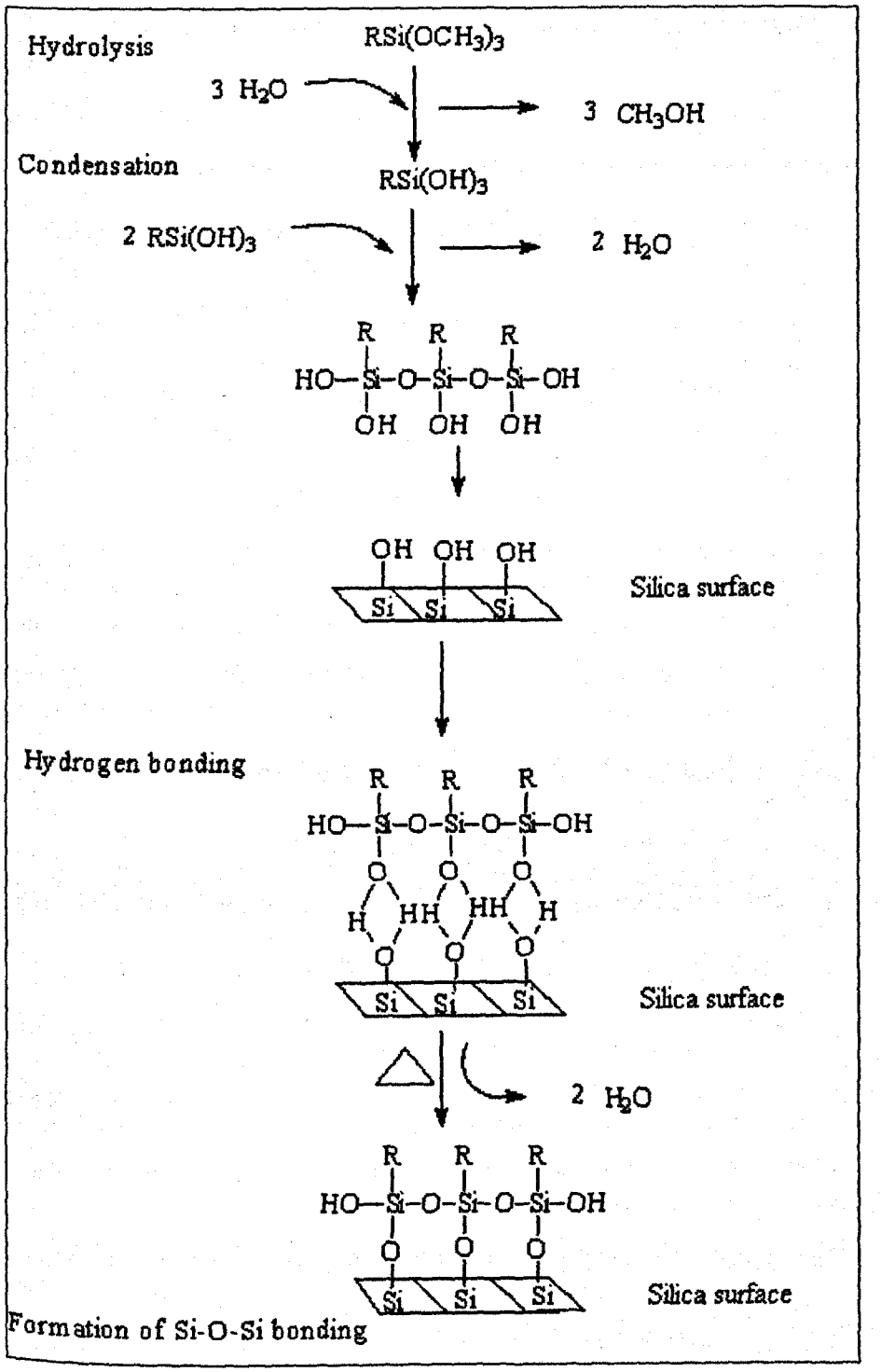


Figure 1.3 Silylation reaction. [68]

The second approach and probably the most documented method, first described by Weetall[74] and later by Hill,[75] for enzyme immobilisation, involves reaction of silica substrate with aliphatic aminosilane (mainly 3-aminopropyltriethoxysilane) to cover the surface with coavalently bonded amino groups. These groups can be further reacted with bifunctional reagents, which have a preference to react with amino groups on the species to be immobilised. Among the bifunctional reagents widely utilised for attachment of ligands or biological substrates/organisms are glutaraldehyde and carbodiimides. This approach is very useful for the attachment of amino acids (e.g., *cystiene*) [76] and organisms (e.g., *alga, saccharoyces cerevisiae*). [77] The bifunctional reagent, however, offers other advantages in addition to the linking. It functions as a spacer between the silica and the immobilised reagent /organism. Another well recognised cross-linking method is the diazo coupling. Oxines such as hydroxyquinoline (HQ) and its derivatives were the first chelating reagents to be immobilised on silica supports using this method. The procedure is adapted from the protocol described by Weetall.[74] The silica surface is firstly silylised using an aminosilane (as mentioned above); then, an aromatic nitro group ($ArNO_2$) is introduced, mostly by reacting the aminated silica with *p*-nitrobenzoylchloride, which is finally reduced to aromatic amine ($ArNH_2$). Sugawara *et al*[78] employed these steps followed by a diazotisation step for the aromatic amine to bring about a diazo coupling to attach HQ to silica surface (i.e., controlled pore glass (CPG)) for the first time. The effectiveness of the resin (assigned as HQ-CPG) was demonstrated by studying the level of Cu and Fe contamination in distilled deionised water. The same procedure has been followed by many investigators to many oxins on silica gel [78,79,80,81] and CPG.[82,83] chelating resins prepared using this method

demonstrated good properties (i.e., capacity, stability) when compared with the properties of many of the commercially available resins.[82,84,85]

1.2.2.5 Miscellaneous resins

As the SPE techniques has established itself as an excellent tool for sample preparation in the analysis of trace metals, the search for new chelating reagents and/or organisms, new supports and efficient chemical or physical transformation for their attachment, is continuously expanding. In this context, Turker and coworkers used the naturally occurring, Sepiolite, to immobilise baker's yeast (*saccharomycescerevisiae*),[86] Asperillus niger[87,88] to produce low cost SPE materials that show efficient performance for the extraction of transition elements. The well known self-assembled mMonolayer (SAM) technology has been adapted to effect the attachment of poly (*L*-aspartate) onto gold minigirds support. Gold was chosen because the reagents can be attached very efficiently to its surface through activated thiol group, and unlike silica, the uncovered gold surface is non-reactive toward metals. Ligand immobilised by this method was found to have higher capacity in comparison with that immobilised on CPG.[89] More recently, the same group was described an adapted procedure to transfer the idea to glass substrate. Firstly, a layer of gold was deposited onto CPG using Electroless from a solution of sodium gold sulphide. Then, the monofunctional metal ligand, 3-mercaptopropionic acid was attached to the gold surface through thiol linkage.[90]

1.3 Performing a SPE process

Classically, SPE experiments can be performed in batch mode using simple apparatus (such as beakers or vessels). In practice, SPE materials (e.g., chelating resin) are suspended with solution containing the targeted metals and left to equilibrate or shaken for certain time period, after which the sorbent beads are filtered and washed while on the filter, subsequently the retained species are desorbed with a minimum amount of a suitable eluting reagent. The applications of batch mode for the preparation of real samples are rather limited as it is a time consuming process and also samples are subjected to contamination from vessels and ambient environment. However, methods based on batch wise are useful in studying many of SPE materials characteristics such as capacity exchange and absorption isotherm.

In a typical SPE experiment the sorbent materials are packed into columns or cartridges where sample containing analyte species is passed through either under gravity or making use of hydrodynamic pumps. The column is then washed, the sequestered analyse are eluted and measured.

Since the concept of flow injection analysis (FIA), as first proposed by Ruzicka and Hansen in 1975[91], interest has been sustained in its application to the development of adequate automated on-line sample preparation protocols, to substitute the labour intensive manual operation techniques. The first sample preparations to be attempted using on-line manifold were based on dialysis and solvent extraction, followed by gas diffusion, on-line precipitation and electrodeposition.[92] The on-line solid phase extraction

(SPE) process using packed minicolumns integrated into flow injection manifolds was introduced in 1983.[93] Following this contribution, on-line SPE has became a fruitful research field which is attracting extensive interest, and has shown to be one which best demonstrates the dramatic effects of revolution of tradition sample preparation practice in the analytical laboratories.[94,95] The proper integration of SPE columns into FI systems offers many advantages including:

1) Simple matrix separation, 2) possible higher enrichment factors, 3) excellent reproducibility, 4) minimal sample manipulation and hence minimised sample contamination, 5) reduced amount of reagents and wastes, and 6) shortened analysis times. Commercial systems providing convenient and effective on-line SPE for trace elements from various matrices have been developed and marketed (e.g., Preplab available from TJA Solutions, Cheshire, UK).[96,97,98] Recently, standard sample preparation protocols for trace metals analysis based on an on-line SPE interfaced with atomic spectroscopic detectors, have been reported. For instance, the environmental protection agency (EPA) official method 200.10 for the analysis of trace elements in marine waters, including estuarine water, seawater, and brines, is based on an on-line SPE using an iminodiacetate functionalized chelating resin.[99]

1.4 Trends toward miniaturised analytical devices

Recently there has been an increased interest to miniaturise analytical instruments and apparatuses. This certainly contributed significantly in a massive reduction in the reagent consumption, saving valuble time and improving the precision of many analytical methods, particularly when automated. The miniaturisation of the SPE process for sample preparation in the analysis of trace metals (as many other separation science processes)

has been approached in three ways, namely, miniaturised columns, lab on chip and lab on valve.

1.4.1 Miniaturised columns

In theory, the production of miniaturised columns can be accomplished by packing small amounts of functionalised solid phase beads inside a short capillary. However, experiments show that it is difficult to obtain a reproducible homogenous packing. Fortunately, the recently emerged monolithic materials, which are characreised by their highly controlled porosity, have simplified this approach. It becomes more efficient to generate this new class of separation media as a monolithic porous material inside capillary columns. Once confined within the capillary, these porous materials can be post-modified (functionalised) with the desired reagents by many *in situ* immobilisation procedures. Monolithic microcolumns fabricated from silica exhibit ideal characteristics as good supports for the attachment of various bonded phases i.e., for capillary chromatography[100] and enzyme micro reactors.[101] It was also proved to be adequate supporting phase in the synthesis of chelating resin. In this context, Lofthouse *et al* [85] have adapted the long-established chemical transformation based on Hill[75] procedure to immobilise HQ *in situ* within the porous structure of monolithic silica (frit). The immobilised HQ-frit showed operational properties comparable with the properties of HQ-CPG for matrix elimination prior to ICP-MS protocol for the determination of trace metals in seawater.

The microcolumns of this type can be implemented within miniaturised flow injection manifolds constructed from the commercially available tubing and capillaries of small diameter. The technology was extensively investigated in chapter two.

1.4.2 Lab on a chip (LOC)

The increased demand for analytical science in many applications such as process analysis and continuous monitoring, calls for the automated analytical processes. Flow injection systems have contributed significantly to the simplification and enhancement of process analysis, as it enables analysts to hyphenate many analytical techniques flexibly, and to perform many procedures with excellent precision in an on-line mode. Automated analytical processes, in which sampling, sample transport and processing, any chemical derivative, separation, as well as detection are carried out, in an on-line manner with minimum or no interfere from the operator, have been recognised as totals analysis systems (TAS) since the early 1980s.[102] TAS has been proven to offer many potential advantages including, improved result accuracy, increased analytical throughput and decreased labour cost. However, the slow sample processing, lack of selectivity, and high reagent consumption, running cost and the amount of generated wastes, have remained issues of increased concern. Therefore it was proposed that many of these problems may be overcome through system miniaturisation. [103]

The first miniaturised analytical device appeared 25 years ago; it was a gas chromatographic analyser fabricated on silicon. Despite its rapid separation capabilities and minute size, this first silicon chip device did not become popular.[104] Ruzicka and Hansen[105] in 1984 used the concept of integrated electronic circuit and the fabricated gas analyser to construct miniaturised FIA systems into a single PVC block. The versatility of this μ-FIA was demonstrated for a number of FIA systems with integrated potentiometric or optical detectors. Gas-diffusion and ion-exchange also have integrated to function as

sample preparation units. The term 'micro TAS (μ-TAS)' was first used by Manz in the Transducers 89 conference.[106] A central objective for μ-TAS is the integration of the whole analytical process, i.e. the sequential operations like sampling, sample pre-treatment, analytical separation, chemical reaction, analyte detection, and data analysis would be performed in one analytical microdevice (thus the origin of expression: lab-on-chip originated).[103] The potential to replace large-scale conventional laboratory instrumentation with miniaturized and self-contained systems offers a variety of advantages including, reduced hardware costs, low reagent consumption, faster analysis speeds, and the capability of operating in a massively parallel scale in order to achieve high throughput.

The initial work in μ-TAS concentrated mainly on establishing the electrokinetic control of flow using electroosmosis, demonstrating fabricating devices [107,108,109] and most of the reported applications were dedicated to separation of simple species.[110, 111] It was quickly realised that these microfabricated systems could be used for a much wider range of applications than simple separations and that the real power of 'on chip' devices lies in the ability to design integrated devices. Evidently, the first examples of lab on chip were based on the applications of known concepts in separation science such as pre-or post column reaction, to improve separation or facilitate the detection.[112] However, much of the recent research endeavours have been oriented to improve other technical issues such a reliable interface for microchip with the real world[113] and device packaging. Such efforts aim to construct fully integrated lab on chip devices, in which all the essential functional components are microfabricated on a single chip.[114,115]

The continued growing interest in miniaturised devices extended the applications beyond μ-TAS to include other chemical processes such as chemical synthesis or biological functions, and hence the terms, lab-on-a-chip, microreactors and microfluidic devices became well recognized in the literature. Since 1998 there has been an exponential growth in the applications of fabricated microfluidic devices, based on lab on a chip technology, in many fields. This is evidenced from the rapidly increasing number of publications and the conferences appearing on the topic, and the high level of investment.[116] The current development and the new applications are well documented in many comprehensive reviews by leading groups in the field[117, 118, 119,120]

1.4.2.1 Microdevice microfabrication

Early microfluidic devices were fabricated from glass or quartz substrates because they are optically transparent and exhibit electroosmotic flow properties similar to those of fused silica. In the past five years or so, a variety of other materials have been successfully employed. These materials include Plexiglas, low temperature ceramics, and many polymers such as polyethyleneterephthalate and polyethylene, poly(dimethylsiloxane) (PDMS) and poly(methylmethacrylate) (PMMA). The generally applied processes for the fabrication of glass and polymeric devices will be summarised briefly in the following sections.

1.4.2.1a Fabrication of glass and silicon

The early microfluidic devices were fabricated from silicon following techniques well-established in the microelectronics industry. Glass has many attractive features that make it a preferable substrate for microchip fabrication including its excellent optical

properties, allowing ultrasensitive detection when optical methods are used for readout. in addition, the surface chemical properties are well understood and modification chemistries extensively documented.[121] More importantly glass can be microfabricated using the same technique as for silicon. Thus glass was the first suitable material to replace silicon in microfluidic devices.

The backbone of silicon glass fabrication is photolithography. Photolithography is basically an extension of photography. One first makes the equivalent of a photographic negative containing the pattern required for the microdivice's feature. This negative, which is called the photomask or master, is then used to copy the structure onto the substrate. Photolithography is the most successful technology in microfabrication. It has been the workhorse of the semiconductor industry since its invention in 1959[122]: Essentially, all integrated circuits are made by this technology.

The photolithographic techniques currently used for manufacturing microfluidic structures are based on a projection printing system (usually called a stepper) in which the image containing all geometrical design information is reduced and projected through a photomask onto a thin film of photosensitive material, called a resist, which has been coated onto the substrate. Where light strikes the resist it undergoes a photoreaction to become either more soluble or less soluble in a selected rinsing solution. The rinsed off parts of the resist leave patterns of exposed substrate which are accessible for etching.
The resolution R of the stepper is subject to the limitations set by optical diffraction according to the Rayleigh Equation (1.4)[122]

$$R = \frac{k_1 \lambda}{NA} \qquad (1.4)$$

where λ is the wavelength of the illuminating light, NA the numerical aperture of the lens system, and k_1 a constant that depends on the photoresist. Although the theoretical limit set by optical diffraction is usually about l/2, the minimum feature size that can be obtained is approximately the wavelength of the light used. As a result, radiating sources with shorter wavelengths such as x-ray or electron beam are progressively introduced into photolithography to generate structures with smaller feature sizes. The photoresist is developed, leaving the areas of the substrate exposed to light open to be microstructured. The defined pattern is then etched away using a wet or a dry etching process.

In wet etching, the substrate is immersed into an appropriate solution at optimum temperature. The geometry of the etched grooves depends on the etchant as well as the crystal orientation of the substrates. Glass substrates are typically etched with an isotropic wet etchant such HF/HNO_3 or HF/NH_4F, and obtained channels are usually V-shaped.

Dry etching can be carried out following various processes; mostly the cost is higher than with wet etching. HF is used in chemical vapour phase etching, and XeF_2 is used to etch silicon and create isotropic etches. However, the most flexible dry etching technology is reactive-ion etching.[123] This technique uses plasma gas to not only physically knock atoms off the surface but also chemically react with the surface. The features of the microchannel can be shaped by adjusting the process: chemical etching is isotropic (etches evenly in all directions, including parallel to the surface under the mask), and physical etching is anisotropic (etches only perpendicular to the surface). A special adaptation to the process, deep reactive-ion etching, allows the etching of vertical-walled

channels (with aspect ratios of 50 to 1) that can be hundreds of micrometers deep. Dry etching also can be effected by blasting a masked substrate with a high velocity powder beam, originating from pressurised nozzle. Using powder of particle size of 30 μm with a well aligned mask minimum feature of 50μm was obtainable.[124]

The fabricated glass or silicon substrates are bonded to another flat substrate from similar materials, typically by thermal annealing at (~600 °C) to enclose the fluidic channels. The cover plate can be made of an electrometric polymer such as PDMS, which can be mechanically clamped to effect reasonable sealing sufficient for many applications.

Glass and its microfabricating methods do present some limitations for microfluidic applications. For example, it is difficult to produce high-aspect-ratio (aspect ratio is the ratio of feature height to its lateral dimension) microstructures in glass due to the isotropic nature of the etching process. While this can be circumvented to a certain degree using reactive ion etching or anisotropic etching in single crystal Si,[125] these techniques can be prohibitively expensive and as it is a sequential process, the production rate of the microfluidic devices is reduced. Moreover, final assembly requires temperatures above 200°C, which precludes any type of chemical modification of the glass surface or inclusion of materials in the fluidic networks prior to assembly. In addition, the relatively expensive substrate materials make these devices unsuitable for disposable uses.

1.4.2.1b Fabrication of polymers

Polymer based microfluidic devices present attractive alternatives to glass counterpart in chemical and biochemical applications because of their lower material cost, wide range of

materials with different physical properties. In addition, their significantly lower annealing temperatures offers more flexibility in the choice of microfabricating techniques conducive to low cost production of devices,.[126,127]

The recent techniques for fabricating plastic and polymeric microfluidic devices have been extensively reviewed by Becker and Locascio.[128] Generally, it is possible to group these techniques into two broad categories: direct machining and replication from a master or stamp.[129] The direct writing of microchannels on plastic substrate is accomplished using controlled milling, X-ray lithography,[130,131] laser-ablation or reactive ion etching.[132] However, methods based on direct writing techniques are limited by their high cost and the long production cycle.

Polymer fabrication based on the known polymer replications offers the potential of low cost production once the infrastructure for the fabrication is exists. For example, a method like elastomer casting allows extremely fast prototyping without the need for sophisticated instrumentation, and is therefore ideal for academic and R&D applications. The first step in the replication microfabrication process of polymeric microdevices is the fabrication of a mould insert (stamp) possessing an inverse microfluidic structure. Most commonly the stamp is fabricated from silicon using lithographic technologies. The device structure is transferred to a silicon substrate coated with photosensitive layer and then etched as a positive structure using an appropriate wet etching solution. Alternatively, a fabricated silicon substrate may be used to construct a stamp in metal.[133] In this process, a metal electroform is produced (typically from Ni) using the etched

microfabricated silicon substrate as the stamp. The first metal electroform is the mirror image of the stamp. Then, a second metal electroform is created using the first electroform as a template. Silicon stamps can be used and are recommended for low aspect ratios. Silicon processing is applicable to fabricate a master from glass but the aspect ratio would be not as good as for silicon. Furthermore metal stamps, produced by milling, can hold geometries with high aspect ratios but they are fairly inexpensive and have high surface roughness.

In recent years, LIGA (from the German Lithographie, Galvanformung and Abformung, i.e., lithography electroplating and moulding) process[134,135], which is charatrised by its ability to produce detailed high aspect ratio structures has been applied to a growing list of metal alloys that could be enlisted as precise masters to fabricate polymeric microdevices. In the traditional LIGA process, X-rays bombard a photoresist on a silicon substrate, creating precise micro-cavities in the shape of the desired parts. The developed wafer is then placed in an electroplating bath, filling the cavities with nickel, copper, or other proper metals. The surfaces of the metal part are finished and the photoresist is dissolved, leaving the finished parts. Once the master is fabricated it can be used to replicate microfluidic devices using an appropriate polymers moulding process such as those in Table 1.1, depending on the type of polymer. The process can be repeated many times depending on the rigidity of the master.

The fabricated microfluidic devices are typically sealed with a cover plate to create closed capillaries, minimising evaporation during analytical applications of the device. Sealing of polymer microdevices is generally much simpler than with silicon or glass and can often be accomplished using low temperature thermal annealing[136]. The same

polymer substrate can be used to form the seal or alternatively, a polymer with a lower glass transition temperature can be used to ensure that there is no deformation of the microchannel during the sealing process[137]. Laser also can be applied to effect an efficient localised thermal bonding process; this process is well known as laser welding. Electrometric polymers such as PDMS have excellent adhesion to a wide variety of substrate materials and can be used to enclose microchannels with a non-permanent seal using simple clamp[138]. To form a permanent seal with PDMS, plasma oxidation of PDMS surfaces[139] has been described to bond the material to itself or to other substrates including glass, silicon, silicon oxide, quartz, silicon nitride, polyethylene, polystyrene, and glassy carbon. It was hypothesized that the permanentbond between two PDMS pieces is a result of a condensation reaction to form a covalent siloxane bond.[140]

Table 1.1 Some moulding processes for polymer replication.

Injection moulding	A moulding process whereby a heat softened plastic resin is forced from a cylinder at high pressure into a cavity containing a stamp that gives the desired structure.
Hot Embossing	A technique in which a mould (stamp) of specific pattern is pressed into a heated, softened thermoplastic film or sheet, followed by a cooling, thereby producing an inverted replica of the mould.
Casting Moulding	In these methods plastic components, typically base and hardener or cure are mixed, and poured in the mould. Heat or UV radiation may apply to speed curing. A typical example is PDMS.
Compression Moulding	A moulding technique, in which a moulding compound, such as heat softened plastic resin, is introduced into an open mould and formed under heat and pressure.

1.4.2.2 Sample preparation on microdevices

Despite the steadily increasing interest, among the scientific community, to downscale analytical systems to microchip level, integration of sample preparation with the rest of the analytical process however still presents a significant challenge that precludes the achievement of a true μ-TAS. In a recent comprehensive review highlighting the state of the art in sample pretreatment on microfabricated devices, Verpoorte and co-workers relate this challenge to two main issues:[141] The first issue to be considered is that it is not always easy to move nanolitre samples around in a branched microfluidic device, an essentially open system in which solutions of different composition must be localised to different areas of the chip. Hence, integration of different sample preparation steps is not just a matter of joining microchannels on a device. It is still more practical to perform some of the sample preparation steps off-chip in many cases. The other issue standing in the way of real μ-TAS is the highly complex nature of many of the samples that need to be analysed. So far, the samples dealt with in microdevices have been quite pure for the most part, having undergone some form of manual sample preparation primarily to avoid clogging of the microchannels.

An inspection of the published work in the area reveals a variety of examples for chip based sample preparation, and it can be realised easily that there is no one universal approach for any sample. This is, however, to be expected since sample preparation protocols have always had to be individually tailored to the type of sample under consideration and the analytical method chosen. Analogous to conventional analytical methods (e.g., TAS), there are however four areas in which research has been focused in

order to achieve an integrated sample preparation for chip-based techniques (μ-TAS): [141, 7] (a) separation of sample from sample matrix, (b) sample preconcentration, (c) derivatisation and (d) biochemical sample preparation. To stay within the theme of this thesis, the discussion in the following paragraphs will be focused mainly on sample preconcentration, particularly the methods that are based on SPE.

Sample preconcentration is an essential operation commonly required for the determination of trace amounts of analytes of interest for which the concentration in the original sample solution is below the detection limit of the chosen detector. This is even more important in the case of microfluic devices given the very small volumes of samples that can be handled within the microchip. Among sample preparation techniques enabling the preconcentration of analyte species, sample stacking[142,143] and isotachoporesis,[144,145,146] have been successfully used in capillary electrophoresis, however, these approaches are only practical for electrodriven systems. In contrast, SPE is a more general method since it enables handling of large sample volumes regardless of the method used for sample flow. In addition to the significant increase in concentration of samples, SPE can be made selective for particular (groups of) analytes.

Three main approaches for on-chip SPE appear in the literature, using either immobilised stationary phases on channel walls, channels packed with coated beads or channels filled with polymeric monoliths. The first method has been more prominent to date, due to its simplicity and compatibility with standard microdevices. The first application of this approach was reported by Kutter *et al.*[147] They demonstrated SPE for neutral coumarin

dye, C460 on microchannels coated with C18. The process showed preconcentration enhancement up to 80 fold, with 160 s sampling time. The significant drawback of this approach is the limited surface area of the microchannel and the difficulty in controlling the length of channel to be coated. However, only a few reports have dealt with the attempts to enhance the limited surface area in channels of microchip. For example, Regnier and co-workers[148,149] fabricated microchip channels containing arrays of ordered tetragonal posts. Ramsey and co-workers incorporated a porous silicate membrane into the microchip. Using this device, electrokinetic preconcentration of DNA from dilute samples was demonstrated.[150]

Placing beads inside microchannel is perhaps the simplest approach to enhance surface area. The use of packed channels is particularly diverse, and most of the reported methods to incorporate beads inside microchannels have been reviewed recently.[151,152] To successfully employ bead in microchannels, a proper means to keep the beads within the channel and not flushed out during operation, is essential. There are currently two methods for restraining beads within a microfluidic device. The first method is similar to the classical method of preparing a chromatography column in which the beads are trapped within a chamber on the microfluidic device. The other method of placing beads within a device involves immobilising beads to the surface, typically using self assembly methods.

Placing bead inside channels making use of the conventional approaches is limited by the technical difficulties associated with the fabrication of frits, similar to that used in capillary electrochromatography (CEC) columns, to retain beads within microfluidic

chip. As an alternative, however, the geometry of the chip is designed to constrain beads within a defined section of the microfluidic channel. In this context, Andersson *et al.* first demonstrated that a series of pillars could constrain beads placed in a chip.[153] The pillars are essentially a microfabricated frit, and were also shown to allow a bead chamber to be placed within a larger cavity. The applicability of this approach has been demonstrated for making devices for single nucleotide polymorphisms (SNP) analysis[154], CEC and solid phase extraction.[155] A similar approach to retain beads into microchannels within microfluidic devices has been to restrict height of the microchannel from 100 μm to 10 μm essentially forming a ''dam'' as demonstrated by Sato *et al.*[156,157] Beads can then be packed into the chamber in a relatively easy procedure. In addition, the beads can subsequently be modified to have various surface chemistries amenable to analytical operations, such as attaching antibodies. It is also possible to fabricate a chamber within a microchip in which the beads are packed from one direction and the analytical flow is applied in another direction (typically orthogonally). Oleschuk *et al.* have used similar approach to prepare systems to perform electrochromatography and solid phase extraction on a microchip.[158] The system feasibility was tested by SPE of 1nM BODIPY dye. Preconcentration factors of 80 up to 500 could be achieved. The same principle used by Wang *et al.* to pack a fabricated large chamber on a chip with 40–50 μm beads.[159] Li *et al.* developed a simple approach making use of a mixed bed (5 μm and 40 μm of beads) to realise the packing of smaller beads into a specific microchannel within microchip.[160] A very practical approach has been introduced by Ceriotti *et al.*, who use a tapered channel in order to trap beads within a microfluidic channel.[161] The tapered

channel allows small beads (3 μm) to be packed into the channel, which was proved to be useful for CEC applications.

The other approaches by which beads are stabilised in microchannels have been introduced for the first time by Andersson *et al.*[162] This method involves modifying the surface of the microchip using a microcontact printing method. The beads are then placed within the microfluidic chip and the beads self-assemble as a monolayer in the chip. The self assembled beads are stable within the chip channels with a flow of water within the channels. Functionalised beads have also been assembled on surfaces using hydrophobic/hydrophilic interactions.[163] Another approach to achieve a self-assembled bead system enabling selective release was recently introduced by Malmstadt *et al.*[164] in this method beads modified with poly (N-isopropylacrylamide) are used. This polymer is temperature sensitive, undergoing a hydrophilic–hydrophobic phase transition at temperatures higher than the lower critical solution temperature. The beads are collected in the channel at an elevated temperature and the beads aggregate on the sides of the channel (eventually greatly filling the channel). The beads can then be labelled within the chip, when the temperature is lowered the beads will then be released from the channel to be detected.

Beads are also utilised as suspensions rather than packed beds. This approach has an advantage from the perspective of analyte transport and delivery to the reactive surface when compared to stationary surfaces. They can be moved through solution

macroscopically using electric fields, pressure-driven flow, gravity, or simply through agitation. [151]

The recently developed monolithic materials show potential advantages as solid phase candidates for microchip based systems. Recently, Yu *et al.*[165] successfully used photo-initiated polymerisation to prepare monolithic porous polymers *in situ* within the channel of microfluidic devices. The preparation of the monolithic material with hydrophobic and ionisable surface chemistries is easily achieved by copolymerisation of butylethyl methacrylate and [2-(methacryloyloxy)ethyl]trimethylammonium chloride with ethelyne dimethacrylate, respectively. The porosity is controlled by the use of a mixture of hexane and methanol as a progenic mixture. This polymerisation approach enables a single step preparation of monolithic materials with wide variety of chemistries and porosities at the desired location. The function of the monolithc preconcentration device was demonstrated using very dilute solution of Coumarin 519, tetrapeptide and green fluorescent protein. The latter was concentrated by a factor of 10^3.

1.4.3 Lab on a valve (LOV)

The newly introduced sequential injection systems with lab on valve (SI-LOV) have been shown to be promising for sample and reagents manipulation. In this approach, the conventional sequential injection (SI) system is miniaturised by integrating the sampling conduit (connectors, microcolumns, sample flow through ports and mixing devices) into a micromachined monolithic structure mounted onto a top of a multiposition valve.[166] Although SI-LOV is an established technique for miniaturised solution chemistry, it exhibits great potential to handle more complicated on-line sample manipulations. In SPE it offers the possibility to use renewable beads, thus the deterioration of reagent will not

be a problem. Many of the recent applications for this technology in sample preparation during the analysis of trace metals and its coupling with atomic spectroscopic instruments (e.g., IC-MS, ETAAS) have been extensively reviewed recently by Wang and Hansen[167] and Economou.[168]

1.5 Detection

As mentioned earlier (in section 1.1 in this chapter), the SPE process has been used for sample preparation in trace analysis of metals to effect their extraction, preconcentration or matrices simplification etc. Sample preparation by SPE is useful with all metal detection, however, most of the applications encountered in the literature, use atomic spectroscopy techniques perhaps because of their excellent selectivity. The low cost and simplicity of optical spectroscopic techniques in the UV-Vis range, particularly with a suitable derivative reaction for ions with colorimetric reagents make them attractive monitoring method. Most frequently this detection mode is employed with electrophoresis/chromatographic separation or flow injection. However, the latter is limited by the availability of the selective reagent (e.g., Ferrozine for Fe (II)). Practically, with traditional FIA manifolds the measurement is performed simply employing a normal flow cell of a standard spectrometer, nevertheless, with miniaturised microfluidic devices and perhaps with some flowing systems, it is a common practice to use fibre optics for optical coupling. Optosensing in which the analye is measured while on the SPE beads is an ideal approach in order to avoid dilution resulted from sample dispersion. Electrochemistry based techniques, particularly amperometry, has been reported mainly with chromatographic separation or to a less extent with flow injection. Chemiluminescence and fluorescence have proved to offer a sensitive detection at trace

level, however, only a few metals (e.g., metals that catalyse luminol reaction such as Fe, Mn and Co), can be monitored.

In this thesis four detectors have been used, namely optical spectrometry in the visible range using colorimetric regents, atomic spectroscopy employing ICP-OES and ICP-MS and direct current DC amperometry (electrochemical).

The essential underlying principals of these detection techniques will be summarised briefly in the following sections.

1.5.1 Optical spectroscopy

Optical spectroscopy in the UV-Vis region has been the mostly applied method for inorganic analysis prior to 1980s. At present the significance of this technique is significantly decreased in favour of instrumental methods (i.e., atomic spectrometric), particularly for routine, trace and automated analysis. The most sensitive spectrophotometric methods are based on complexes, chelates or ion-pairs (ion-association complexes) of metals or non-metals, especially with dye (colorimetric reagent). [169] In general, these procedures are of limited selectivity and need prior separation of the analyte or sophisticated masking agent.

1.5.1.1 Theoretical concept of optical spectroscopy in metal complexes

Light absorption by molecular species or metal complex in general takes place when electromagnetic radiation (e.g., in UV-Vis rage) causes bonding electrons to move from their ground state to an excited state (anti bonding). In metal-complex there are three

types of transitions giving rise to UV-Vis spectra that can be identified, although it may not be possible to distinguish between them clearly; these transitions are:[16,170]

1. Excitations within transition metal ions, generally associated within states of a d^n configuration, and referred to d-d transitions. Similarly, in the rare earth, f-f transitions can occur.
2. Excitations within the ligand
3. Charge transfer transitions, involving the transfer of electrons from being mainly on the ligand to being mainly on the metal, or vice versa.

Excitation due to d-d and f-f transitions in metal complexes provide bands in visible, the near ultraviolet, or the near infrared regions, with molar extinction coefficients (ε) ranging from 0.1 to 100, and are responsible for the colours of most of the complexes of these metals. Although they are the best understood spectra of metal complexes, their intensities are too low for analytical applications.

Electron transfer within the ligand results in absorption bands of very important applications in the determination of metals, especially, for the complexes of non transition elements. They are very intense, corresponding to π-π^* or n-π^* transitions and depend on the nature of bonds in the complex. If the bond between a metal ion and ligand is essentially electrostatic, any metal ion will influence the spectrum of the ligand similarly as does its dissociation, and the shift in absorption maximum (λ max) in the complexation corresponds to the shift in λ max in the dissociation scheme of the ligand. The complexation does not much affect the shape of band or ε value.

The absorption band originate from charge transfer are formed by the electron transfer from ligand to metal (L-M) or from metal to legand (M-L). They are usually very strong (ε ~10^2-10^4) and have particular importance in analytical applications. L-M charge transfer in the complexes of transition metals, are often associated with the transition of electrons from a σ bonding orbital or π orbital within the ligand to the intibonding t_{2g} and e_g in metal ion in a higher oxidation state. M-L charge transitions take place when electrons are excited from t_{2g} orbital within metal ion in the lower oxidation to ligand contains the π electron system. Of analytical importance are the tris complexes of 2,2`-bipyridyl,1,10-phenanthroline, and 2,4,6-tripyridyl-1,3,5- trizine regents, which have a distinguished selectivity for Fe(II).

1.5.2 Inductively coupled optical emission and inductively coupled plasma mass spectrometry

Inductively coupled optical emission (ICP-OES) and inductively couple plasma mass spectrometry (ICP–MS) have been established, among the various analytical techniques, as the most powerful that are widely used for trace element determinations. They are characterised by (a) multielement capability, (b) high sample throughput, (c) low detection limits of the order of 1-100 ng ml^{-1} for ICP-OES and 1-10 pg ml^{-1} for IC-MS (i.e., quadrupole instruments), (d) wide linear dynamic ranges (up to 8 order of magnitude) because of its optical thinness; (e) few chemical interference compared with other atomic emission sources (such as flames) because of the high temperature that atomise completely most of samples and, (f) high selectivity and the capability for isotope analysis in ICP-MS.

These techniques have attracted the interest of many researchers in the field of spectroscopy and analytical chemistry and at present they are well established for research and routine trace element determinations in many fields. For example, isotope dilution ICP-MS is indispensable for the study of the formation of methyl mercury in the environment and its accumulation in organisms.

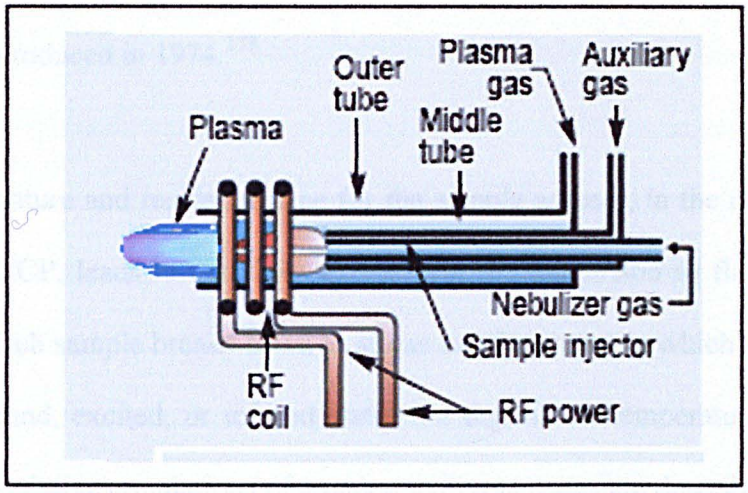
1.5.2.1 Inductively coupled plasma (ICP)

Plasma is defined as "*any luminous volume of gas having a fraction of its atoms or molecules ionised*".[171] An inductively coupled plasma (ICP) [172], is an electrical discharge usually sustained at atmospheric pressure in a noble gas in which high thermal energy is supplied by electrical current, which is induced by fluctuating magnetic fields.

Usually ICPs are generated in a torch which consists of three concentric tubes with a maximum outer diameter of 30 mm. A part of the torch is surrounded by an induction coil transmitting a 40 or 27 MHz RF power to induce a fluctuating magnetic field inside the end of the torch. Three different gas flows of a noble gas, usually argon, are directed through different compartments of the torch (see figure 1.4). During ignition, electrons are 'seeded' inside the torch from an external spark/discharge such as a Tesla coil. The fluctuating magnetic field induces an electrical current, known as eddy current, by accelerating ions and electrons in a closed annular path. The eddy region (induction zone) is thereby heated to a high temperature, up to 10,000 K, because of ohmic resistance.[173, 174] Once the ionising temperature of the noble gas is reached, the ICP is selfsustained by maintaining a partially ionised support gas. To prevent the outer tube of the torch from overheating, it is cooled by a tangentially introduced high gas flow. A sample in the form

of a gas, fine droplets or solid particles is transported by a nebuliser gas *via* a narrow injector tube to a central channel surrounded by the annular shaped induction region. There the sample undergoes desolvation, vaporisation, atomisation and ionisation. Normally, the energy is transferred from the induction zone to the central channel by conduction, convection, and radiation. Because the sample is normally confined within the central channel through the skin effect, interference effects of aerosols or sample constituents in the energy coupling between the load coil and the induction region are minimised. Thus, the fundamental plasma properties are only slightly altered when the sample composition introduced is changed within defined limits.

(A)



(B)

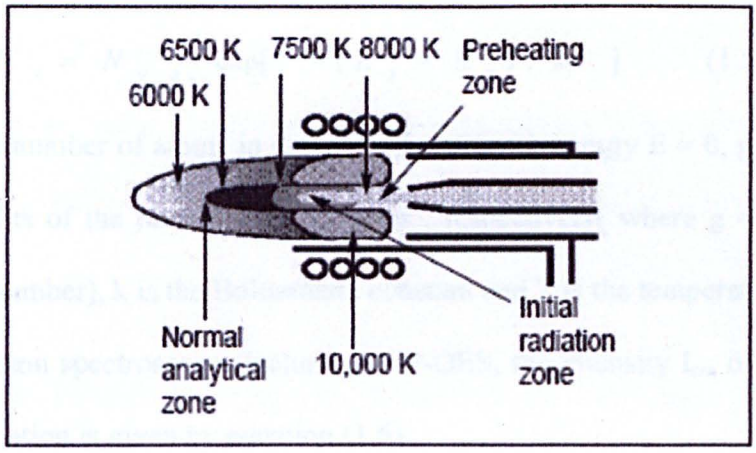


Figure 1.4 A) Detailed view of Plasma torch showing the main components; B) The different temperature zones in plasma. [175]

1.5.2.2 Fundamentals of ICP-OES

The first ICP appeared in 1961 when Reed wrapped an induction coil around a flowing stream of argon, forming an electric arc without the need for electrodes. The unique potentials of the ICP as a high temperature atomisation/excitation source for spectrochemical analysis were first recognised by Greenfield *et al* [176] in England and

Fassel and Wendt at Iowa State University[177] in 64/1965. The ICP-OES instrument was commercially introduced in 1974.[178]

The high temperature and residence time for the sample aerosol, in the chemically inert environment of ICP, leads to complete atomisation in comparison to flame. Generally, the degree to which sample breaks down to atoms and the extent to which a given atom is found in its ground, excited, or ionised states are depend on temperature. At thermal equilibrium, the relative populations of atoms at different states are described using Boltizmann distribution (equation (1.5)) [179]

$$N_j = N_o \frac{g_j}{g_o} \exp[\ -(E_j - E_o) / kT\ ] \qquad (1.5)$$

where N_o is the number of atoms in the ground state with energy E = 0, g_j and g_o are the statistical weights of the jth and ground states , respectively(where g = 2J+1, J is the third quantum number), k is the Boltizmann constant and T is the temperature.

In atomic emission spectroscopy, including ICP-OES, the intensity I_{em} of a spontaneous emission of radiation is given by equation (1.6)

$$I_{em} = A_{ji} h\nu_{ji} N_j \qquad (1.6)$$

where A_{ji} is the transition probability for spontaneous emission , h is Plank's constant, ν_{ji} is the frequency of radiation and N_j the number of atoms in the exited state. For a system in thermodynamic equilibrium, and if self-absorption is neglected substituted equation (1.6) in (1.5) ,I*em* will becomes.

$$I_{em} = A_{ji} h\nu_{ji} N \frac{g_j}{g_o} \exp[\ -(E_j / kT\ )] \qquad (1.7)$$

Equation (1.7) shows the critical effect of temperature on the intensity emission. Therefore, the high temperature (6000-7000K) sustained in ICP makes it preferable source for atomic emission spectroscopy. When sample reach this temperature it exists as exited atoms and ions representing its original elemental composition. The excitation of the outer electron of a ground state atom to produce wave length-specific photon of light is the fundamental basis of atomic emission. The selectivity in OES, as well as other atomic emissions spectroscopic techniques, is based on the fact that the excited atom or ion releases energy as light of characteristic wavelengths during reversion to the ground state, the positions and intensities of which can be measured. The energy transfer for electrons when they fall back to ground state is unique to each element as it depends upon the electronic configuration of the orbital. The energy transfer is inversely proportional to the wavelength of electromagnetic radiation as in equation (1.8),

$$E = \frac{hc}{\lambda} \qquad (1.8)$$

where h is Planck's constant, c the velocity of light and λ is wavelength, and hence the wavelength of light emitted is also unique.

The wavelengths used in OES ranges from the upper part of the vacuum ultraviolet (160 nm) to the limit of visible light (800 nm). As borosilicate glass absorbs light below 310 nm and oxygen in air absorbs light below 200 nm, optical lenses and prisms are generally fabricated from quartz glass and optical paths are evacuated or filled by a non absorbing gas such as argon.

The optical system (spectrometers) used for ICP-OES consists of a monochromator system to separate the individual wavelength and focus the desired wavelength onto the

detector. The older systems used two types of spectrometers to measure the intensity of light emitted by the analyte ions or atoms: the sequential and the simultaneous multi channel instruments. The simultaneous multichannel has a photomultipllier tubes set up at preselected wavelengths. It is capable of determining simultaneously a number of preselected elements but does not permit a choice of wavelength. The sequential spectrometer has a scanning monochromator and measures one element after another. Therefore, it requires more time for the analysis but it offers an unrestricted choice of wavelengths.[180] In modern instruments, solid-state detectors based on charge coupled devices (CCD) are used, providing very flexible systems and eliminating the need for numbers of single photomultiplier detectors. The data in both types of the instruments is handled by a dedicated computer system. In the sequential instrument the computer has to do extra work as it is also responsible for controlling the wavelength drive of the spectrometer.

1.5.2.3 Fundamentals of ICP-MS

The application of inorganic mass spectroscopy in the analytical chemistry was started in 1950s. The ions' source was RF spark.[180] The ability of ICP as source for ions was observed in 1975.[181] The first investigations of the properties of the ICP for producing charged particles for mass spectrometer were in 1981, and the first ICP-MS was built in 1983. Since then, ICP-MS has grown to be one of the most important techniques for the elemental analysis.[182,175]

ICP-MS theory is based on the fact that, the high energy in the ICP is sufficient to remove an electron from its outer orbital to generate an ion. It is the generation,

transportation, and detection of these ions that give ICP-MS its characteristic ultra trace capability. For systems existing in thermal equilibrium, the degree of ionisation of an atom is given by the Saha equation (1.9) [179]

$$\frac{n_i n_e}{n_a} = 2\frac{Z_i}{Z_a}(2\pi mk\ \frac{T}{h^2})^{2/3}\exp(\ -E_i / kT\ ) \qquad (1.9)$$

where n_i, n_e and n_a are the number densities of the ions, free electrons and atoms , respectively , Z_i and Z_a are the ionc and atomic partition functions respectively, m is the electron mass, k is the Boltizman constant, T is the temperature ; h is Plank's constant and E_i is the first ionisation energy. Ionisation is effected by ion-atom and atom-atom collisions, where the energy required for ionisation is derived from thermal agitation of the particles. The extent to which atom is ionised is dependent on the electron density, the temperature and ionisation energy of element in question. For argon ICP in which the average electron number density around 4×10^{15} cm^{-1} and the ionisation temperature reach 8730 K, the degree of ionisation as a function of first ionisation energy, predicted by the Saha equation. Apparently, most of the elements in the Periodic Table have first ionisation energies less than 9 eV and thus over 80 % are ionised in ICP. The remaining third are ionised to a lesser extent depending on their first ionisation energy, with the most poorly ionised element being He, Ne, F, O, N< 1% ionised, Kr, Cl 1% to 10% , C, Br, Xe, S 10% to 30% and P, I, Hg, As, Au, Pt 30% to 80% . Such thermal ionisation is probably the most dominant ionisation mechanism in ICP. However, ionisation may result via electron impact when electron of high kinetic energy is formed, and also because of charge transfer expected between atoms and ions.[180]

A major problem in the coupling technology is the fact that ICP operates at atmospheric pressure while the mass spectrometer requires a high vacuum. However, this problem can be tackled using certain type of interfaces. The ICP-MS systems employ mass spectrometer as a mass filter, transmitting ions with a preselected mass/charge ratio, most commonly, quadrupole or double-focusing high-resolution are used. The transmitted (filtered) ions are then detected with a channel electron multiplier. This detector (usually used with quadrupole instruments) operates in a similar way as in photomultiplier, apart from the absence of dynodes. In addition it requires vacuum conditions.

1.5.3 Electroanalytical techniques

Electroanalytical techniques have a long history in metal analysis; more than 200 years have passed since the first example of quantitative electrochemical analysis (for copper) was performed by Cruikshanks.[183] There are three basic modes of electrochemical detection used in metals detection, namely, conductimetry, potentimetry, and voltammetery.

Potentiometery is based on the measurement of cell potential in the absence of applied current (zero current condition); ion concentration can be measured directly from the potential an ion-selective membrane. The selectivity to a particular ion is established by the use of a proper ion selective membrane. More often than not, membranes are made of glass, sparingly soluble inorganic salts such as LaF_3 crystal or $Ag_2S/AgCl$ powder, polymer immobilised ionophore membranes incorporating complexing agent or ion exchange, and gel-immobilised and chemically bonded enzyme membrane for enzyme catalytically reaction.[184] In fact, apart from glass membrane which used in the pH meter,

no perfect ion selective electrode exists, thus the technique has a limited application in the analysis of trace metals as there are only a few ions (e.g., Ag, Cd, Pb) that can be monitored with rational selectivity.[185]

Conductimetry is a simple method based on the measurement of solution (elyctrolyte) conductance. Conductance is a measure of the ability of a solution to carry current and depends on the concentration, ion mobility, charge of species in solution, and temperature. Conductimetry is not selective, however, it can be useful in ion chromatography to monitor ions i.e., alkalis and alkaline earth metals after being separated, or for use in situation where it is necessary to ascertain for example, whether the total ion concentration is below a certain maximum level. [186]

Voltammetry comprises a group of electroanalytical methods in which information about analyte is obtained from the measurement of current as a function of variable potential applied between a working and reference electrodes in an electrochemical cell containing a high concentration of conductive solution or supporting electrolyte. Polarography with dropping mercury electrode, which has been an important tool for inorganic ions and certain organic species for many years, is a subset of voltammetery. In trace analysis, however, stripping voltammetery is the most useful because it is possible to preconcentrate analytes and identify them based on their characteristic stripping potential. Perhaps the simplest voltammeteric experiment is that in which the current is measured at fixed working potential; **amperometry**. This working potential is usually determined from a plot of current-potential (voltammogram).[186] As the analye species approaches the working electrode (WE), electrons are catalytically transferred from the

analyte to the WE (oxidation) or to the analye from the WE (reduction). The direction of flow of electrons is dependent upon the properties of analye and can be controlled by electric potential applied to the WE. The charge transfer is measured in current. Some extent of selectivity may be obtained by judicious selection for the applied potential to the electrode by the potentiostat. Moreover, selectivity may be enhanced by modifying the working electrode surface with specific reagents.

1.5.3.1 Theory of amperomeric measurement

The theoretical concept of amperomeric measurement is ultimately based on Faraday's law (equation 1.10): [186,187]

$$Q = nFN \qquad (1.10)$$

where Q is the number of coulombs used in converting, N mole of materials , n is the number of electron equivalents lost or gained in the transfer process per mole of material, and F is Faraday's constant (96,500 C equiv^{-1}). Differential of equation (1.10) with respect to time yields current (I), which is the measure of the rate at which material is converted.

$$\frac{dQ}{dt} = I = nFA\,\frac{dN}{dt} \qquad (1.11)$$

when the amperometric detector is set at a sufficient potential to oxidise or reduce all species once they reach the electrode, current would depend on mass transport. This is determined by diffusion coefficient, D and by the concentration gradient, the change in concentraton with distance (x) evaluated at the electrode surface (x = 0):

$$\frac{dc_{x,t}}{dx_{x=0}} \qquad (1.12)$$

The rate of mass transport (mol cm^{-2} s^{-1}) is given by

$$\frac{dN}{dt} = -D\left(\frac{dc_{x,t}}{dx}\right)_{x=0} \qquad (1.13)$$

or in terms of the current response,

$$i = -nFAD\left(\frac{dc}{dx}\right)_{x=0} \qquad (1.14)$$

Under constant flow or controlled hydrodynamic conditions the concentration gradient is constant because the diffusion layer, δ is non-varying:

$$I = nFAD\ \frac{c_o}{\delta} \qquad (1.15)$$

where c_o is the unperturbed concentration of reactant. Equation (1.15) relates a measurable quantity, the current to the concentration of analyte passing the electrode. Flow-through mode has the advantage of enhanced S/N ratio and thus, the detection limit is improved through the increased mass transfer rates. It is called the hydrodynamic approach for improving the S/N ratio. An increase in the flow rate increases the signal (faradaic current), while the background current changes less as its main component is the charging current, which is flow-independent.[188]

Over many years amperometry has gained a recognised usefulness as a monitoring tool in liquid chromatography and flow injection technique. The simplicity and flexibility to fabricate miniaturised electrochemical cells makes it appropriates for the microchip format because microelectrodes can be fabricated using many of the same photolithographic techniques that are already used to construct microchips. [189,190]

1.6 Thesis aims and objectives

The work in this thesis has three fundamental objectives concerning various aspects of miniaturised solid phase extraction systems for sample preparation in the analysis of trace metals. The aims of the investigated areas, however, are in harmony with the recent research trends. Firstly, the work aims to develop a rapid and green chemistry compatible chemical synthetic procedure for the immobilisation of the reactive chelating reagents, oxines, onto silica surfaces. Also, the possibility of improving resin selectivity, for certain transition metal ions, via the use of masking agent has to be taken into account. Secondly, it intends to examine the feasibility to synthesis silica porous monolithic materials making use of low cost materials and a simple gelation process, and then demonstrates the application of these materials as new solid phase formats instead of the long-established packed beads in the production of miniaturised SPE apparatus (e.g. micro columns). Finally, it further points towards the use of the newly emerged lab on chip technology to downscale SPE tools to the level of microchip based devices to function as an integral part of microfluidic device. Thus, the fabrication of true micro total analytical systems (μTAS) would be realised.

Chapter 2

A rapid immobilisation for 8-hydroxyquinoline onto silica materials and its application for on-line solid phase extraction of transition metals

Chapter 2 – A rapid immobilisation for 8-hydroxyquinoline onto silica materials and its application for on-line solid phase extraction of transition metals

2.0 Aims

This chapter aims to highlight a rapid immobilisation procedure, compatible with the new trends twards green chemistry, to anchor 8-HQ onto silica surfaces via its silylation with aromatic aminosilane, aminophenoxypropyltrimethoxsilane, followed by direct diazotisation. Then, it intends to examine the applicability of this product, which has an analogous structure to that synthesised according to the common procedure, for on-line preconcentration/ matrix elimination of trace metal from sediment extract prior to their detection and quantification using inductively coupled plasma optical spectroscopy (ICP-OES). Finally it addresses the effect of major transition metals (e.g., Fe, Al and Mn) on the solid phase extraction process and investigates the effectiveness of the suggested protocols in minimising such effects by determination of trace elements in sediment reference materials.

2.1 Introduction

8-hydroxyquinoline (oxine), which has the chemical structure represented in Figure 2.1, behaves as a bidentate (N, O) univalent anionic ligand with high tendency to form uncharged chelates with a wide range of metal ions. When the coordination number of metal is greater than 2n, the uncoordinated sites of the metal ion are often occupied by water.[191]

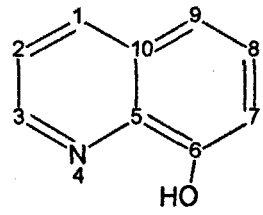


Figure 2.1 Chemical structure of 8-hydroxyquinoline.

Since the introduction of oxine as an analytical reagent by Berg in 1927[192], it has been accepted as one of the most common organic reagents for metal analysis. It has been extensively employed as a precipitating, extracting and photometric reagent for a wide variety of metal ions other than univalent cations.[191] By proper control of the pH of the solution or oxine concentration or by using masking reagents, a greater degree of selectivity for group of ions can be achieved.

Recently, Ohashi *et al.* [193] highlighted the function of oxin and its derivatives as valuable reagents in analytical chemistry with particular attention given to their applications in liquid-liquid extraction.

With the shift toward solid phase extraction (SPE) during the past years, oxines have been the most extensively employed chelating reagents in the preconcentration and separation of trace metals from various matrices, particularly in flow injection where sample preparation is carried out on-line. Good examples emphasising the new applications are well represented in two recent comprehensive reviews by Prasada *et al.*.[194,195]

As stated in chapter one, SPE for metal ions is usually carried out using one of two processes. In the first approach, metal ions are allowed to react with specific chelating agents, either in batch or in a flowing stream, to form a metal-chelator complex, which is subsequently captured onto appropriate solid materials such as C18, Amberlite XAD, activated carbon, activated alumina and PTFE knotted reactors etc. In the other approach, the chelating reagents are attached onto suitable solid materials and metal ions are trapped as complexes onto the immobilised chelating agent. The reagent can be attached either by physical impregnation or by covalent chemical bonding. Chemical bonding of a chelating reagent with a desired functionality offers a unique advantage since detachment of the grafted agent is prevented due to the strong covalent bonding of the molecule to the substrate. Therefore it is possible to reuse an immobilised reagent many times.

Oxine and its derivatives are among the first chelating reagents that were successfully immobilised. There is an enormous quantity of literature on the chemical attachment of these reagents onto a variety of supports. There are, however, only three types of supports, frequently encountered in the literature. These are naturally occurring polymers (mainly celloluse), organic polymers and inorganic polymers (mainly silica). Some examples demonstrating the applications of these supports, alongside the chemical transformations that are mostly utilised in the synthesis process, are presented in the following paragraphs. It is noteworthy that most of the reported transformations involve an initial surface activation with an amino group.

Bauman *et al.*[196] described a procedure for the diazo coupling of hydroxyquinoline and dithizone to benzidine carboxymethylcellulose. These materials were used to recover trace metals from seawater with high efficiency. Csanay *et al* [197] coupled 8-hydroxy-quinoline-5-sulphonic (sulphoxine) acid to cellulose by converting carboxymethyl cellulose into aminocellulose followed by Mannich reaction with formaldehyde. A similar procedure was utilised for the covalent attachment of sulphoxine to chlorodeoxy and ethylenediamine-cellulose. The product was used in a FI-GFAAS system to preconcentrate trace metals.[198] 8-Hydroxyquinoline (8-HQ) immobilised onto polystyrene-divinyl benzene (DVB) was synthesised by Vernon *at al* [199] and investigated by many workers. [200, 201] Other vinyl polymer materials (e.g., Fractogel TSK) have also been used. [202,203] The synthesis process involves an attachment of nitrobenzoyl groups, followed by reduction of the nitro group, which was then diazotized to allow oxines coupling. Another route for the addition of nitro groups is the direct nitration of the phenyl ring using a mixture of concentrated nitric and sulphuric acids.[204] A novel simplified procedure for producing 8-HQ covalently bonded to a vinylpolymer agglomerate resin via an amino link between the resin and the 8-HQ functional group was described by Landing and co-workers.[205] This linkage was reported to be more stable to acid or base hydrolysis than the ester linkage employed in their original method [202], and greatly reduce the bleeding of the 8-HQ functional groups from the resin. The new resin was used to extract trace metals from natural water. Recently, 8-HQ has been successfully immobilised onto polyacrylonitrile (PAN) hollow fibre membrane and used to preconcentrate rare earth elements (REEs) metals from seawater prior to their detection using ICP-MS. The immobilisation involved two steps. Firstly, PAN was reacted with

hydrazine at 90-94 °C, and then 8-HQ was coupled via diazotization.[206] Reagents were immobilised onto cellulose and polymeric supports, although they exhibited an acceptable exchange capacity and capability to extract metal from solutions over a definite pH range. However, it has been proven that these materials suffer from many serious limitations, such as slow kinetic exchange, swelling and shrinking, and lack of mechanical stability in dynamic systems. Cellulose and its derivatives have the additional problem of degradation due to microbial action.

Silica gel and glass materials are known to be almost free from the above limitations. In addition they have a reactive surface that can be activated easily. Most frequently, the surface activation prior to the attachment of oxins is carried out using amino silane. Several chemical transformations have been used to achieve the attachment of oxins on the amenated silica, including; (1) Mannich reaction via condensation with formaldehyde[207,208] , (2) the formation of Schiff's base by reacting a reactive derivative such as 5-formyl-8-hydroxyquinoline[209] and (3) the aminated silica surface, and diazo coupling.

Essentially, most of the reported procedures for covalent attachment of oxines onto aminated silica surfaces have been achieved using diazo coupling following a transformation procedure firstly developed by Hill[210] for enzyme immobilisation. In this procedure, the silica surface is aminated via its silylation with aminoalkylsilane, generally 3-aminopropyltriethoxysilane. The resulting aminoalkylated silica is reacted with *p*-nitrobenzoylchloride, and then the nitro group is reduced to give a *p*-aminobenzoyl

derivative. This intermediate is then diazotized to the diazonioum derivative, and coupled with oxines. This procedure, however, suffers from being too long as it involves many preparation steps and is more likely to generate hazardous wastes. Thus, it does not fulfil the new requirement of green chemistry.[211] For instance, during the benzoylation step, the substrate is heated for several hours in chloroform. Marshall and Mottola[212] have used an aminophenyl silane '(aminophenyl)triethoxysilane' to activate the silica surface, which is then diazotised to facilitate oxine coupling in one step, thus significantly simplifying and shortening the immobilisation procedure; however, steric hindrance effect is more likely to distress resin functionality as the short linkage is not sufficient to distant the functional group away from the solid support.

In the work throughout this chapter, the silylation reagent, *p*-aminophenoxypropyl-trimethylsilane, was considered as a good candidate. The presence of phenoxypropyl chain reduces steric effect at the support, allowing more chelating groups to be available away from the silica surface for reaction with metal ions. In addition, the final product has a chemical structure and stable linkage identical to that synthesised according to the common procedure modified by Hill.[75]

2.1.1 The solid supports (controlled pore glass)

For several decades, silica materials have been widely used as a stationary phase in separation science including solid phase extraction and chromatography. These materials are generally commercially available as silica gel. Controlled pore glass (CPG) is a particular form of silica gel prepared from a quaternary glass mixture, of typical composition of 50-75% SiO_2 1-10% Na_2O, and the remainder is B_2O_3. This mixture is

melted and formed into the desired shape, and the molten glass is phase separated by cooling to between 500 and 750 °C. The time taken for this treatment determines the extent of phase separation and the resulting average pore size. The borate phase is leached out by acid solutions at high temperatures. The remaining glass contains also colloidal silica particles, which are removed by a treatment with NaOH followed by washing with water. The final glass has porosity between 50% and 75%, and an average pore size between 4.5 nm and 400 nm. CPG has a surface area ranging between 10 and 350 m^2/g, depending on the pore size.[213]

Many chemical and physical features such as high surface area, efficient mass transfer, excellent mechanical properties and chemical inertness, have made CPG preferable substrates for the covalent attachment of numerous chelating regents, particularly when the immobilised materials operated in hydrodynamics systems, e.g., for on-line applications. Probably of greatest importance, however, is the rapid metal exchange, the lack of which is an obstacle to the use of many organic polymer-based chelating resins.

Nonetheless, silica materials have some disadvantages. These are principally the degradation of silica above pH 9 due to alkaline hydrolysis and, in the case of immobilised chelating regents, low exchange capacity due to the rigid structure of the silica matrix. This prevents expansion of the materials with increasing analyte uptake, resulting in satiric obstruction of the reactive site.[214] Steric obstruction on the surface also reduces the number of chelating groups that can be attached during the

immobilisation. These facts could explain the inherently lower capacity of silica compared to their polymeric counterparts. [215]

2.2 Experimental work

2.2.1 Chemical reagents

All reagents used in the immobilisation or analytical process were of analytical grade. The solid support controlled porous glass (CPG) (mesh size 120-200) and sodium nitrite ($NaNO_2$) were purchased from Sigma (Poole, UK). The silylation reagent (*p*-aminophenoxy-propyl)trimethoxysilanae was obtained from ABCR (Manchester, UK), anhydrous toluene and ethanol from Fisher Scientific (UK), hydrochloric acid from (Philip Harris, UK) and super purity nitric acid from Romil, (Cambridge, UK). Elemental stock solutions (1000μ ml^{-1}, SpectrosoL, Merck, Poole, Dorset, UK) were used in the preparation of standard solutions of the studied metals. Ammonium acetate buffer was prepared from the solid and purified by passing through a column of Chelex-100 (BioRad, Hemel Hempstead, UK). The pH was adjusted to the required value with acetic acid or liquid ammonia. The masking agent pyrophosphoric acid (Sigma Aldrich) was added to the buffer immediately prior to the analysis procedure. High purity water (18 Ωcm resistivity, Elgastat UHQ PS, High Wycombe, UK) was used throughout.

All glassware were thoroughly cleaned with Lipsol detergent (LIP, Shipley, UK) and soaked overnight in 5% HNO_3. Immediately before use, all acid soaked glasses were rinsed with high purity water.

2.2.2 Instrumentations

Elemental analysis was performed with a CHN Fisons Instrument EA1108 (CE Instruments Ltd., UK). The ICP-OES system used consisted of Perkin-Elmer Plasma 40 Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) (Perkin Elmer, Beaconsfield, Buckinghamshire, UK) controlled by an IBM XT-486 personal computer using Plasma 400 software . The spectrometer output was connected to Kipp & Zonen Type BD 111 chart recorder (Environmental Monitors Ltd., UK). The ICP-OES operated at the conditions summarised in Table 2.1.

Table 2.1 Operating parameters for the ICP-OES.

ICP Instrument-	
Forward Power/W	1350
ReflectedPower/W	0
Aerosol gas flow rate/l min^{-1}	0.8
Intermediate gas flow rate/l min^{-1}	0.6
Outer gas flow rate/ l min^{-1}	12
Nebulizer	cross-flow
Spray chamber	Ryton
ICP date acquisition-	
emission wavelength	
Element	Wavelength/ nm
Co	238.892
Ni	221.647
Cu	324.754
Zn	213.856
Pb	220.353
Mn	257.610
Chart recorder	0.05- 2 V f. s d, 1 cm min^{-1}

The solid sediment samples were digested using a temperature-pressure controllable microwave oven (MDS 2100, CEM Corporation, USA).

2.2.3 Immobilisation of the 8-HQ onto the CPG

0.50 g of controlled porous glass (CPG) was boiled gently in 10 ml of 5% (v/v) nitric acid (HNO_3) solution for about 30 minutes. The CPG was washed with distilled water over a Büchner funnel until the filtrate attained natural pH 7, then washed with water and dried in an oven at 95 °C for three hours. The dried beads were silylised with a 5% (*p*-aminophenoxypropyl)trimethoxysilane solution prepared using anhydrous toluene for 4 hours at room temperature. The silylised beads were then filtered and washed with toluene and acetone before being dried at 110 °C in an oven overnight to ensure complete curing. This resulted in the formation of arylamine CPG. The cured arylamine CPG beads were left to cool at room temperature before being treated with 2% sodium nitrite in 2 M HCl at 0-4 °C for 30 minutes. This reaction results in the formation of diazonium salt-CPG which was quickly filtered, washed with three 10 ml portions of cold distilled water and added to 10 ml of a 2 % solution of 8-HQ in ethanol. Appearance of a deep red colour indicated the formation of the azo-coupled 8-HQ. This coupling reaction was carried out for 30 minutes, after which the CPG-immobilised 8-HQ was filtered and washed sequentially with ethanol, 0.1M HCl, and water. This material was then left to dry over a Büchner funnel for 2 hours and stored in a desiccator for subsequent use.

The overall reaction is presented in figure 2.2

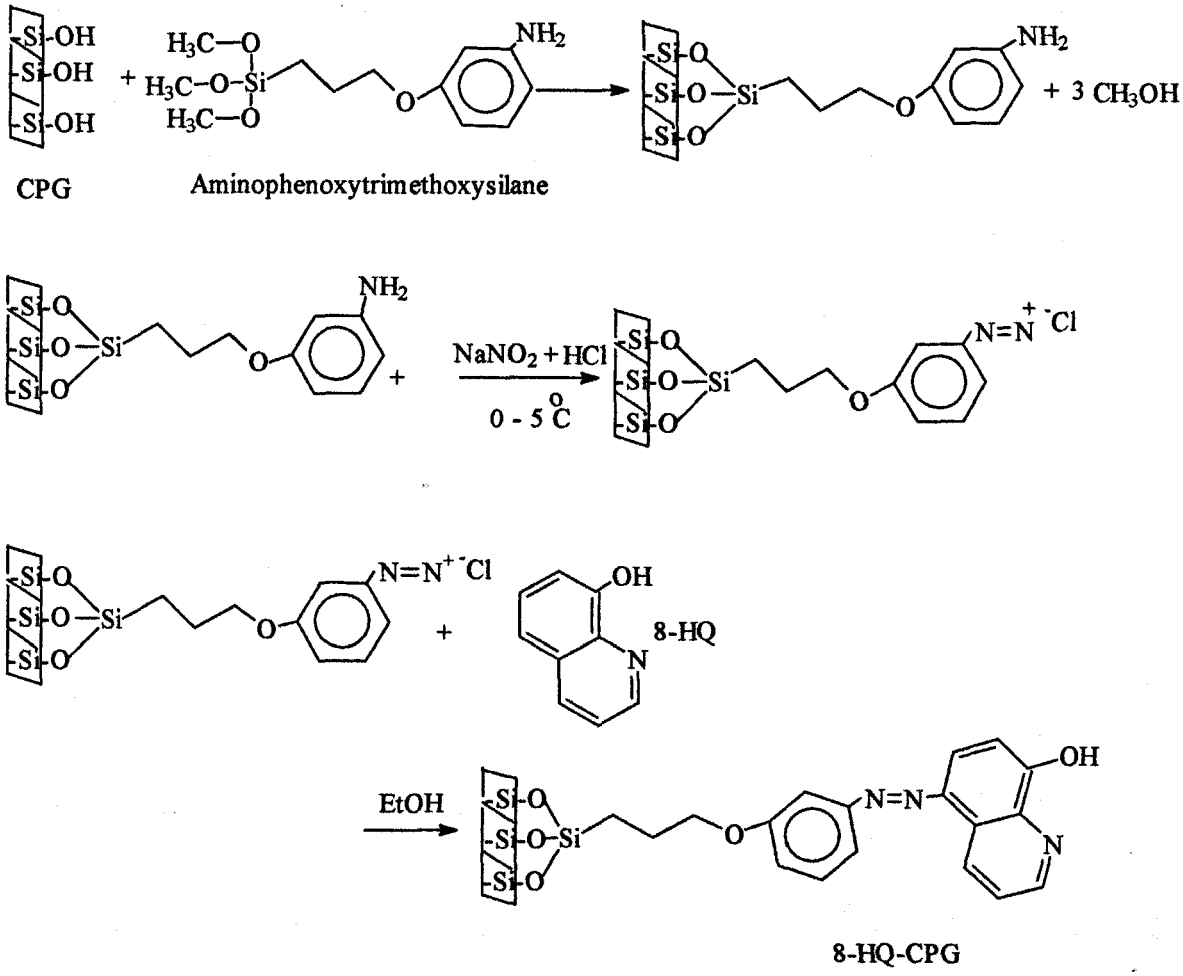


Figure 2.2 Reaction scheme for the covalent attachment of HQ onto silica (CPG).

2.2.4 Flow injection manifold for the solid phase extraction

The flow injection manifold for the solid phase extraction process is illustrated in Figure 2.3. It was designed to be simple in order to be operated manually and to facilitate rapid processing in order to increase the sampling frequency. A glass column (3 mm i. d. and 25 lingth) (Omnifit, Cambridge, UK) was used in place of an injecting loop in a 6-port rotary valve (Omnifit). CPG-HQ material was packed into the column; at both ends 25 μm pore size PTFE frits (Omnifit) were fixed to prevent material losses. A single four-

channel peristaltic pump (Gilson Minipuls 3, Anachim, Luton, UK) was utilised to pump the sample and reagents throughout. The transport lines and mixing coils for sample and buffer delivery were made using 0.9 mm i.d. PTFE tubing and the eluent (nitric acid) delivered using 0.4 mm i.d. tubing. The pump speed was adjusted to deliver the eluent at a flow rate equal to the consumption rate of the nebulizer (1.0 ml min^{-1}) and the column outlet could therefore be connected directly to the sample uptake device of the ICP-OES

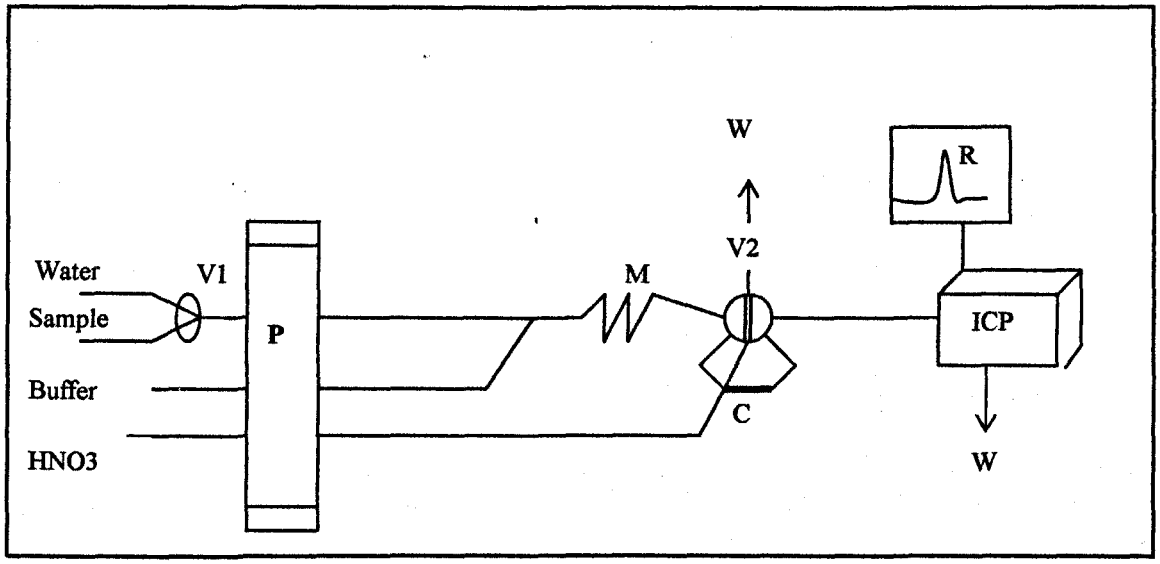


Figure 2.3 Flow Injection manifold; A SPE system for heavy metals coupled on-line with ICP-OES, where C; column, M; mixing coil, P; pump, V, selection valve, W, waste, R; chart Recorder.

instrument. At this optimum speed, the sampling flow rate was 4 ml min^{-1}. Prior to operation, 2 M nitric acid was pumped throughout the FI system for 15 min to remove any metal impurity and then the system was flushed with deionised water to remove residual acid.

2.2.5 Solid phase extraction process

For conditioning the first three way selection valve (V1) was switched to the water position and the second 6-port selection valve (V2) switched to the elution position to permit the water/buffer mixture to pass through the column while acid flow was maintained continuously to the ICP instrument. After column conditioning, the valve V1 was switched to permit standard/ sample loading onto the column at 4 ml min^{-1} for the desired loading time. When reaching the duration of loading, V1 was switched back to a position that allowed water/buffer to flow in order to wash the column and transport lines from residual metals and weakly bound calcium and magnesium. By switching the V2 to elution position, the chelated trace metals were eluted off the columns with the eluent (nitric acid), countercurrent to the loading step. Opposite flow direction of loading and elution avoids problems due to the tendency of clogging, which would affect column performance, as well as maximizing preconcentration.[216] The column was prepared for the next run by its conditioning with buffer for 30 seconds.

2.2.6 Determination of exchange capacity

The capacity of the immobilised 8-HQ was determined for Cu and Mn by both a static and a dynamic method as described by Nelms *el al.*[84]

2.2.6a Static method

100 mg of dry resin was placed into polyethylene sample tubes and 10 ml of 40 μg ml^{-1} solution of each metal prepared in acetate buffer (0.1 M, pH 6) added. The mixture was allowed to equilibrate overnight. The solution was filtered and the metal concentrations in the supernatant liquid (unextracted metals) were measured versus the original

concentration. The decrease in concentration was used to calculate the capacity using equation (2.1).

$$C_d = \frac{(c_o - c)v}{1000mM_r} \qquad (2.1)$$

where C_d is the static capacity in $mmolg^{-1}$, c_0 and c are the concentrations of the metals before and after the equilibrium, v is the sample volume ml, m is the mass of immobilized chelate in g and M_r is the relative atomic mass of the metal under study.

2.2.6b Dynamic method

For the determination of dynamic capacity, 50 mg of the dry resin was packed into the column and 50 μg ml^{-1} standards of Cu and Mn loaded into the column for various loading times (1-20 min.). The sorbed metal was desorbed with 2 M nitric acid into the ICP. Three repeated measurements were made at each loading time and the results were evaluated in terms of the eluted peak height. The volume beyond which no further increase in peak height was obtained was deemed to represent the dynamic capacity limit. This was evaluated based on equation (2.2).

$$C_d = \frac{cv}{1000mM_r} \qquad (2.2)$$

where C_d is the dynamic capacity in $mmolg^{-1}$, v_s is the volume of sample loaded (calculated based on loading time), c is the concentration of the metal at the dynamic capacity limit, m is the mass of immobilized CPG packed into the column in grams (g).

2.2.7 Sediment digestion

The procedure described in section 2.2.5 was validated by analysing Cu, Co, Mn, Ni, Pb and Zn in three sediments reference materials: MESS-1 (Canada), GBW07309 (China)

and CRM-ES (estuarine sediment solution). The solid sediments MESS-1 and GBW07309 were digested in microwave apparatus according to a protocol validated by Kowalewska *et al.*[217] A portion of 0.50±0.02 g sediment powder was placed into a 250 ml dry polytetraflouro-ethylene (PTFE) vessel then a mixture of 4 ml HF, 3 ml of HCl and 3 ml of HNO_3 was added. The vessel was covered loosely with a lid and left for 16 hours to allow organic gases to release. The microwave was then operated at a pressure of 120 psi and a temperature of 170±5 for 25 minutes. After venting and cooling, the digest was transferred into a 50 ml polyethylene centrifuging tube with 2% HNO_3, centrifuged using (Cenrtmic S-577 Lap-Plant, UK) at 300 g for 20 minutes. The supernatant liquid was decanted into a 50 ml volumetric flask, the volume was completed with 2% HNO_3, then, transferred immediately into a dry polyethylene container and kept at 4 °C prior to the analysis. This protocol was carried out for a batch of 5 vessels, including one as a blank. The sediment solution CRM-ES was used as supplied. The standard solutions for the calibration were prepared in 5% or 2% HNO_3 to match the acidity of sample digests.

2.3 Results and discussion

2.3.1 Immobilisation reaction

A two step chemical transformation developed to immobilise 8-HQ onto controlled pore glass (CPG) for the work in this chapter is shown schematically in figure 2.2. In the first step, *p*-aminophenoxypropyltrimethoxysilane is employed to aminate the CPG surface by the usual silylsation reaction in dry toluene. The second step in the sequence used to couple HQ to the aminophenoxypropyl-CPG involves diazotization coupling. As stated in chapter one, the silylsation reaction is believed to proceed throughout hydrolysis of the

methoxy group of the silane, by the aid of surface adsorbed water, followed by condensation of a surface silanol with a silanol of the silylasing agent[218]. Residual silanol groups of the silane can then be condensed by curing at 90-120 °C.[219]

The use of aminophenoxypropyltriethoxysilane as a silylasing agent allows the attachment of aromatic amine in one step and also provides a spacer arm of similar length as with the common procedure. Therefore the attachment of oxines by diazo coupling can be accomplished straightforwardly with no need to attach aromatic amine as usually required with conventional protocol. Aromatic amine attachment to the traditional silane, aminotriethoxysilane, attachment involves 24 hours condensation with nitrobezoyl chloride in chloroform followed by reduction of the nitro group to an amine with sodium dithionite. The reduction step itself has been reported to be problematic.[220] The introduction of aromatic amine in the silylation step, however, shortens the time required to complete the conventional protocol (2-3 days)[221] and eliminates the limitations associated with the reduction step. This synthesis procedure enables an easily controllable immobilisation to be completed in less than a day in ambient temperature. In this chapter the feasibility of this procedure was demonstrated by immobilising 8-HQ onto CPG; however, it will be more appropriate for the attachment of oxines and similar reagents to silica surfaces within the porous structure of silica based monolithic materials (see chapter three). Moreover, it is green chemistry compatible, since no hazardous chemicals are used; therefore no harmful wastes are generated. [80]

2.3.2 Capacity study

The capacity of the materials was investigated using nitrogen micro-determination by elemental analysis [222] and by examining the Cu and Mn metal uptake in static and dynamic mode.[84]

The nitrogen content can be taken as an indication of the surface converge and the success of the immobilisation. The capacity value based on nitrogen determination was found to be 0.267± 0.029 $mmolg^{-1}$. Static and dynamic capacity values for Cu were 0.098±0.014 and 0.106±0.011 $mmolg^{-1}$ respectively and for Mn were 0.091±0.016 and 0.098±0.013 $mmolg^{-1}$. The value obtained by nitrogen determination is reasonably consistent with that elicited by metal uptake methods and serves as evidence indicating that both metals form a 1:1 complex with the bound oxine.

There may be an argument as to the validity of the capacity values which are obtained by both the metal capacity and nitrogen methods. It has been reported that the free amine site in the silica immobilised 8-HQ product would apparently react with Cu ion at higher pH and may thus result in higher capacity values than expected. To ensure that metals were extracted only by the immobilised HQ, the aminophenoxypropyl-CPG (the product of the first step in the immobilisation process) blanks were run during metals capacity determination.

A similar situation may happen for the determination of nitrogen and carbon by elemental analysis. Kolstad *et al* [223] showed that many N and C containing by-products,

as impurities covalently bound to the silica surfaces, may be left when the conventional immobilisation protocol is employed to effect the attachment of oxins to silica materials. Subsequently, it will result in an erroneously high value of the bound reagent based on calculation of the measured carbon and nitrogen contents. However, in this short immobilisation procedure, the possibility of residual organic by-products being bound to the silica surface would be considerably reduced. Capacity measurement by nitrogen determination on this resin can, therefore, be taken as a true capacity, taking into account the two nitrogen atoms involved in the diazo linkage of the reagent which are expected not to contribute to the chelating process.

2.3.3 Optimising the SPE procedure

2.3.3.1 The effect of buffer pH

Since the solution pH affects the extent of complexation, which in turn determines the percentage of metal retained by the resin, the influence of the pH of 2 M ammonium acetate buffer on the recovery of the selected metals was studied in the range from 3 to 9. Standard metal solutions (200 ng ml^{-1} of each metal) were prepared in 2% nitric acid and buffered on-line using the FIA manifold. The recovery values for ions in study were evaluated from calibration curves obtained by direct injection of a series of standards prepared in 1.5 M nitric acid. The results calculated as the average of three determinations are presented in Figure 2.4. The effect of pH on sorption process may be explained as follow. Metal sorption starts when the pH rises to the range where most acidic ion exchange sites start to exchange hydronium ions (H^+) for metals and the capacity reaches maximum value in the pH range where all the ion exchange sites take

part in the reaction and the functional groups able to form chelate rings with metal cations. As the figure shows, all ions exhibited linear range with upward trends before reaching the maximum because the degree of dissociation increases and H^+ concentration in solution decrease. Nearly all metals studied reach maximum sorption around pH 5.5. At this pH value, the recovered value for the studied metals ranged from 100% for Mn, and Zn, and 78% for Ni. Therefore, this pH value was considered for all the subsequent work.

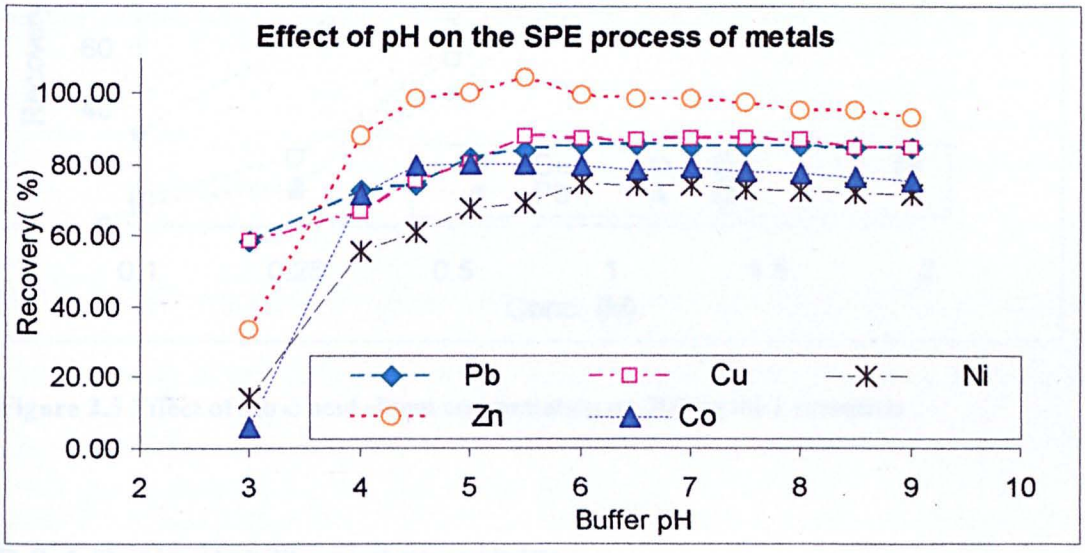


Figure 2.4 Effect of buffer pH on metals sorption.

2.3.3.2 The effect of eluent concentration

The effect of nitric acid on metal desorption was evaluated by increasing the acid concentration from 0.1 to 2 M. The results were evaluated based on the recovery values and represented in Figure 2.5. Although some of the investigated metal ions could be eluted efficiently using low concentration of acid (e.g., Cu), others required a concentration of 1.5 M for quantitative recovery. The acid concentration required for

each individual element increased as the formation constant of metal-complex increased. In this study, acid concentration of 1.0 M was considered to be sufficient to elute (recover) the maximum amount of all metals.

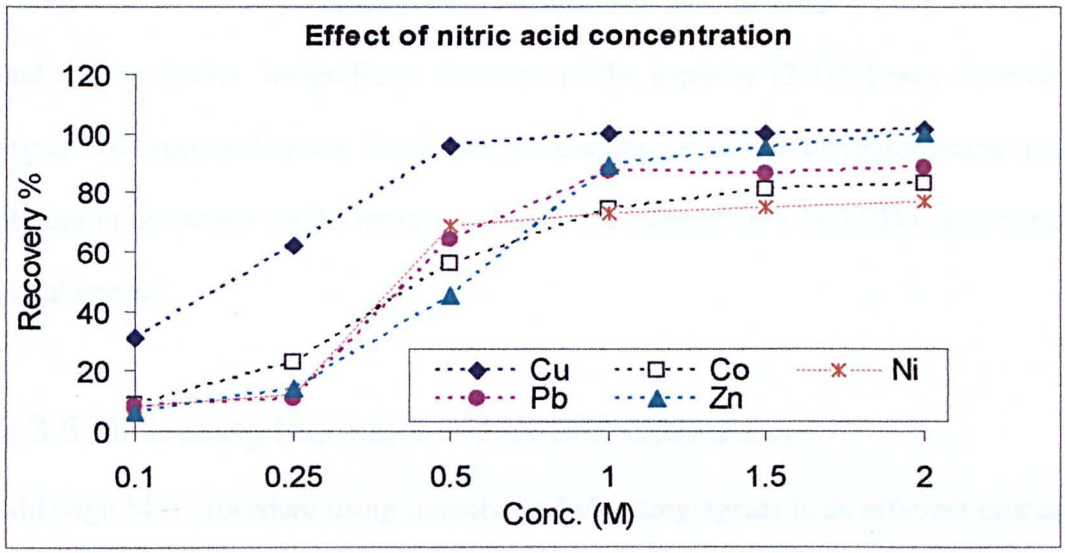


Figure 2.5 Effect of nitric acid eluent concentration on 200 ng ml-1 standards.

2.3.4 Resin stability and reusability

The stability of the prepared HQ-CPG materials was investigated in acid (0.50-4.0 M HNO_3). A portion of 0.5g of resin was shaken with acid solutions of varying concentrations for 5 h and filtered. The solid was washed with distilled water until free from acid, dried at 120 °C in an oven and its sorption capacity was determined using the procedure described in section 2.3.2. The sorption capacity for chelating resin treated with acid (4 M) was found to be similar (variation $\leq$2.5%) to that of the untreated one. Thus the present resin can withstand 4.0 M HNO_3, but a light orange coloration was immediately observed in the acid solution when the resin was placed in 5.0 M acid

solution. This could be attributed to the cleavage of the ether group of aminophenoxypropyl within the linker arm in highly concentrated acid. The sorption capacity of the resin was also reduced significantly after such treatment. Similarly, when the material was placed in solution of pH >9, again an orange colour developed in the solution. The exchange capacity for the metal ions was determined after several loading and elution cycles. Insignificant decrease in the capacity (2.41%) was observed after around 100 repeated cycles. Therefore, the chelating resin can be reused many times. On storing in dessicator for 10 months the sorption capacity of 8-HQ-CPG does not change significantly.

2.3.5 Minimising the major matrix and calibration

Although SPE procedure using immobilised chelating agents is an efficient procedure to eliminate matrix including alkaline and alkaline earth elements in trace metal analysis, the fact that the concentration of some major co-existing metals such as Fe, Al, and Mn is many times higher than those of trace elements of interest in environmental samples such as sediment and soil digests, is a significant drawback. This is because the major metals compete with the targeted metal ons for the complexing sites on the resin materials. [223,224] The elimination of this problem was attempted by controlling the pH of solutions during the extraction together with the use of masking reagents. The masking agent should bind the major ions (e.g., Fe, Al) selectively forming highly stable complexes, thus preventing them from being taken up by the resin. In this context, citric acid has been used to remove Fe and Mn during the SPE of trace Cd, Cu and Pb by the sorption of their diethylammonium-N-Ndiethyldithiocarbamate (DDDC) or ammonium

diethyldithiophsphate (DDPA) complexes on C18 from soil and sediment digests.[224] The same procedure was made more selective for the extraction of Zn using dialkyldithiophosphates in the complexation at pH 3.[224] The low pH e.g., 2-3, however, limited the application of this procedure with the immobilised HQ, which works better in neutral pH. Recently, Dionex Corporation advertised a procedure for the removal of major elements (Fe, and Mn) prior to the ICP-OES analysis of trace metals. In this procedure trace metals were trapped onto the IDA-based resin (MetPac CC-1) and the major element masking was affected by adding pyrophosphoric acid to the buffer.[225] It believed that pyrophosphate not only prevents the precipitation of Fe and Al, at high concentrations, but also allows an effective removal of these ions from the column. [225] The relatively stable metal-pyrophosphate complexes formed during the on-line neutralization step (*in situ*) do not interact with iminodiacetate groups and are not retained in the column. As this masking agent operates preferably with IDA based resin which has an optimum pH range similar to that of the immobilised 8-HQ, it was suggested that it would be effective to mask major metals from real sediments sample. The effectiveness of this procedure was investigated using standard solutions containing 100 $ngml^{-1}$ of the studied trace metals in presence of 100 μgml^{-1} of the major elements (Al, Fe and Mn) at the optimum pH. The optimum concentration of pyrophosphoric acid was established by experiment. The results obtained with 8-HQ were similar to these reported with immobilised IDA.[225] As can be seen in Figure 2.6, 40 mM of the reagent could remove more than 90 % of the original amount of Fe and Al, and about 80 % of Mn, while the recovered percentages of all other elements almost remained unaffected.

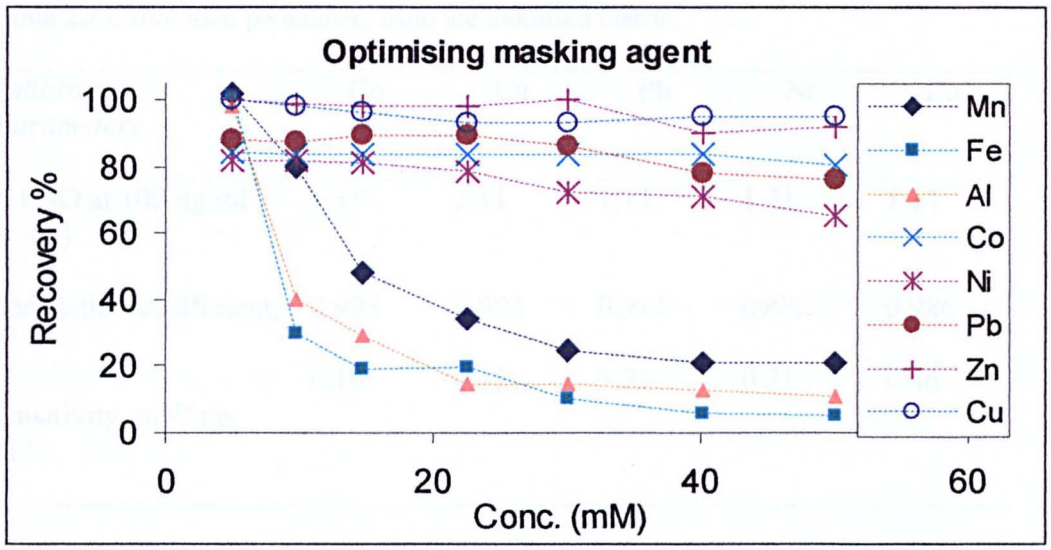


Figure 2.6 Masking the major elements (Fe, Al, Mn).

Therefore, this concentration of masking agent has been used as optimum to mask major elements during the analysis of trace metals in the certified reference materials (sediments) in the subsequent work (see the following section). For the reason that there is no commercial synthetic matrix simulating the composition of reference sediment, calibrations were obtained in a home made sysnthetic matrix to resemble the real sample. This has been accomplished by the addition of calculated amounts of the most important matrix elements encounter in sediment samples, including Fe Al and Mn (as matrix modifiers) to acidified standards. The standards contained individual metal ions over the range 50-300 $ngml^{-1}$. The analysis below this level would require long sampling (loading) time which may not be cost effective as the ICP instrument will be idle during that period. Also too long sampling time will reduce sample throughput.

Table 2.2 Calibration parameters from the modified matrix.

Calibration Parameters	Co	Cu	Pb	Ni	Zn
% RSD at 100 ng ml^{-1} (n = 4)	1.35	2.11	1.75	1.21	1.14
Correlation coefficient, R^2	0.995	0.994	0.991	0996	0.986
Sensitivity, mV/ ng ml^{-1}	0.16	0.42	3.21	0.31	0.46

2.3.6 Analysing sediment reference materials

The digests of sediment samples were analysed using the FI manifold described in the experimental section. 2 M ammonium acetate was used to buffer the standards and real sample on line before it passed the column. On-line buffering enables sample mixing and transportation to be carried out within a closed system and hence reduces the chance of contamination of analytes from the environment. It also offers an additional benefit for the application in this work. Because sediment samples contain high level of Fe and Al, off-line buffering to around pH 5.5 would result in iron and aluminium being precipitated immediately as hydroxides. These hydroxides, when formed, will work as co-precipitating agents for trace metals from the solution, thus reducing their concentration to very low levels. On-line buffering minimises this phenomenon because the solution passes quickly to the column. The calibration standards were prepared in aqueous solution of 1% nitric acid (containing the matrix modifier) covering the dynamic range shown in the table 2.2 used to determine the targeted metals in the real samples, sediment

reference materials. Table 2.3 shows the concentration of the analysed metals in reference materials with the certified values. Apart from Cu and Ni ions in the solution (extract) of estuarine sediment, CRM-ES, all the analysed metals were quantified with satisfactory results. The low recovery of these ions could be ascribed to the presence of high level of competing matrix ions in this sample or due to the digestion procedure that being employed for the solid samples. However, the obtained values are still in agreement with the certified ones.

Table 2.3: Analysis of reference materials (in μg/g) ,(at *90 % confident limit, n=3*).

Sample	Metals									
	Co		**Cu**		**Ni**		**Pb**		**Zn**	
	Found (Recovery%)	Certified	Found (Recovery%)	Certified	Found (Recovery%)	Certified	Found (Recovery%)	Certified	Found (Recovery%)	Certified
MESS-1	11.7±1.50 (108.20%)	10.8±1.90	22.62±3.62 (90.10%)	25.1±3.80	26.9±3.07 (91.10%)	29.5±2.40	31.58±5.61 (92.90%)	34.0±6.10	196.62±14.75 (102.90%)	191.0±17.00
GBW07309	15.3±1.32 (106.10%)	14.41	30.7±2.41 (95.60%)	32.10	29.3±2.94 (90.70%)	32.30	21±1.54 (91.30%)	23.00	79±7.24 (101.30%)	78.00
[a] CRM-ES	0.11±0.03 (110.10%)	0.10	0.17±0.23 (85.20%)	0.20	0.22±0.09 (73.20%)	0.30	0.32±0.03 (106.60%)	0.30	1.71±0.34 (114.20%)	1.5

[a] μg/ml

2.4 Conclusion

The work in this chapter reports on a simple, rapid and green chemistry compatible chemical transformation for the covalent chemical attachment of oxines onto silica surfaces (controlled pore glass (CPG)). The procedure allows the synthesis of an 8-HQ-CPG resin possessing an identical chemical structure and chelating characteristics as that synthesis according to the common procedure. The use of aminoaraylsilane, aminophenoxypropyltrimethoxysilane, to activate silica makes it easily achievable to attach oxines by direct diazotisation, thus the immobilisation process could be completed in a short time in comparison with traditional process, which is usually takes up to 3 days to be completed. Since only few synthetic steps are involved, this rapid process is an attractive for the immobilisation of oxines and similar reagents within the porous structure of monolithic silica materials, as the traditional method is impractical. For instance since reagents are propelled through the microstructure, controlling of reaction conditions during prolonged steps (e.g., overnight heat) not possible as with batch methods.

The 8-HQ-CPG resin produced exhibited excellent performance as SPE materials for on-line sample preparation of trace metals proceeding to ICP-OES quantification. The method performance was examined by analysing sediments certified reference materials. The presence of high level of major transition metals (i.e., Fe, Al and Mn) in sediments was found to limit the applicability of the SPE process for sample preparation during the analysis of trace metals in sediments; as they can saturate resin, leaving a smaller amount of the reactive chelating centre available to retain trace analytes. Therefore, methods that can reduce their effect are essential. The work in

this chapter demonstrated that the addition masking agent, pyrophosphoric, to buffer solution is an effective simple method to minimise the effect of these ions.

Chapter 3

Fabrication of miniaturised monolithic silica columns and their applications as solid supports in the synthesis of chelating resin for SPE of transition metals

Chapter 3–Fabrication of miniaturised monolithic silica columns and their applications as solid supports in the synthesis of chelating resin for SPE of transition metals

3.0 Aims

Initially, this chapter aims to highlight the extraordinary properties of the recently developed porous monolithic materials, together with their ever increasing applications as alternative solid phases to packed beads, particularly in separation science. The chapter goes on to demonstrate a synthetic process for the production of porous monolithic silica, describing the chemistry process involved. The applicability of these versatile materials to fabricate miniaturised apparatus for solid phase extraction in trace analysis of transition metal is then illustrated by functionalising these materials with chelating regents. Finally, it demonstrates how these miniaturised SPE columns can be integrated into FIA manifolds to allow sample preparation (by SPE) and metals quantification to be carried in on-line.

3.1 Introduction

3.1.1 Microporous monolithic columns

Monolithic materials or continuous stationary phases (porous rod) are defined as materials consisting of continuous pieces of solid that fill the entire volume of a cavity such as a column or disc. This piece of porous material (polymers or silica) contains two interconnected networks of pores, the macropores and the mesopores. The macropores, also called the throughpores, have dimensions in the 1.5–2-μm range.

The macropores form a network of pores ensuring a low pressure drop over the monolithic, hence dramatically reducing separation time in chromatographic applications. The mesopores form the fine pore structure of the column interior and create a very large surface area, ~300 m^2/g, onto which adsorption of the target analyte take place. [226,227] It is generally agreed in the literature that the macropore network accounts for approximately 80% of the total porosity and the mesopore network represents 10–15% of the total porosity with an average size range between 10 and 20 nm.[228,229,230].

Since the early 1990s the number of applications of monolithic columns in separation science has continuously increased. They have become an attractive alternative to the classical packed beads in various techniques, as they have successfully resolved many technological limitations widely encountered with packed columns such as irregularities in packing density and excessive channelling, column back pressure, and dead volume.[231,232]

3.1.2 Synthesis of monolithic materials

There are four main approaches to prepare continuous beds: [233]

(i) polymerization of an organic monomer with additives,

(ii) formation of a silica based network using a sol–gel process,

(iii) fusing the porous particulate packing material in a capillary by a sintering process, and

(iv) organic hybrid materials.

At present, the first two quoted procedures are the most popular. Monolithic materials with different surface functionalities and geometrical designs produced from inorganic and organic materials have become available on a commercial scale from

various manufacturers. Some of the commercial monolithic materials and their features are summaried in Table 3.1. The fourth approach is being thoroughly studied by organic chemists and seems to be promising.

In the following sections, the synthetic protocols of microporous organic and inorganic monolithic materials will be introduced briefly.

Table 3.1 Some of current commercial monolithic stationary phases for HPLC separations.[234]

Product	Shape	Producer	Chemistry	Separation modes
CIM disc	Disc	BIA Separations	Modified polymethacrylate or polystyrene copolymers	Ion exchange, hydrophobic interaction, reversed phase,
CB Silica plate	Disc	Conchrom	Modified silica	Reversed phase, normal phase
SepraSorb	Disc	Sepragen	Modified cellulose	Ion exchange
CIM tube	Tube	BIA Separations	Modified polymethacrylate	Ion exchange
UNO	Cylinder	BioRad	Polyacrylamide-based copolymers	Ion exchange
Swift	Cylinder	ISCO	Modified polymethacrylate or polystyrene copolymers	Ion exchange, reversed phase
Chromolith	Cylinder	Merck	Modified silica	Reversed phase
Monoliths	Cylinder	LC Packings	Polystyrene copolymer	Reversed phase

3.1.3 Organic monolithic materials

The first organic monolithic materials were synthesised by *in situ* polymerisation of polyurethane in the form of open-pore polyurethane foams. These polyurethane foams were investigated in the early 1970s as stationary phases for both high performance

liquid and gas chromatography, but their use was limited as they exhibited excessive swelling and softening in some solvents.[235,236] Interest in these new formats was revived in the early 1980s as a novel approach to monolithic separation media , after the successful efforts by Hjerten to prepare porous organic monolithic materials by *in situ* polymerization of an aqueous solution of acrylamide derivatives.[237] Nowadays, porous organic monolithic materials with a variety of chemistries and geometries (e.g., column, disc) are produced by the polymerisation of various monomers.[238, 239] Practically, the preparation of polymeric monolithic columns is similar to that used for macroporous polymer beads. It can be accomplished by a straightforward moulding process. Typically, an empty mould, usually column or capillary, is filled to the desired length with a polymerisation mixture consisting of monomer, initiator, and porogen agents (precipitators) and then sealed at the other end. Traditional porogens include inert diluents that may either be solvating or non-solvating solvents for the polymer being produced, soluble linear polymers, or mixtures of the above. [240] The presence of porogenic agents during the polymerization can greatly affect the solubility of the polymer molecules in the polymerization system, as described elsewhere, [241] and resulting in matrices with different structures in terms of pore structure, pore size distribution, and porosity.

The polymerization is then triggered, most often by heating in a bath at a temperature of 55–80 °C or by UV light. The seals are then removed, the tube is provided with fittings, attached to a pump, and a solvent is pumped through the monolith to remove the porogens and any other soluble compounds that remained in the polymer after the polymerization was completed. Moulds for the preparation of monoliths of the desired sizes can be made of various materials, such as stainless steel, poly(ether ether ketone) (PEEK), fused silica, and glass tubes or capillaries.[242] Polymerisation usually

proceeds with a nucleation and growth mechanism. The decomposition of an initiator to form radicals initiates the polymerisation of the monomers. The growing polymers produce nuclei (precipitate) to which the porogens and the non polymerised monomers are distributed. Continued polymerisation in swollen globules increases the size, allowing coalescence to form clusters, which in turn, join to form an integrated structure. These interconnected clusters of globules form the porous monolithic materials.[243]

3.1.4 Inorganic monolithic materials

The synthesis of monolithic silica columns is more complicated than that of its polymeric counterpart; however, many properties make them a very attractive stationary phase in separation science. Porous monolithic silica materials are generally prepared via sol-gel methods. The overall sol-gel process, as the name implies, usually involves two stages:

Precursors initially form high molecular weight but still soluble poly-intermediates, a sol, and the intermediates further link together to form a three-dimensional network, a gel.[244]

The precursor for a sol-gel reaction could be inorganic salts, alkali silicates, or metalo-organic compounds, known as metal alkoxides. The formation of a gel from both types of precursors will be illustrated in the following, with silica as the primary example, since most of our understanding of the sol-gel process is derived from silica composition.

3.1.4.1 Metaldo-organic (alkoxide) precursors

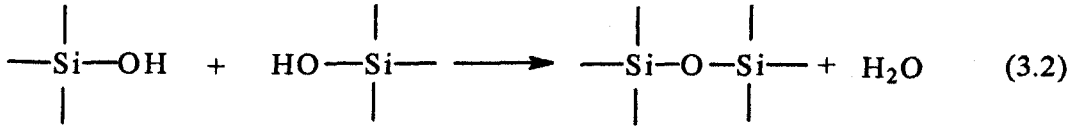
Silica gel synthesis following this route involves the hydrolysis and condensation

reactions of an alkoxide such as tetraalkoxysilanes in the presence of water and appropriate catalysts.[244]

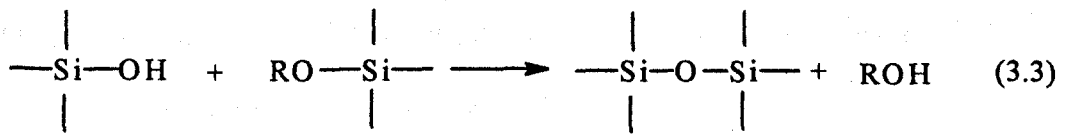
Firstly, silicic acids are produced by hydrolysis of an alkoxysilanes $Si(OR)_4$, as in reaction (3.1).

(3.1)

The silicic acids then undergo either self-condensation, reaction (3.2), or condensation with an alkoxide as in reaction (3.3). Tetraethoxysilane (TEOS) and tetramethoxy-silane (TMOS) are the most commonly used alkoxide precursors in the preparation of silica containing gels, due to their controllability in reaction kinetics.



(3.2)



(3.3)

An enormous literature has emerged in the last few years showing an obvious progressive increase in the application of this route to synthesis monolithic silica materials with porosity in the micrometer range. An elegant and flexible means for the preparation of such types of materials has been described in a series of papers by Nakanishi and co-workers.[245, 246] A comprehensive discussion on this process and its applications are well documented in the recent reviews by Nakanishi[247, 248] and Nakanishi *et al.* [249, 250] Briefly, this method relies on the hydrolytic polymerisation of metal alkoxides such as tetramethoxysilane in acidic aqueous solution and in the presence of a water soluble polymer such as polyethylene glycol (PEG),

poly(ethylene oxide), PEO, poly(vinyl pyrrolidone), PVP and poly(acryl amide), PAAm. These polmers also known as porogens and their role is to induce phase separation by controlling the condensation step in order to maintain microporosity in the micrometer range (normally 1.2-1.5 μm). The mesopore size can be altered by post treatment with aqueous ammonia and with a proper temperature control. Typically the process is initiated at 0°C and kept at this temperature for the first hour then the temperature allowed to rise to 40 °C for 24 hrs or more. Monolithic materials prepared according this process in moulds of wide diameter (e.g., column or disk) usually show a significant shrinkage in the volume of the whole structure as the gel solidifies during the drying process. Thus, the resulting silica monoliths are normally encased inside a heat shrink tube, PTFE tubing or PEEK resin to restrain the silica mass in place and prevent it from being dislodged during operation. [251,252]

3.1.4.2 Inorganic salts (alkali silicates) precursors

Porous silica gels can be prepared from inorganic salts, such as sodium silicates or potassium silicates (also known as colloidal silica) as precursor materials.[253] The method involves two steps of chemical reaction:

Initially silica acid $Si(OH)_4$ is generated by acidic neutralization of alkali silicate in water soluble silicates reaction (3.4)

$$Na_2SiO_3 + H_2O + HCl \longrightarrow Si(OH)_4 + NaCl \qquad (3.4)$$

then a polymeric condensation of the silicic acid is progressed following reaction (3.5).

$$\begin{array}{c} | \\ -Si-OH \\ | \end{array} + \begin{array}{c} | \\ HO-Si- \\ | \end{array} \longrightarrow \begin{array}{c} | \quad | \\ -Si-O-Si- \\ | \quad | \end{array} + H_2O \qquad (3.5)$$

The extensively applied method to synthesis monolithic silica with microporous structures from inorganic salts is based on a simple process originally developed by Shoup [254,255,256] to manufacture porous glass from colloid silicates. It is based on the hydrolysis of a mixture of potassium silicate and colloidal silica (SiO_2 and K_2O) solution in the presence of a drying control chemical additive e.g., formamide at room temperature. Formamide which is also called latent acid-forming reagent reacts in solution producing ammonia; therefore it increases particle size by reducing the rate of silica hydrolysis. Silica species, being hydrolysed, react and expand into a silica gel network containing some alkalis in the form of reaction by-products. These by-products have to be leached (removed) in order to obtain pure silica monoliths. The leaching operation usually consists of successive washing with ammonium nitrate or mineral acid solution and water, preferably using hot solutions at 70-90°C for few hours.

The porosity of the monolithic materials prepared following this method is effected by several parameters include; silicate precursor ratio SiO_2/K_2O (i.e., as the ratio reduces the pore size increase), temperature, pH, aging and drying conditions, among which pH and the drying condition are the most significant ones.

As a comparison, homogeneous multicomponent gels may be formed more easily from hydrolysis of alkoxides than from alkali silicates. On the other hand, the alkali silicates method has several advantages: [244] first, the preparation of these materials can be attained at ambient conditions using simple and low cost resources. Second, drying in this method takes much less time, since the average pore size is larger and therefore the capillary force which is generally the primary causes of crack initiation during drying is less severe. In addition, the large pore size of the monolith allows the

solutions to be pumped throughout the monolithic under moderate pressure. Third, these gels can be cast easily in small-diameter fused silica capillaries. Fourth, the resulting surface of the gel is amenable to chemical modification.

3.1.4 Applications of monolithic materials for solid phase extraction

Although the main area in which monolithic materials have been applied extensively is chromatographic separation, there is an increasing interest among the scientific community to extend the applications of these materials to many other fields such catalyst, microrectors and solid phase extraction. During the last few years, monoliths have been successfully used as sorbents for SPE in hydrophobic, ion exchange, and immunoadsorption modes. The ability of concentrating highly diluted compounds makes these materials suitable for the pretreatment of various environmental, food and biological samples. The unique properties of monoliths including high surface area, surface reactivity, high permeability for liquids, low mass transfer resistances, low cost and ease of preparation together, with availability of many simple means to control shape, porosity and selectivity, meet the requirements of modern SPE materials specially designed for rapid, sensitive, accurate, and miniaturised devices.[257]

To the best knowledge of this researcher, only a few articles reporting the application of monolithic silica in the SPE and separation of trace metals have been published so far. The first report was published by Lofthhouse *et al.* [85] These authors adapted the well recognised immobilisation protocol for the attachment of 8-HQ to allow its covalent attachment within the microporous structure of these monolithic materials. The immobilised monolithic column was used for preconcentration and matrix elimination in analysis of trace metals in seawater by ICP-MS. When compared with

HQ immobilised onto CPG and the commercial resin, Chelex 100, the materials show no drawback. In another report, by Paul and co-worker[258], iminodiacetic acid (IDA) was covalently bonded to a monolithic column, commercially available from Merck KGaA (Germany). The functionalised column (chelating ion exchange) has been applied effectively to separate and determine alkaline earth metals at trace level. In this chapter, miniaturised monolithic silica columns were synthesised from alkali silicate, and used as solid support for the covalent immobilisation of HQ and *L*-Cystiene. The produced materials were incorporated into flow injection manifolds to perform SPE of some trace metals from standard solutions, and then used to analyse Fe from real samples.

3.2 Experimental work

3.2.1 Chemicals and reagents

Microporous monolithic silica materials were fabricated from formamide (Avocado Research Chemical Ltd., Heysham, Lancashire, UK), potassium silicate (21% SiO, 9% K2O, Prolabo, Manchester, UK) and acetamide (BDH, Poole, UK).

The reagents required for immobilisation of L-cystiene included; 3-aminopropyltri-ethoxysilane (Sigma, Poole Dorset, UK), gluteraldehyde (25%) and *L*-cysteine (Lancaster Chemicals, Lancashire, UK). The 20% solution of 3-aminopropyltri-ethoxysilane used in salination was prepared in toluene from Fisher Scientific (Loughborough, Leicestershire, UK). In the immobilisation process of 8-hydroxy-quinoline the reagents described in chapter two were used.

The metal standard solutions were prepared by successive dilution of 1000mgl-1 standard solutions obtained from BDH, (Poole, UK) and the chromogenic reagent 4-(2-pyridylazo) resorcinol (PAR) (Lancaster Chemicals, Lancashire, UK) prepared in a

buffered aqueous solution containing 0.04% of the surfactant Tween-80 (Folka Biochemika), which is required to enhance the hydrodynamic parameters of the system, and a mono-sodium salt of 3-(2-pyridyl)-5,6-bis-(4-phenylsulfonate)-1,2,4-triazine) (Ferrozine) (Aldrich, Gillingham, England) prepared in acetate buffer containing 0.02 % ethanol and 20 nM reducing agent, Hydroxylamine hydrochloride (Aldrich) . A dilute solution of DL-dithiothreitol (DTT) (Lancaster Chemicals, Lancashire, UK) in ammonium acetate buffer of pH 7 was used to reduce the columns prior to use and between each 20 cycles.

All the other reagents were analytical grade chemicals and high purity de-ionised water (18 MΩ resistivity, Elgstat UHQ PS, Elga, High Wycombe, UK) was used throughout.

3.2.2 Instrumentations

A peristaltic pump (Ismatec SA, MS-Reglo, Laboratoriumstechnik, Switzerland) with 0.42 ml accurated pump tubing (Elkay Lab Products Ltd., Hampshire, UK) was used for solution delivery throughout the preparation of the miniaturised monolithic silica columns and during the immobilisation processes. The monolithic materials were prepared in glass capillaries of 500 μm id (LIP Equipment & Services Ltd., West Yorkshire, UK).

The flow injection systems were constructed from a peristaltic pump (minipuls 3, Gilson, Villiers-le-Bel, France) or HPLC pump (SA103 HPLC pump, Speack Analytical) to effect reagents propulsion, 4-way injection valves for sample and effluent direction and/or injection, and PTFE tubing (Anachem, Luton, UK) for all transport lines. The integration of the functionalised miniaturised columns into the

flow injection manifold was attained by holding them inside a 2-way tubing connector (Omnifit) with the aid of O ring rubbers. The connector was modified in house to enable straightforward replacement and ensure tight connection free of leakage.

The detection was achieved using a spectrophotometer with a 70 µl sample flow cell volume and 10 mm path length (Cecil Instruments Ltd., Cambridge, UK)

3.2.3 Preparation of microporous monolithic silica columns

The microporous monolithic silica columns were synthesised according to a procedure similar to Shoup's method [254, 255] previously adapted by McCreedy and co-workers [101] for the manufacture of microporous silica support in immobilised enzyme microreactor. Briefly, a measured portion (0.04-0.1 g) of acetamide (BDH, Poole, UK) was added to an aliquot of formamide (Avocado Research Chemical Ltd., Heysham, UK). The two chemicals were mixed thoroughly and 300-600 µl of distilled water was added followed by aliquot of potassium silicate 350-700 µl (21% SiO_2, 9% K_2O, Prolabo, Manchester, UK). The solution was mixed thoroughly for about 30s, and then drawn into capillary tubes by means of a peristaltic pump pre-set at its minimum speed. To enhance the bonding of silica to the capillary surface, the capillary was etched with alcoholic KOH solution, washed with distilled water and flushed with nitrogen immediately prior to use. For the duration of filling, the tubes were held vertically, closed from one end using blu tack® and held upright to gel at room temperature for 1 h then placed in the oven at 60 °C for another hour. After that the capillaries were removed and allowed to cool at room temperature and cut into short pieces of 10-12 mm length. A solution of 2 M HNO_3 hot (80 °C) was pumped through these short columns to leach out alkali metals, followed by a thoroughly washing with distilled water until the pH of drainage became netural, then washed

with 50:50 water: methanol solution, and methanol then water again.The microcolumns were flushed with nitrogen at low pressure to remove water from the microstructure and placed in an oven to dry at 150 °C overnight. The dried microcolumns were then allowed to cool to room temperature and kept in a desiccator until subsequent use.

3.2.4 Immobilisation of chelating reagents

The chelating regents used in this chapter and the immobilisation routes applied to accomplish their covalent attachment onto silica surfaces, were chosen to fulfil the following conditions: (1) the immobilisation procedure should be efficient and time saving; (2) has to be adaptable to permit *in situ* immobilisation of the regents within the micro/meso-porous structure of the monolithic materials; (3) the immobilised materials had to be reasonably stable to withstand the hydrodynamic pressure when used in flow injection manifolds; (4) and the application of harsh reaction conditions and the use of toxic reagents or solvents had to be avoided or minimised as possible.

Among the chelating reagents that have been extensively immobilised onto silica, oxins, (e.g., 8-hydroxyquinoline (8-HQ) and the synthetic amino acid, cystiene, attract special attention to produce stable solid phase extraction materials having high capacity exchanges and greater selectivity for heavy/transition metals. [259,76,260,261] The combination of soft and hard donor sites present in cysteine is expected to result in a resin of wider applicability and high capacity, as complexes of both hard and soft metal ions would stabilise.[262] In this chapter, the immobilisation procedure that has been already developed for the attachment of oxines onto CPG (chapter 2, section 2.3.1) was adapted to facilitate *in situ* immobilisation within the microstructure of the monolithic silica. The covalent attachment of the synthetic amino acid, *L*-cysteine,

was accomplished via the modification of a well documented method.[76,260] In this method, the silica surfaces are aminated (silanised with aminosilane), then coupled to the amino acid through the bifunctional reagent, gluteraldehyde. The coupling of cysteine in this immobilisation is believed to take place through the amino group, leaving the thiol and carboxyl groups available for chelating bonding.[83] The immobilisation was found to be straightforward and proposed to be easily modified to suit with the microstructures of monolithic silica microcolumns. The experimental details of the procedure utilised for the attachment of the two reagents 8-HQ and *L*-cysteine monolithic silica microcolumn are presented in section 3.2.4.1 and section 3.2.4.2.

3.2.4.1 Immobilisation of 8-HQ

The immobilisation procedure developed for the covalent attachment of 8-HQ onto CPG (in chapter two), was adapted here to allow its covalent attachment within the microstructure of the monolithic silica. The presence of the solid supports *in situ* necessitates that the reagents be pumped or sucked through using a peristaltic pump. A 10 mm length monolithic silica microcolumn was cleaned and activated using a hot solution of 10% HNO_3 drawn through for a period of 30 min, flushed with distilled water and purged with nitrogen for 30 min. to remove water. The activated microcolumn was then dried at 120 °C in an oven for 4 hours. After that, the oven was turned off and the microcolumn was left to cool to about 30 °C then washed with an anhydrous toluene and a solution of 10% (*p*-aminophenoxypropyl) trimethoxysilane in anhydrous toluene was drawn through the monolithic microcolumn for about 3 hours to silanise the silica microcolumn. Then, the microcolumn was washed with anhydrous toluene and acetone, and purged with nitrogen at low pressure before being

placed in the oven to cure at 115 °C for 4 hours. The silanised mirocolumn was allowed to cool to room temperature and placed in an ice bath at 0 °C then a cold solution of 2% sodium nitrite was sucked through (at a very slow flow rate) for about 30 min. to yield the diazonium intermidaite. The microcolumn was washed quickly with cold water and cold ethanol and a solution of 8-hydroxyquinoline (4% m/v in absolute ethanol) was drawn through the microcolumn. A deep red colour in the microstructure indicated that the immobilisation was successful and the diazo compound had been formed. The immobilised monolithic microcolumn was finally washed with 2 M HCl and water, and stored in a desiccator until use.

3.2.4.2 Immobilisation of *L*-cysteine

The procedure for the immobilisation of *L*-cysteine was adapted from a former procedure used for the covalent attachement of cystiene [76] and poly-cystiene[90,260]. Here, the procedure that has been developed for the immobilisation of cysteine onto CPG in batch mode was modified to allow *in situ* immobilisation within the meso/micro pores of monolithic silica microcolumn in which the reagents need to be pumped or drawn through to access the attachment sites. In summary, *in situ* immobilisation of *L*-cysteine within the microstructure of the monolithic silica microcolumn was carried out as follows: Firstly, hot 10% v/v nitric acid was drawn through the frit to clean and activate the microporoous silica structure. The microcolumn was then washed with distilled water, purged with nitrogen and dried in an oven at 80 °C, and subsequently silanised by reaction with 15%v/v *3*-aminopropyltriethoxychloride in anhydrous toluene for 3h. The microcolumn was then washed with anhydrous toluene, acetone and purged with nitrogen, then oven dried overnight at 60 °C, after which it was allowed to cool at room temperature. A

solution of 5% v/v gluteraldehyde in pH 7.0 phosphate buffer was then drawn through the microcolumn for 1.5-2 h with nitrogen bubbling into the solution to prevent oxidation. The microcolumn washed with phosphate buffer and distilled water and a solution of 0.2%m/v *L*-cysteine in pH 6.0 phosphate buffers pumped through the microcolumn with slow nitrogen bubbling through the solution during the first two hours. The *L*-cysteine solution was drawn through the microcolumn for further 24h. Finally, the microcolumn was washed with phosphate buffer, water, dried with nitrogen and then kept in a desiccator for later use.

3.2.5 Procedure for SPE of metals

The efficiency of the immobilised monolithic column to function as SPE materials for transition metals was examined in dynamic mode (see the detailed results and discussion section). Briefly, aliquots of standard solutions and samples of studied ions, Fe, Cu and Co, were loaded into the miniaturised column in the flowing system. The column was then washed with a buffer to remove any residual metals within the pores or transport lines, and subsequently the ions sequestered by the immobilised reagents, were stripped off the column with minimum amount of mineral acid (50 μl of 1.5 M of nitric acid or 1.0 M hydrochloric acid in the case of Fe) into a flowing stream of chromogenic reagent to react inside a reaction coil producing coloured complexes, before being passed through the flow cell for spectrophotometer measurement. The column was washed with acids between samples. As thiol groups of *L*-Cys-Si might be oxidised during the immobilisation process or during SPE process, a dilute solution ($10 mgl^{-1}$) of DTT in ammonium acetate buffer of pH 6 was pumped through the monolithic columns for 40 minutes before use the new column to convert maximum amount of thiol groups into the reduced form.[263] The same solution used to re-reduce the column between each set of 20 cycles.

3.2.6 Capacity study

The dynamic capacities exchange of the functionalised monolithic columns for the studied elements was determined according to Greenway and co-workers procedure [84], as previously described in chapter 2. In this study, standard solutions of metals were prepared in the range 10-1000 μgml^{-1} in ammonium acetate buffer at the optimum pH. Three repeated analyses were made at each concentration and the results were evaluated in term of peak height. The concentration beyond which no further increase in peak height was observed was considered to represent the dynamic capacity limit. The dynamic capacity limit was evaluated using the equation (2.2) as described in chapter 2. All the parameters are similar except the mass; *m* is the mass of the functionalised monolithic silica materials inside the capillary in gram. It is estimated by comparing the weight of a column containing the monoliths with its weight after the monolith being removed mechanically, or compared with empty capillary. The elemental analysis was also used to calculate the total amount of immobilised reagents. For this purpose, the immobilised silica mass was removed from the glass capillary in which it had been prepared by the application of high pressure from one end. The material was powdered, weighed and kept in dry desiccator.

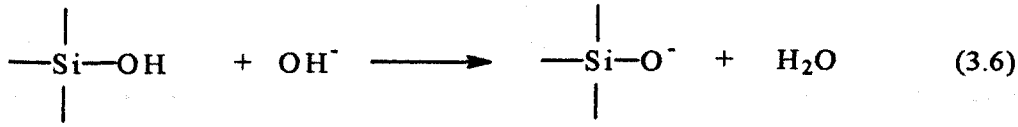
The immobilised HQ monolithic silica was characterised by measuring the nitrogen content in 0.1 g portion using CNH elemental analysis performed with a CHN Fisons Instrument EA1108 (CE Instruments Ltd., UK) as in described in chapter two for immobilised CPG. Sulphur content was determined to estimate the amount of immobilised *L*-cystiene.[264]

3.3 Results and discussion

3.3.1 Preparation of microporous monolithic silica

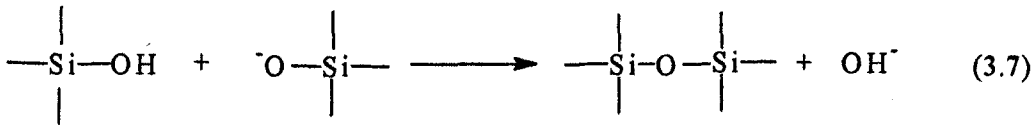
The polymerisation of potassium silicate solution resulted in a monolithic structure of white solid materials with ceramic appearance. It was chemically bound to the wall of the capillary column through surface silanols. The examination of the silica structure by electron microscopy (see Figure 3.1) showed that these materials possessed sponge-like structures consisting of a rigid silica backbone having pore size distribution from 0.3 to 2.4μm with a distinct maximum near 2.0 μm. The physical geometry was random, without any regular lattice structure. Chemically, the synthesis of these monolithic materials is believed to take place in two stages.[253] In the first, the initially formed $Si(OH)_4$ polymerises (condenses) to form colloid particles, ideally at pH 10-11. In dilute solution, a slow increase particle size can take place, but at a concentration of about 1 % of silica, these primary particles are able to polymerize further to form a gel with porous continues structure, extending throughout the medium, thus imparting a certain degree of rigidity upon the gel material. In both stages of polymerisation, the mechanism is the same, that is, condensation to form Si-O-Si links, but in the first stage, condensation leads to particles of large silica, while in the second stage, the number of Si-O-Si linkages between particles is fewer in number than those within the particles themselves, because it is difficult to fit two particles accurately together over a common face. Thus, the adjacent particles exist in a fixed position relative to one another, in order to form a tangled network of branching chain and hence a porous rigid structure is attained. The process is proposed to take place according to the following mechanism. Hydroxyl ions in the

solution react with silanol groups to create negative charges which prevent particles from encountering each other. The reaction probably takes place as follows: [265]



(3.6)

As the solution pH drops, particle charge decrease so that condensation of silanol may occur , leading to the formation of cross-linkages siloxane cluster and eventually a three dimensional gel.



(3.7)

The rate of reaction (3.7) is dependent of the concentration of ions produced from reaction (3.6). If the pH is too low (i.e. < 5), only few SiO^- become available to assist aggregation of colloidal particles and the sol becomes more stable once again. At higher pH, more SiO^- ions will be formed and consequently the gelation is more likely to proceed.

Formamide and acetamide compounds are well known as drying control chemical additives (DCCAs) which are often added to the sol to shorten the reaction times and to enhance the drying behaviour of the gel network. [266] Investigations using Raman and small angle x-ray spectroscopy proved that the textural and structural characteristics of gel formed in the presence of formamide are highly dependent on the concentration of fomramide. The particle size, pore volume, and average pore radius increase as the concentration of formamide increases.[266] In addition the gel was found to exhibit a better resistance towards cracking upon drying. Spectroscopic studies using FTIR revealed that in formamide containing sol gel, a two-step structural evolution was observed. Condensation first preferentially leads to small

oligomers of less than six Si atoms. These oligomers then condense together, resulting in a highly interconnected structure which rapidly evolves towards the vitreous silica structure.[267] It has been noted that in the presence of formamide, the Si-O-Si bonds are stronger as a result of a more cross-linked structure. This could be ascribed to the nature of formamide as a protic solvent with high tendency to form hydrogen bonds with the reactant species, thus, provides, more extensive steric shielding around silicon preventing efficient condensation.[267] This line of reasoning appears in consistent with the results of FTIR spectroscopic study, which shows more extensive condensation in the formamide system. The contradiction may be explained by the partial hydrolysis of formamide to produce ammonia and formic acid.

$$HCONH_2 \longrightarrow HCOO^-NH_4^+ \longrightarrow HCOOH + NH_3 \quad (3.8)$$

This reaction leads to a progressive increase of the solution pH with time. Because this type of condensation is generally observed to be first-order in [OH^-], the presence of ammonia should increase the condensation rate.[268] However, adding a large amount of formamide or water leads to extreme dilution which will drive the reaction (3.8) to the right, therefore releasing an excessive amount of ammonia which may inhibit particle growth because of its adsorption on the colloidal surface.

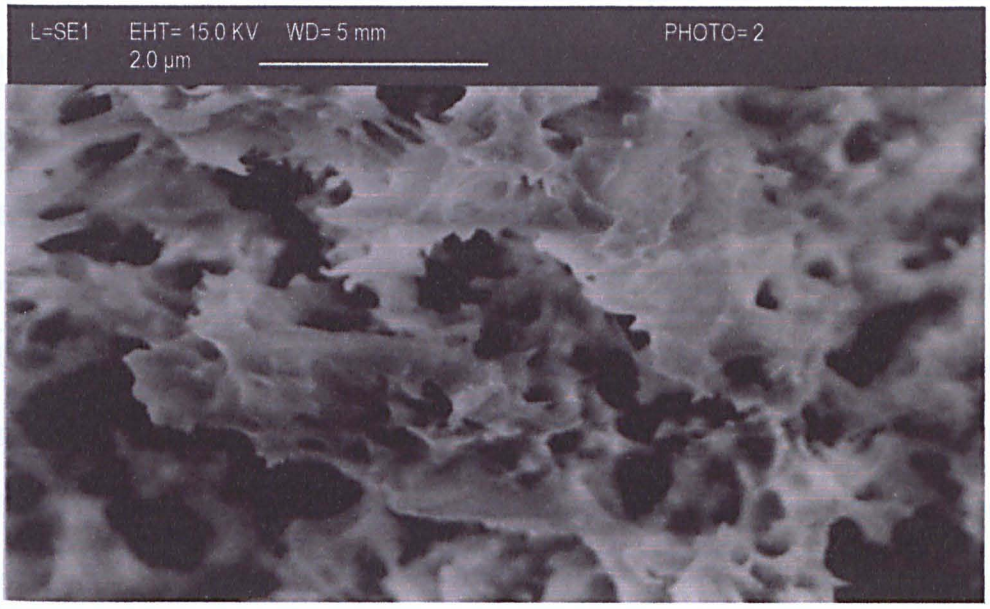


Figure 3.1 Scanning electron microscopy image of the monolithic silica materials.

3.3.2 Evaluation of monolithic silica microcolumns

The monolith's porosity was assessed by estimating the weight of water that fills its pores. A 10 mm length microcolumn was filled with water and then its weight was measured. Subsequently, the microcolumn was purged with nitrogen and dehydrated at 120 °C and weighed again. The process was repeated 4 times, and the ratio of water mass relative to dry mass was used to estimate the porosity. The porosity of the monolithic silica microcolumns used in this study was in the range 84-90%. These values are slightly higher than a previous ones reported by Lofthouse *et al.*[85] for monolithic materials prepared with 50% potassium silicate diluted 1:1 with water but comparable with that reported by McCreedy and co-worker. [101]

3.3.3 Modification of immobilisation procedures

The covalent immobilisation of cystiene within the porous structure of the miniaturised monolithic silica was carried out according to a modified version of a previous procedure originally used for batch immobilisation onto controlled pore glass (CPG). [76] The 8-HQ attachment was effected by adapting the procedure described in chapter two. Where the support sites are presented *in situ,* the reagents had to be cycled throughout the materials. It was reported that reagents could be moved through the support more effectively if they were drawn rather than pumped because the effective pressure built up, that may cause leaks, becomes less significant.[101] Moreover, drawing reagents through the monoliths would make key experimental parameters such as heating and oxygen removal more accessible. It is believed that the time dependent stages of the procedure and reagents concentrations have to be increased in order to enhance the interaction (contact) opportunity between reagents and the immobilisation sites within the support.[85] Nevertheless, the early results from this work showed that a prolonged silanisation reaction and/or extended time for coupling with glutaraldehyde decreased material porosity significantly. This is due to the fact that silane and glutaraldehyde tend to polymerise forming multilayers of oligamers that could build up and partially block the meso and micro porous structure of the support.[269] Thus, moderate concentrations of reagents and short treatment periods with reversing the flow, were considered as a compromise resolution. Additionally, the distressing of the elastic property (deformation) of peristaltic pump tubing by organic solvents such as toluene necessitates the use of solvent resistant tubes.

The progress of the two immobilisation procedures can be traced visually by the naked eye. In case of *L*-Cys-Si, the spread of light brown colouration down the entire microcolumn during treatment with glutaraldehyde, which is subsequently turned into reddish/brown when *L*-cystiene solution was drawn through the monolithic columns in the final step, indicated the accomplishment of the immobilisation. Similarly, the immobilisation of HQ within the porous structure of monolithic silica can be distinguished by the observation of the development of reddish brown colour during the silanisation using aminophenoxy- propylsilane, which turn into a deep red colouration in the final step.

3.3.4 The initial flow injection manifold

The functionalised monolithic silica microcolumns were produced with the aim of being incorporated into FI systems to examine their ability to perform on-line SPE process in the analysis of transition metals. Initially, the flow injection system, in Figure 3.2, was constructed employing a HPLC pump to overcome any high back pressure that might result because of the nature of material microstructure.[101] This set up was designed originally to study the hydrodynamic properties of the microcolumns for aqueous solutions (e.g., buffers and acids), as it is flexible enough to apply different pressure over an extended range. To use this manifold to carry out the SPE process, buffered standards and sample, and eluent were injected into buffer stream (carrier) via two injection loops. The remarkable advantages of this manifold are the construction simplicity and the capability of introducing accurate volumes of sample and eluent with excellent repeatability. However, in this manifold, samples have to be buffered off line, as it does not permit on-line buffering. In addition, the analysis time was rather long because the system size is still somewhat large, since another pump (i.e., peristaltic) was needed to deliver the colorimetric reagent required for

spectrophotomeric derivative of the eluted metal ions. Also it is not simple to remodel it to operate in an automated manner to suite continues monitoring.

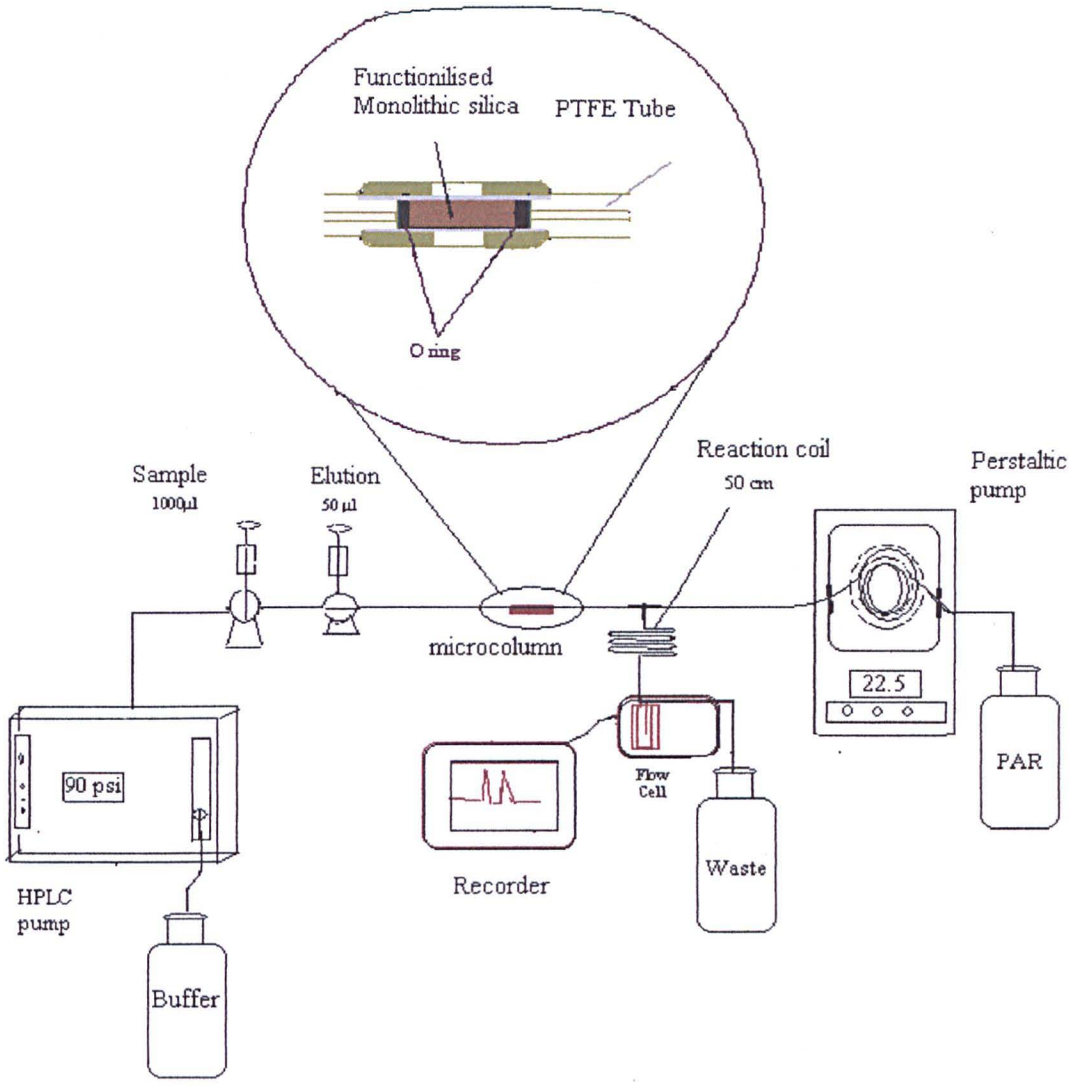


Figure 3.2 Initial flow injection manifold (High pressure).

The flow injection manifold was coupled with UV/Vis spectrometer as a compact and low cost detector for metal ions. The FI manifold allows the eluted metals to be mixed on-line with selective chromogenic reagents to form coloured complexes prior to the spectrophotomeric measurement. For this purpose, 4-(2-pyridylazo)-resorcinol (PAR) was considered to be adequate due to its general selectivity for transition metals. This

reagent is characterised by the ability to form coloured, water-soluble complexes with many transition metal ions with molar absorptivity in the order of 10^4 at maximum absorbance (λmax) in the range 520-540 nm at neutral or alkaline pH.[191,270] It was employed in this work as a general chromogenic reagent to monitor metal ions throughout the investigation of resins characteristics (e.g., capacity exchange and optimisation of pH) from standards solution. The applicability of the developed system in the analysis of real sample was demonstrated using the highly specific and sensitive reagent, ferrozine, (a monosodium salt of 3-(2-pyridyl)-5,6-bis-(4-phenylsulfonate)-1,2,4-triazine). This reagent reacts specifically with Fe (II) at pH 5.5 to form an intensely coloured magenta complex with iron, $(Fe(FZ)_3$, where FZ is ferrozine); its absorption band has a maximum value at 562 nm (molar absorptivity is 2.8×10^4 L $mol^{-1}cm^{-1}$).[260] Since this reagent is selective for the determination of iron (II) (i.e., Fe (III) does not form coloured complexes with ferrozine), Fe (III) has to be reduced to Fe (II) when the determination of total iron is required. In this work this was ensured by the addition of 2 mM of reducing agent, hydroxylamine hydrochloride, to the colorimetric reagent (ferrozine). Thus the eluted ions were converted into Fe(II) and simultaneously derivatised on-line in the reaction coil before being passed the flow cell for the spectrophotometeric measurement.[271, 272] Figure 3.3 shows the absorption spectra of the reagents with the studied metals at the optimum pH values along, with their chemical structure.

The detection system was optimised in respect of the length of reaction coil. Coils made of 0.5 mm i.d. PTFE tubes of different length in the range 15 to 100 cm (from the microcolumn to the spectrophotometric cell) were examined. As Figure 3.4 shows, a length of 25 cm is sufficient for the colourimetric complexation reaction to occur. Longer coils may enhance the dispersion and resulted in broader peaks. Thus this

length was used as the optimum length in all investigations followed throughout this chapter.

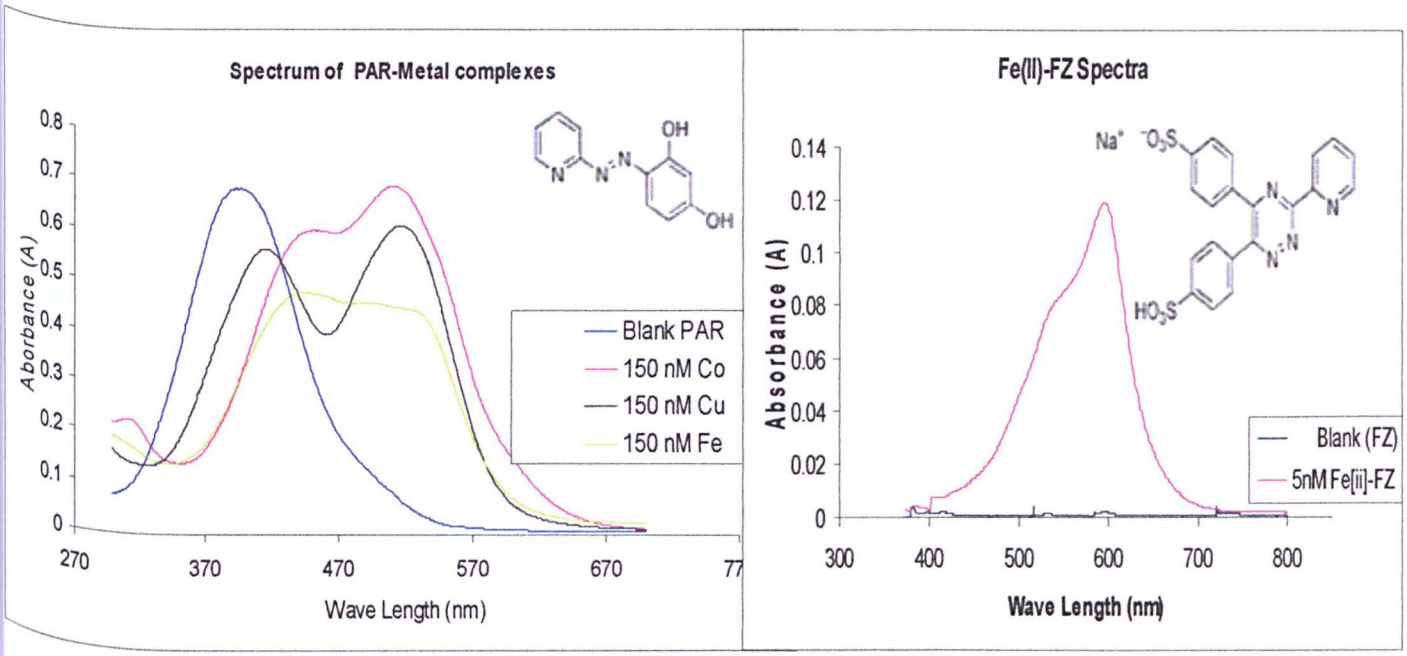


Figure 3.3 Absorption spectra of metal complex with the chromogenic reagents.

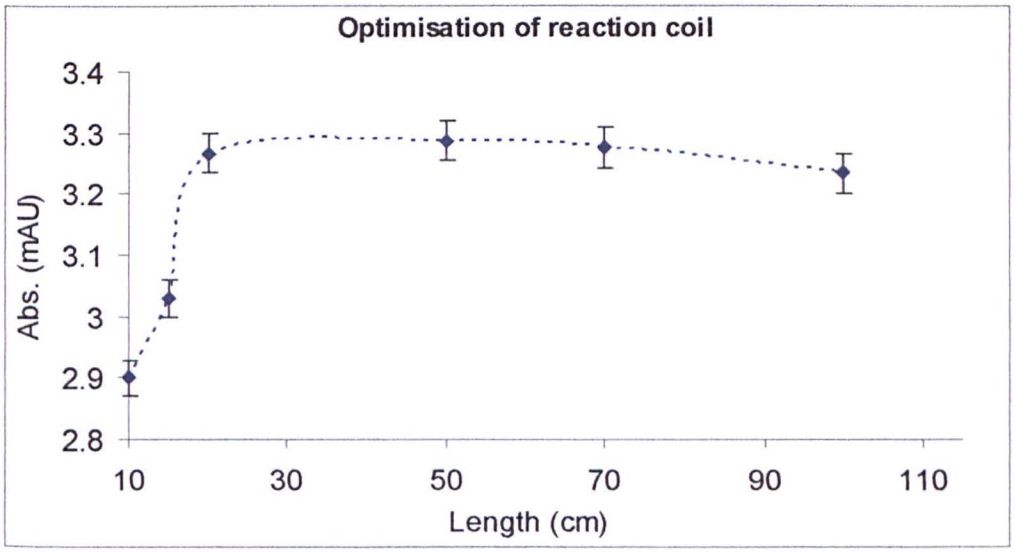


Figure 3.4 Effect of reaction coil length on complexation reaction (evaluated using the reaction of 0.4 μ gml^{-1} Cu with 50 nM PAR).

3.3.5 Capacity exchange and hydrodynamic studies

The exchange capacity of the functionilised microcolumns, *L*-Cys-Si and 8-HQ-Si was determined for the studied metals, Cu, Co and Fe in hydrodynamic mode using the high pressure manifold. The measured values are represented in Table 3.2 and compared with capacity values calculated based on elemental analysis. *L*-Cys-Si shows lower capacity for the tested ions when compared with 8-HQ-Si. This could be related to the coupling procedure; as it is believed that the bifunctional coupling agent, glutaraldehyde, exhibits self-polymerisation [269], which could diminish the porosity of the monolithic material. This may explains the low capacity of this resin for Cu when compared with the reported values for other silica materials. For example the capacity values for the poly-*L*-cysteine[260] and cystiene[66,76] immobilised on CPG were found to be 0.14 and 7.86 $mmolg^{-1}$ respectively. On the contrary, the calculated capacity for 8-HQ-Si for Cu is consistent with the earlier reported values for similar materials. In this context, Greenway and co-workers reported a dynamic capacity value of miniaturised monolithic silica 0.102 $mmolg^{-1}$ for 8-HQ immobilised within microporous silica (frit) [85], and a batch value of 0.086 $mmolg^{-1}$ for the reagent immobilised on CPG.

Table 3.2 Exchange capacity of the functionlised monoliths; 8-HQ-Si and *L*-Cys-Si.

Resin	**Calculated capacity based on;**				
	Metal uptake ($mmolg^{-1}$)		Elemental Analysis ($mmolg^{-1}$)		
	Cu	Fe	Co	N	S
***L*-Cys.Si**	0.052±0.004	0.056±0.007	0.074±0.004		0.058
8-HQ- Si	0.127±0.003	0.134±0.005	not tested	0.118	

The capacity value also agrees with that calculated for the same reagent immobilised on PAN fibre membrane (i.e., 0.715 $mmolg^{-1}$).[273]

The flow resistance of the miniaturised monoliths was evaluated under aqueous solution.[231,274] Figure 3.5 depicts the pressure needed to deliver liquids through the microcolumns *vs.* flow rate for the bare monoliths as well as the functionalised columns. The graph reveals that the bare microcolumn demonstrates a linear relation up to 0.6 ml/min, (R^2 = 0.9935, slope = 304 $psi.min.ml^{-1}$) and proves there is no compression or dislodging for the monolithic materials out of the column, even when high pressure up to 700 psi is being applied. The relatively low flow resistance indicates that these materials have satisfactory porosity, and it is possible to sustain a reasonably high flow rate at low hydrodynamic pressure. Therefore, a low pressure pump such as a peristaltic pump can be used instead of an HPLC pump.

Similarly, the pressure *vs.* flow rate for the immobilised miniaturised columns show good linearity up to 0.5 ml/min and 0.3 ml/min with R^2 values of 0.9956 and 0.9978, with slopes values of 600 and 392 $psi.min.ml^{-1}$ for the two columns *L*-Cys-Si and 8-HQ-Si respectively. Obviously the flow resistance of *L*-Cys-Si is twice higher relative to Bare-Si. However a reasonably low pressure would be sufficient to pump solutions through this porous column. Again this elevated flow resistance confirms that the material porosity is reduced during the course of immobilisation process.

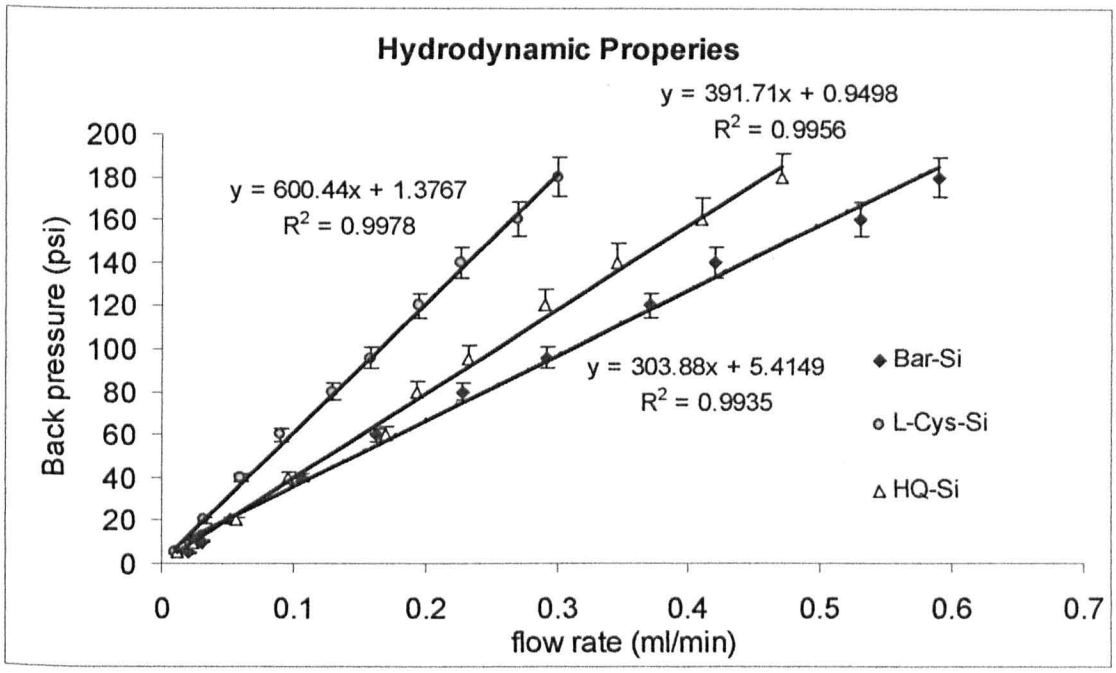


Figure 3. 5 Hydrodynamic properties of monolithic microcolumns.

3.3.6 The developed flow injection manifold

Hydrodynamic studies have proved that the monolithic materials synthesised using optimum ratio of constituents, preserve excellent porosity. The 8-HQ-Si material almost sustained its original porosity during the immobilisation better than *L*-Cys-Si, however the back pressure was relatively low for both materials. Therefore it was suggested that these microcolumns could be integrated into a miniaturised low pressure FIA system exploiting a single peristaltic pump (see Figure 3.6). Peristaltic pumps are usually preferred in FIA systems because of their ability to deliver multi streams of solutions simultaneously, hence offering more flexibility in terms of sampling and on-line sample mixing. In order to maintain the original porosity it was essential to implement a suitable filter or guard column to protect the microcolumn from any particles that might be found in standards or samples which could block the micro pores.

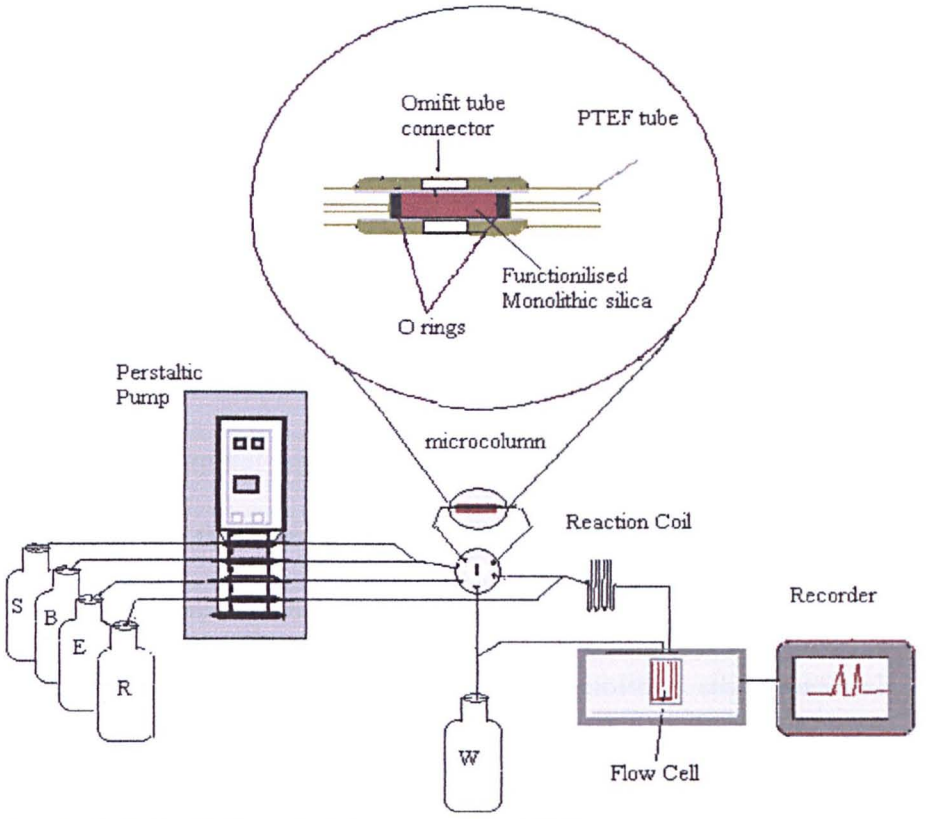


Figure 3.6 The developed flow injection manifold (Low pressure manifold).

3.3.7 Buffer effect study

The influence of buffer concentration on signal in the high pressure manifold in which standards were buffered off-line was not optimised since no real samples were analysed using this system, and standards can be prepared in buffer of the desired strength. In the case of the low pressure manifold which was used to analyse real samples, where samples and standards are buffered on-line, the buffer concentration was studied to identify the optimum concentration i.e., that sufficient to buffer the samples (usually acidic) to the right pH before being loaded into the SPE columns. This was carried out by measuring the pH of the sample before and after on-line

mixing with buffers of different strength. It was found that acetate buffer of concentration 1.5 M and pH 5.6 was sufficient for efficient on-line buffering of sediment samples to pH 5.4 (this pH also the optimum for iron determination using ferrozine). Consequently, this concentration was used throughout the rest of the work reported in this chapter.

The effect of pH on metal uptake by the two resins (Si-L-Cys. and Si-8-HQ) was assessed by studying the change in the recovery of Cu, Co and Fe (II) ions from standard solutions contain 150 $ngml^{-1}$ of each ion at different pH values in the range 3.5-7.5 in 0.5 M ammonium acetate buffer. As monolithic silica materials might be dissolved at higher pH values (alkaline media) more basic buffers have not been investigated. Roughly speaking, the studied metals showed similar trends on the two resins. The slight drop in recovery percentages beyond pH 6.5 may be due to the expected partial oxidation of thiol groups. . As can be seen from figure 3.7, Cu could

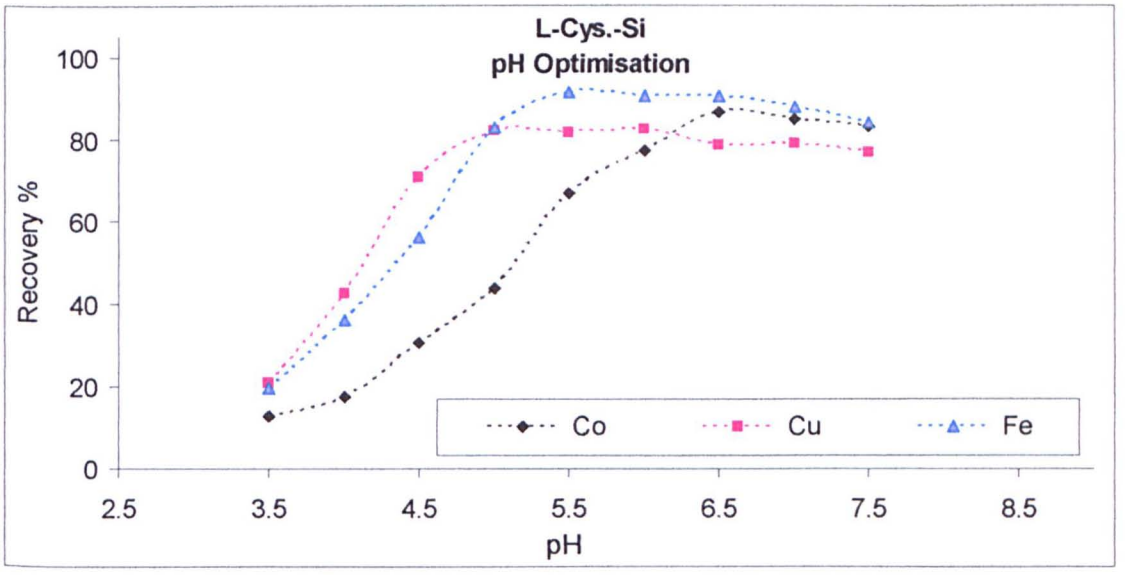


Figure 3.7 Optimising pH of buffer for SPE process on *L*-Cys- monolith.

be retained efficiently on Si-L-Cys at pH 5.0 while Co required a higher pH value, nearly 6.5. The optimum pH value at which maximum amount of Fe (II) could be retained is around the usual value; 5.5.

The pH effect on 8-HQ-Si showed general trends similar to that immobilised on CPG (see chapter 2). In general, the optimum pHs for the recovery of all metal ions were almost similar with the two resins.

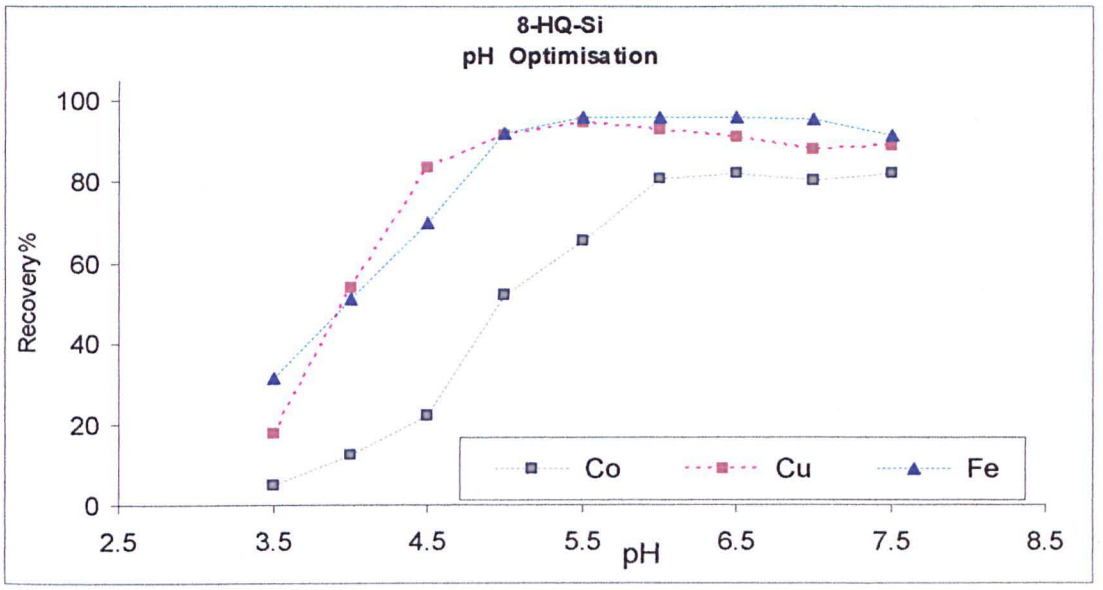


Figure 3.8 Optimising pH of buffer for SPE process on 8-HQ-monolith.

3.4 Application of the developed manifold for the SPE of transition metals

The developed manifold was used at its optimised variables and conditions established in the above sections to preconcentrate the studied ions from standard solutions. Calibrations were obtained for the three metal ions using the immobilised microcolumns operating at their maximum flow rate; 0.5 $mlmin^{-1}$ for 8-HQ-Monolith and 0.3 ml min^{-1} for *L*-Cys-Monolith. Tables 3.3 and 3.4 detail the linear range, slope

and correlation coefficients for the three ions in the standard solutions and the limit of detection (calculated as three times the standard deviation of the blank).

Table 3.3 Calibration results for the *L*-Cys-Monolith.

	Cu	Co	Fe(II)
Linear Range ng ml^{-1}	20-240	10-200	5-180
R^2	0.9943	0.9983	0.9955
RSD at 50 ng ml^{-1} (%, n=5)	2.92	2.51	2.13
Slope	0.146	0.161	0.167
LOD/ng ml^{-1} ($3\sigma_{n-1}$)	8.4	5.35	2.32

Table 3.4 Calibration results for the 8-HQ-Monolith.

	Cu	Co	Fe(II)
Linear Range ng ml^{-1}	15-300	10-250	5-250
R^2	0.9961	0.9923	0.9885
RSD at 50 ng ml^{-1} (%, n=5)	2.92	2.33	2.63
Slope	0.153	0.167	0.182
LOD/ng ml^{-1} ($3\sigma_{n-1}$)	7.1	4.23	3.12

Both microcolumns show acceptable calibration parameters and can be used effectively to analyse real samples; however 8-HQ-Monolith allows a higher flow rate; therefore, it was used for the analysis of iron in estuarine sediment solution

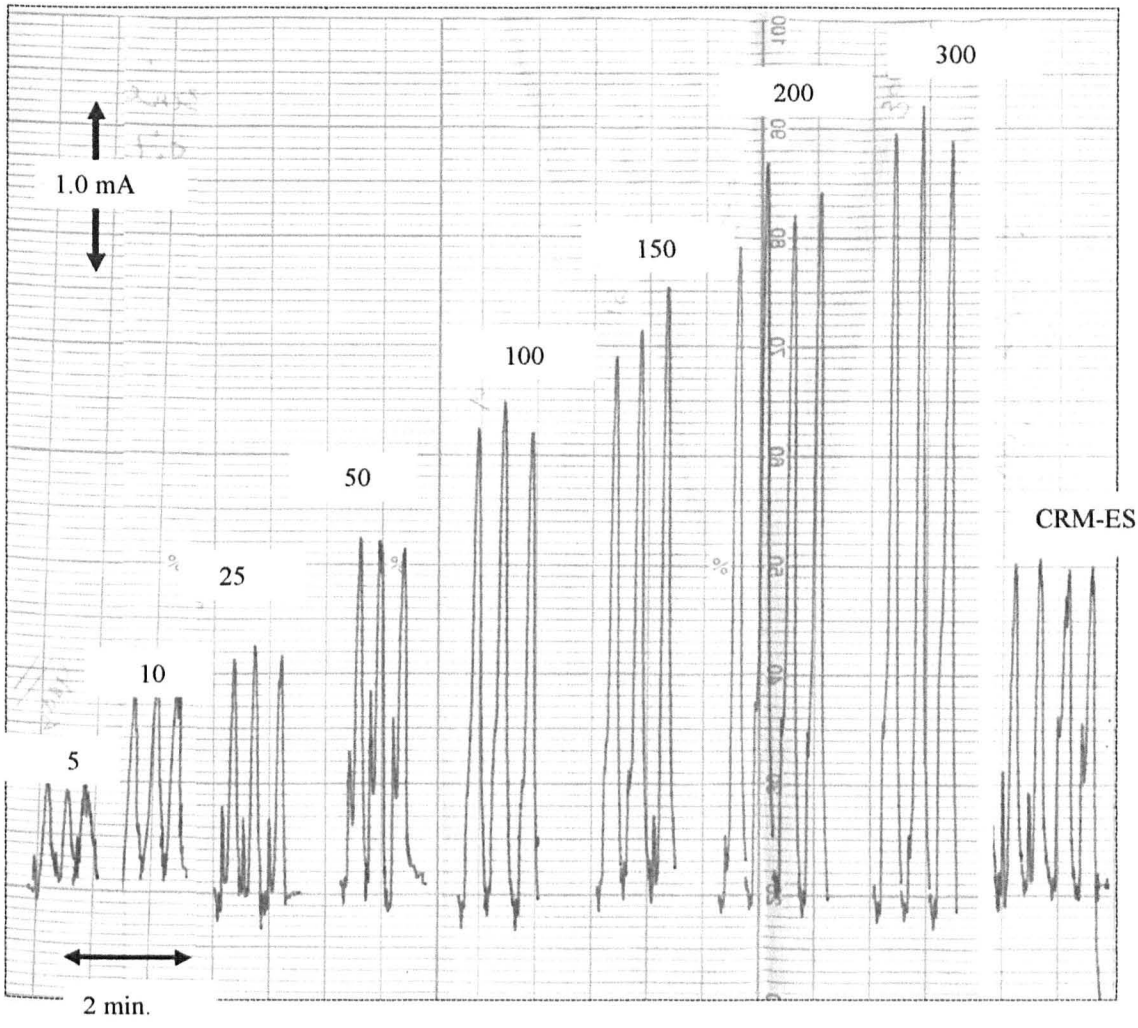


Figure 3.8 Signal profiles for standards of Fe (II). Numbers above signals are in unit of $ngml^{-1}$, and RM-ES are the signals obtained from diluted real sample.

(CRM-ES) reference materials. Since that iron concentration in the reference materials fell outside the dynamic range of the calibration graph, the sample was diluted with 0.01 M HCl prior to the analysis. The determined total iron in the reference materials (calculated as Fe (II)) from the method described in this chapter

was found to be 351.20 ±1.12 μgml^{-1}. This value agrees with the certified value *ca* 350 μgml^{-1}.

3.5 Conclusion

The development in monolithic materials over the last several years resulted in the production of materials with precisely engineered porosity from various substances. However procedures based on the controlled polymersation of organics monomers or sol-gel technology for silica, are the most common. In separation science (including SPE) porous monolithic materials are attractive because of their high surface area, good hydrodynamic for flow and mass transfer, and the simple techniques to retain them inside capillaries of microchannels without frits. Although there are many sol-gel approaches to fabricate porous silica monoliths, the process reported in this chapter is rapid and simple and allows the production of miniaturised monolithic columns of controlled porous structure. It is based on a simple gelation process (hydrolysis) for potassium silicate colloid in the presence of formamide and acetamide inside silica capillaries. The porous structure and high surface area possessed by these materials made it possible to use them as adequate supports to synthesis monolithic silica-based chelating resins that function as SPE materials for transition metals. This feasibility was demonstrated in this chapter by modifying two immobilisation techniques for the attachment of two common chelating reagents, 8-HQ and *L*-cysteine, within the meso/micro pores (*in situ*) of these miniaturised monolithic columns. The functionalised columns showed comparable capacity exchange, relative to other silica-base chelating resins, and exhibited low flow resistance for aqueous solutions in hydrodynamic systems. Therefore, it was possible to integrate them into miniaturised flow injection systems operated by peristaltic pumps, using house-built

connector, with no significant technical difficulty. The FI systems were coupled with UV/Vis spectrophotometer to facilitate on-line spectrophotometeric monitoring. The optimised FI system, incorporating the miniaturised SPE column, was demonstrated a potential applicability for the analysis of total iron in sediment solution reference materials (CRM-ES).

Chapter 4

On-chip solid phase extraction for matrix elimination prior to inductively coupled plasma-mass spectrometry analysis of transition metals

Chapter 4–On-chip solid phase extraction for matrix elimination prior to inductively coupled plasma-mass spectrometry analysis of transition metals

4.0 Aims

Initially this chapter aims to investigate the possibility of using microfabrication technology to construct a microdevice with multi channels, which can be used as a SPE device for the analysis of trace metals. Then, it demonstrates a simple technology to interface this device with the real world to effect sampling and sample preparation, and with ICP-MS to perform an on-line monitoring/quantification.

4.1 Introduction

Despite the potential advantages associated with on-line sample preparation, as mentioned in the previous chapters, there are however indispensable arguments about the cost and sample throughput, especially when instruments with high operating cost such as ICP-MS and ICP-OES are used. This is because the measurement period is only a small fraction of each analysis cycle, during the length of which the instrument is idle waiting for the next sample, thus wasting valuable resource (e.g., argon gas). To make the process cost effective, some workers recommended the use of flow injection manifold for sample preparation processing, but instead of on-line analysis, samples are collected in small vessels then introduced to the instrument by conventional means for off-line analysis.[85, 275] The process is sometimes called semi

online SPE.[276] Rationally, this procedure is expected to reduce the quantification time and hence save the instrument running cost. However there is a high risk of contamination from equipment and the valuable advantage of preconcetration could be lost by the intrinsic dilution. Also, the actual time to complete the whole analytical process might increase.

In recent years, it has been suggested that the applicability of the versatile FIA can be extended to carry out sample preparation in the field simultaneously during sampling, by making use of SPE apparatues (e.g., columns).[277] The technique was originally used to solve problems associated with preserving species during sampling and sample storage in speciation studies of metal ions.[278,279] Recently it gained a considerable recognition as a remote sample preparation tool in the field as the portable analytical instrumentation is still far behind the laboratory based instrument in term of selectivity and sensitivity. The approach is well identified nowadays as 'microcolumn field sampling' (MFS).[280,281,282] It offers many potential advantages such as, reduction in time and cost spent on sample transportation and storage, and minimisation of loss or change of sample species during transportation and storage. In this technique, samples such as sea water are processed using a simple flow manifold at the sampling site and trace metals of interest are accumulated onto the SPE materials in the micrcolumns. The microcolumns are then returned to the central laboratories and again implemented into a FI system for on-line elution and quantification.[278,279] Thus, in addition to the previously mentioned potential advantages, the running cost of laboratory based instrument employed for the monitoring and quantification can be significantly reduced because the instrument is required to operate only for a short time during the monitoring period. The principle is

also valuable to reduce the cost of elemental analysis process in laboratory based work. However, because this technique makes use of a simple single channel FI manifold incorporating a single column, the replacement of microcolumn with another during quantification requires flow interruption. Therefore, the method's precision and reproducibility would not be ensured. Also the quantification time might be lengthened because of column replacement.

In this work it was proposed that the capability of the microfabrication technology to engineer miniaturised microfluidic devices with a choice of geometrical architectures into small size substrate, can be used to miniaturise a SPE device to the level of lab on chip. The potential advantage of this device lies in the ability to fabricate as many microchannels as needed with exact similarity onto a single microdevice. Once connected to a flow injection manifold either during sample loading or throughout the subsequent desorption and quantification, the flow does not need to be disrupted as with a single column. Thus, in addition to the improved sample throughput, excellent reproducibility can be achieved.

4.2 Experimental work

4.2.1 Reagents and chemicals

All reagents used were of analytical grade. High purity water (18 Ωcm resistivity, Elgastat UHQ PS, High Wycombe, UK) was used in solution preparations and super purity nitric acid (Romil, Cambridge, UK) and elemental stock solution (1000µl ml^{-1}, SpectrosoL, Merck, Poole, Dorset, UK) were used in the preparation of standard solutions. 2 M stock solution of ammonium acetate buffer was prepared from the solid and purified by passing through a column of Chelex-100 (BioRad, Hemel Hempstead,

UK). The pH was adjusted to the required value with acetic acid or ammonia solution (20 % ammonium hydroxide, Romil).

All glasswares were thoroughly cleaned with Lipsol detergent (LIP, Shipley, UK) and soaked overnight in 5% HNO_3. Immediately before use, all acid soaked glassware rinsed with di-ionised water. Reagents and standard solutions were freshly prepared each day before the experiment was carried out.

4.2.2 Fabrication of glass microfluidic devices

The glass microfluidic devices used in this study were fabricated in house according to a rapid procedure developed by McCreedy[283] for the microfabrication of glass microchips in the general laboratory. This process uses photolithography to transfer pattern (microchannel network) to the surface of a precoated glass, followed by a wet etching to form the microchannel.

Superwhite Crown B270 glass was obtained precoated with a chromium film and photoresist layer (photronics, Bridgend, Wales). The required channels network was drawn using a CAD package or CorelDraw. The mask was produced by photo reduction of laser printed drawing onto Kodalith orthofilm type 3 (Kodak professional) at Hull University's photographic services. The mask was then placed over the glass substrate and the channel pattern was photolithographically transferred to the photoresist using radiation using a standard UV exposure unit (Mega Electronics, Linton, Cambridge, UK). The substrate was immersed in a developer solution consisting of 50% microposit developer and 50% deionised water until the pattern was clearly seen, then rinsed with distilled water to expose chromium which was then etched away with chrometch solution. Once the chips had been patterned, they were hard baked for at least 4 h at 120°C; however, more consistent results are

obtained if a minimum of 24 h is employed. After hard baking for sufficient time, the exposed pattern on glass substrate was wet etched using 1% hydrofluoric acid (BDH) buffered with 5% solution of ammonium fluoride at 65 °C for 1 h to engrave the channels pattern. The etched substrate was washed under a running tap until channel were free from deposit and debris and the remaining photoresist could be removed with microposit remover solution and the exposed chrome then removed with chrometch. The clean glass was then washed in detergent solution and dried. This fabrication approach was found to result in channels with good precision.

Thermal bonding was used to bond the top plate of Superwhite Crown B290 borosilicate (Instrument Glass Enfield, UK) incorporating 2 mm predrilled holes, used for tubing connection. In some applications the holes for tubing interface were drilled at the edge after bonding using a diamond drill. The wafers are cleaned, dried and aligned properly with the aid of a magnifying lens then placed in a furnace. Increasing the temperature slowly to below the glass transition temperature, around 600 °C, was found to result in appropriate bonding if the surfaces are smooth enough and well cleaned. A weight was also placed on the wafers pair to facilitate bonding.

The fabricated microchannels were sealed and packed with chelating resin. The wide sections of channels where SPE materials was packed from the end of the side drilled 1.6 mm i.d. hole, to the beginning of the narrow channel, indicated in Figure 4.1 as bold brown lines, was 350 μm wide and 80 μm deep in each channel. The *in situ* miniaturised sample preparation units were created by introducing etchant solution (1% HF/NH_4F) into the closed channels to fill the wide sections. The etchant solution was left in the channels for approximately 10 min. and then it was pumped out via the side drilled 1.6 mm i.d. holes. As the etchant was slowly advanced along the channel

from channel's inlet, the tapered geometry was created with the tapering occurring in both the width and depth. The process was optimised experimentally to achieve the best packing conditions.

4.2.2 Microchannel packing

The chelating agent, 8-HQ, immobilised on the CPG (prepared as in chapter two) was dispersed into acetate buffer solution containing 0.01% surfactant (Tween 80, Fulka) to enhance particulates suspension, to form a diluted homogenous slurry. A 5 ml plastic syringe equipped with pieces of 50 µl plastic pipette tip was used to force slurry into the wide channel through the side-drilled 1.6 mm i.d. inlets. To prevent small beads from accumulating into the beginning of the tapered channel, which could lead to high pack pressure within the channel or blockage, a tiny amount of large porous CPG beads (CPG-240, 80-120 mesh) was carefully placed at the beginning of the channel to work as a weir.[160] It was observed that the quality and density of the packing was improved by applying occasional sonication for short periods of 5 seconds throughout the packing process. Once the wide sections were homogenously packed to the same length, another minute amount of large beads was placed at the end of the packed large channels, and a 1.6 mm o.d. PTFE tubing, which was used to interface the microdevice with the real world, was inserted in the drilled 1.6 mm id inlets and fixed in place with epoxy glue. The CPG beads could easily be removed by disconnecting the tubing and reversing the process and this meant that if the resin was exhausted, the microdevice could be repacked with new reagent. Flushing the microchannel with a few microlitres of diluted solution of KOH in ethanol was found to be effective in dissolving the CPG beads without any obvious effect on widening the channels. The chip could be repacked as many times as required without the system collapsing. If the microchannels were visibly packed correctly, without gaps or

air bubbles, the results obtained from the packed resin were consistent. Sometimes the packing failed, leaving visible channels and gaps and the beads then needed to be removed and repacked. Figure 4.2 shows a photograph for a microdevice with the glued connected tubing ready for use.

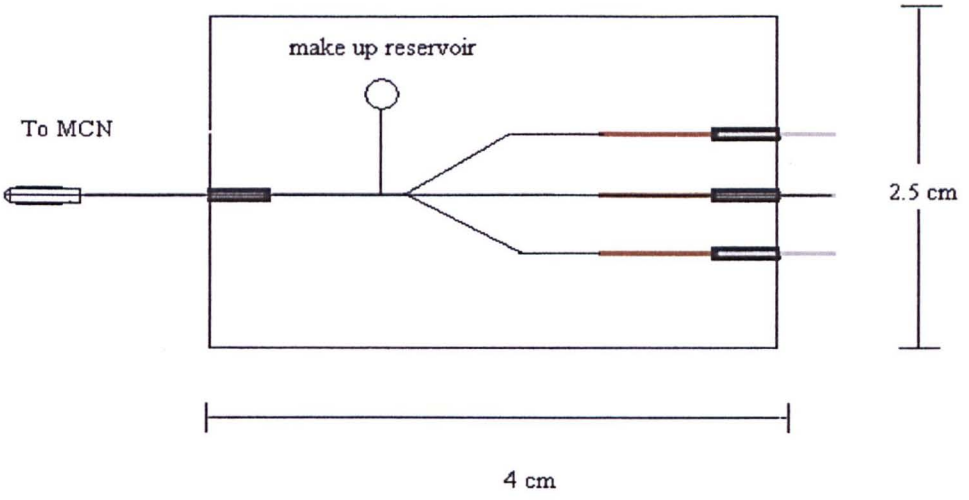


Figure 4.1 Schematic diagram for the microfluidic device with three packed microchannels (brown lines) and the glued connection PTFE tubing for its coupling with FI and the interface with ICP-MS.

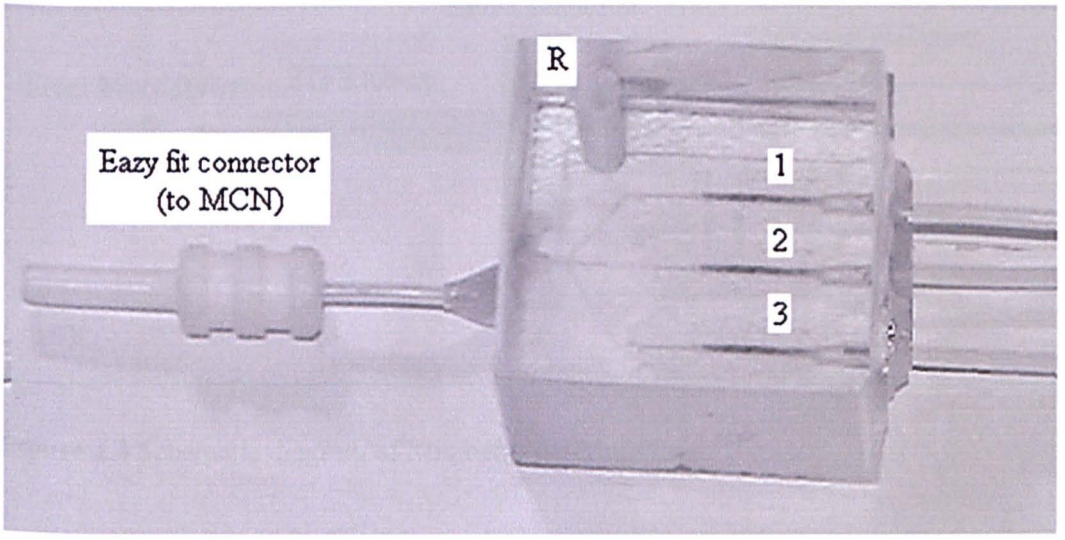


Figure 4.2 Photograph of the microfluidic device; 1, 2 and 3 indicate the packed microchannels; R , make up reservoir. The photo shows the glued connection PTFE tubing to interface the devices with the ICP and the FI manifold.

4.2.3 Microchip-ICP and FI-microchip interface

The microchip was interfaced to the ICPMS (Thermoelemental PQ2+) *via* a commercially available, low flow rate concentric nebuliser (Micromist, Glass Expansion, Switzerland).This was achieved by using a simple method similar to that previously reported by Song *et al.*[284] to interface ICP with microchip for speciation analysis by electrophoresis. Briefly a piece of 30 mm length fused silica capillary (195 µm, o.d. 75 µm i.d., Polymicro Technologies, LLC, Ilkley, West Yorkshire, UK) supported by being encapsulated inside a PTFE tube (1.6 mm o.d., 200 µm i.d., VWR International Ltd., Lutterworth, UK), was inserted into the 1.6 mm i.d. drilled hole at the exit port of the channel and held in place with a thin layer of epoxy glue (UHU plus, Germany). The other end was then directly connected to the nebuliser by using a Teflon connector (EzyFit, Glass Expansion, Switzerland) as shown in Figure 4.3

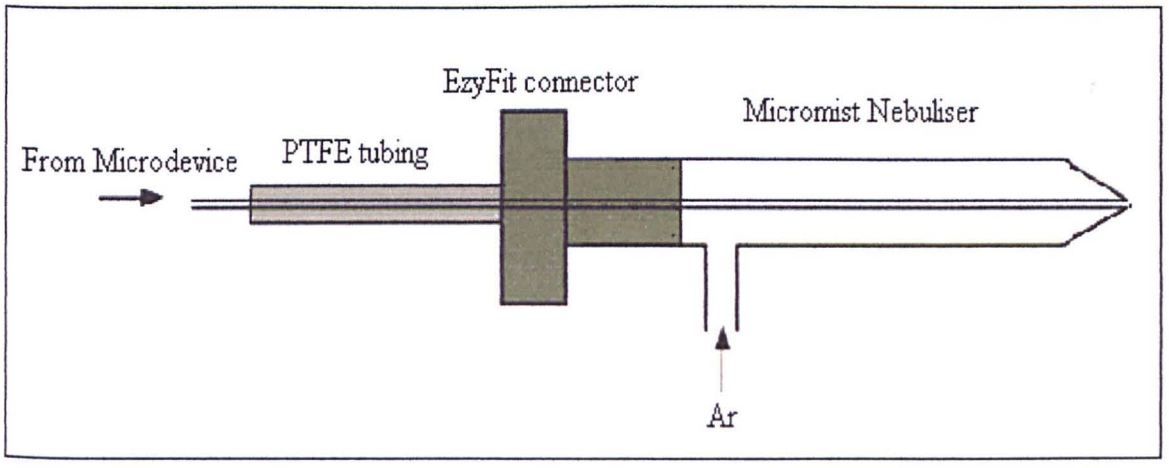


Figure 4.3 Schematic diagram of Microchip-ICP interface. [284]

A miniature jacketed (4°C) cyclonic spray chamber (Cinnabar, Glass Expansion, Switzerland) was used to obtain high sample introduction efficiency with the low flow rate.

The manipulation of solutions (i.e., on-line buffering, sample loading and elution) in this study was performed using simple flow injection manifolds constituted of a four channel Gilson Minipuls-3 peristaltic pump (Gilson, Inc., Middleton, USA) fitted with PVC pump tubing, PTFE transportation tubing (0.3 mm i.d., 1.6 mm o.d., Omnifit) and 4-way selection valves (Omnifit). To match the low flow rate allowed by the microchannels, a restriction valve was implemented in the manifold immediately behind the microchip connection junctions. Once this valve was partially open, the main stream could be split in controllable ratios with excellent precision. To minimize the dead volumes within the connection fitting, the FI-microchip interface (tubing from the microchip to the valves) was constructed from 40 mm length fused silica capillary (195 μm o.d., 75 μm i.d) supported by being inserted inside a PTFE tube (1.6 mm o.d., 200 μm i.d.). One end of each PTFE tube was inserted into the drilled hole and fixed in place with epoxy glue while the other end was furnished with a standard screw fitting and connected to the main stream via three 3-way 'T' valves (Omnifit, UK). Thus the microchip could be simply replaced with another with minimum connection fitting points.

The lowest stable uptake flow rate of the utilised nebuliser was about 42.5 μl min^{-1} whereas the flow rate through the packed channels was controlled by the splitting valve and the peristaltic pump (Minipuls 3, Gilson) to give an appropriate flow rate in the range 10-20 μl min^{-1}. The makeup solution was delivered by peristaltic pump to the makeup reservoir. These manifolds are schematically presented in Figure 4.4.

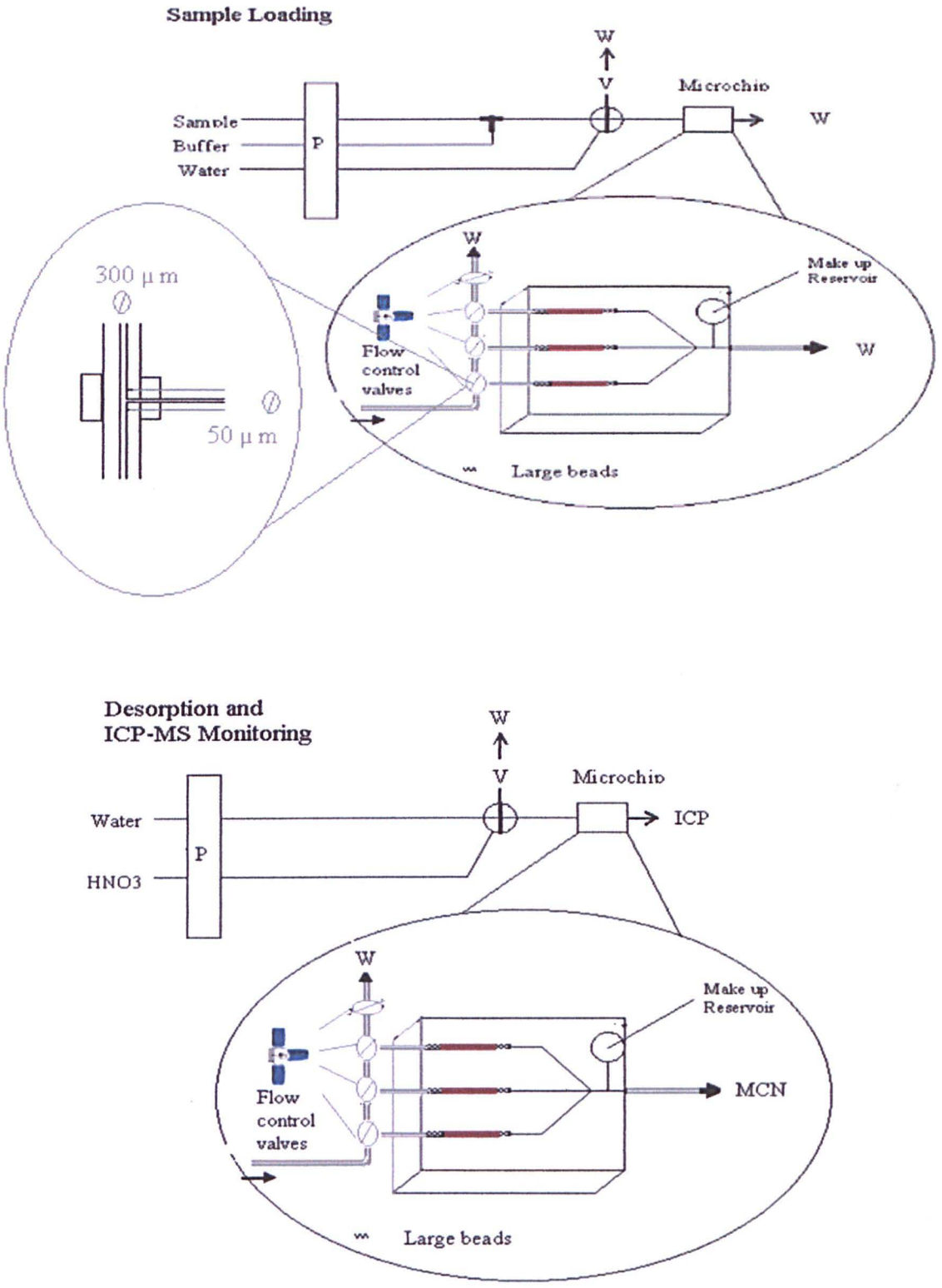


Figure 4.4 Flow injection manifolds for trace metals sample loading, and desorption and monitoring.P, peristaltic pump; V flow selection valve; W, waste; MCN, mico-concentric nebuliser; V flow selection valve; ICP, inductively coupled plasma.

4.2.5 Analytical procedure

The microfluidic device was implemented into the FI system (Figure 4.4) and a solution of 1.0 M HNO_3 was propelled throughout the system to flush any residual trace metals within the connection tubing or the microchannels for at least one hour. Afterward, the system was cleaned by running deionised distilled water through for 30 min. Once the whole system was cleaned, sample or standard was buffered on-line and loaded through the packed microchannels at a flow rate of 20 ± 2 µl min^{-1} for the required periods, then the selection valve was switched to allow water to wash the microchannels to strip off any residual non retained metals within packed microchannels or the connection tubing. The loaded microdevice was then transferred to the ICP-MS laboratory where it was incorporated into another FI manifold interfaced with ICP-MS. Once it was incorporated into the elution manifold, and a stable signal from internal standard solution (50 ng ml^{-1}) in the make up reservoir was obtained, the selection valve was switched to the 1.0 M HNO_3 position to elute the sorbed trace metals from the packed microchannels sequentially into the ICP-MS for monitoring and quantification. TRA software from Thermo Elemental was used for off-line data processing. The operating conditions during elemental analysis for ICP-MS were as in Table 4.1.

Table 4.1. The operating condition of ICP-MS system during the analysis.

Forward Power/ W	1350
Reflected Power/W	1-3
Cool Gas/ l^{-1}min	14
Auxiliary Gas /l^{-1} min	1.2
Nebulising Gas / l^{-1}min	0.890
Spray Chamber	glass, water cooled at 4 °C
Data acquisition mode	peak jumping
Point per peak	3
Dwell Time	10.24 ms
Detection mode	pulse counting
Isotopes	^{111}Cd, ^{59}Co and ^{60}Ni,
Internal standard	In

4.3 Results and discussion

4.3.1 Initial development of the microchip with packed channels

In this work it was proposed that sample preparation systems relying on the use of SPE equipments (e.g., columns), can benefit from the new development in miniaturisation technology based on lab a chip concept. In this context, the potential of microfabrication techniques like lithography and chemical etching to construct many identical features on a single glass microchip, with excellent precision, could be utilised to fabricate microchip incorporating many integrated SPE apparatus. This will make it possible to replace the conventional systems based on single column, such as these described previously in this thesis (see chapter two and three), with a single

miniaturised microfluidic device incorporating many SPE microchannels. Therefore, besides the potential advantageous that can be accomplished from device miniaturisation, multiple and parallel processing could be easily achieved.

Initially, many attempts were made to craft a short section of porous monolithic silica inside a single microchannel fabricated within a glass microfluidic device using sol gel process as described in chapter three. However, these efforts did not succeeded because it was not possible to localise the silicate solution to polymerise at the desired location within a confined short section. To alleviate this limitation it was proposed to attach the chelating reagents onto the inner surface of microchannels. Even though this method was easily practised and permitted a successful immobilisation, it was restricted by the insufficient surface area, as the preliminarily results proved that the enriched ions on the chelating reagents immobilised on the inner surface of a 30 mm length microchannel could not be quantified using the best excellent available detector (i.e., close to the detection limit of ICP-MS for many metals). Therefore, it was suggested that increasing the surface area by generating a thin layer of porous silica could resolve this dilemma. This was undertaken making use of a short capillary (50 mm length and 0.3 mm id) following a reported method designed to generate a layer of silica whiskers on the inner wall of glass capillary for open capillary chromatography.[285] In this process, a solution of ammonium hydrogen fluoride is allowed to flow through glass capillary at a very low flow rate ($\approx$10 µl min^{-1}) for about four hours in order to etch the surface. The dissolved silica in the etching process may redeposit on the inner surface because the hydrodynamic flow is not sufficient to flush it away. A baking step at 400 °C in an oven for about 8 hours is subsequently applied to affix the deposited silica layer to the capillary wall. The

successfulness of this method was evidenced from the appearance of a thin layer of white silica material on the inner wall of the capillary. The deposited porous material covering the capillary inner surface was functionalised with 8-hydroxyquinoline following the immobilisation protocol previously described for monolithic silica (as in chapter three). This coated silica capillary, however, exhibited good stability in aqueous buffered solution or organic solvent during the immobilisation process but dislodged from the capillary with acidic solutions during metals' desorption. This phenomenon may be due to the effect of acidic solution on breaking the bounding attaching the deposited silica to capillary surface. Thus, this approach was considered to be impractical for on chip applications and was not investigated further.

As both of the previous attempts failed to produce adequate solid support within the channels or capillaries, the packed channel was considered to be a good alternative. Thus, glass microchips incorporating three microchanels were fabricated by means of lithography and wet chemical etching as in the experimental section. Within each individual microchannel, a small section that could be packed with SPE materials such as immobilised bead was fabricated. Initially, a single channel was fabricated in a glass microchip device using a design developed for an immobilised enzyme packed bioreactor.[286] However, the process of packing the glass microchip was not straightforward; frequently, larger beads accumulated in the side channel and the corners, and therefore blocked the microchannel. Unlike glass microchips with PDMS cover which can be removed and cleaned and packed again, the blockage of glass microchannel covered with a glass top can be a terminal problem. Therefore, a simple straight channel was suggested to be more practical as bead slurry could be injected more easily into the straight channel than pushed from a side channel then

sucked into the packed section.[286] The straight channel design is more useful in fabricating microfluidic devices with multi channel facility as many identical channels can be etched in small substrate. Once packed and interfaced properly with the system for fluidic handling, this device will be a useful miniaturised means of sample preparation in the analysis of trace elements.

In this work the feasibility of fabricated miniaturised devices to operate as remote systems was demonstrated by carrying out sample preparation and the subsequent monitoring in different locations and at different times to resemble field work. In other words, sampling and sample preparation were undertaken at the research laboratory and then the microdevices either taken to the ICP laboratory for quantification or stored in the cold room for later analysis. This was confirmed further by stability study (see section 4.3.6).

4.3.2 Interfacing the microfluidic device with real world and ICP-MS

The microdevice was interfaced with a simple FIA system for solution manipulation. Thus the washing, conditioning and sample loading through packed channels could be accomplished simply by directing the flow through them sequentially by means of 3-way valves. The low flow rate in the $\mu l min^{-1}$ range required for sample manipulation within micro fluidic devices was achieved using the flow splitting principle. This was made possible by using a two way valve on the main stream but close to the micro devices. The valve can be used (partially opened or closed) to establish a stable splitting ratio up to 1:10 for the original main stream. All transporting lines constructing the FIA manifold were made from PTFE tubing of 170 μm id to

minimise the dead volume within the manifold or in the connection points. Also the use of small diameter tubing would be useful in order to sustain minuscule flow rates compatible with miniaturised microdevices, either directly or throughout simple splitting tools.

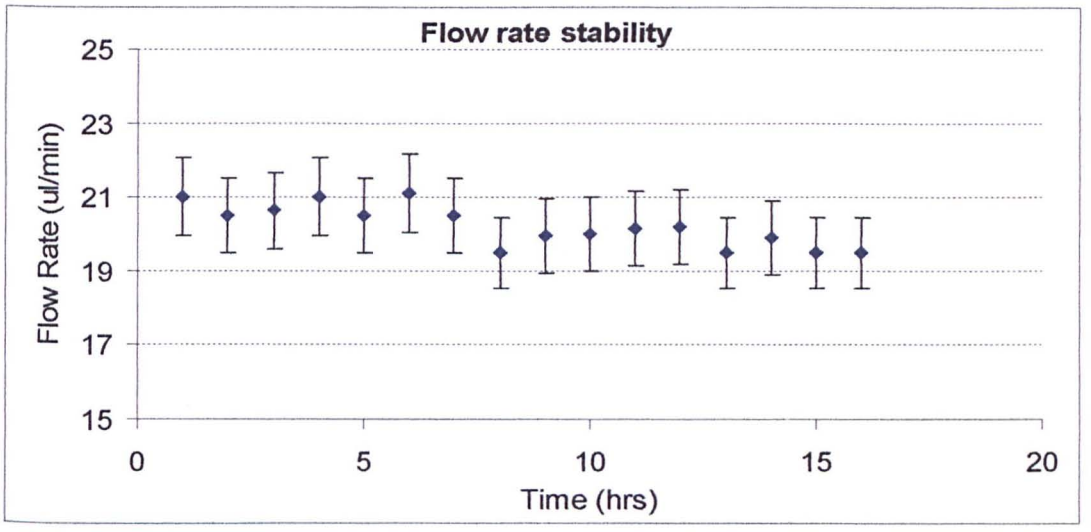


Figure 4.5 Flow rate stability at 20 μl min^{-1} maintained by splitting the main stream using a controlling valve.

Figure 4.5 shows the long term stability of the flow rate at 21 ±2 μl min^{-1} through the microhannel. The original stream propelled by peristaltic pump at 80 μl min^{-1} was split up by proper adjustment of the controlling valve. Although it is possible to use higher flow rate with packed microchannels, *ca.* 100 μl min^{-1} [286], the flow rate (21 ±2 μl min^{-1}) was chosen to avoid progressive tightening of the packed beads in the narrow ends of the tapered microchannels, which could influence flow stability.

The feasibility of packed microchannels to function as miniaturised SPE apparatus for transition metal ions was studied by the use of three metal ions, Cd, Co and Ni, as illustrative examples. These metal ions were chosen because of theire low blank. The SPE studies were conducted with standard solutions containing 5 ng ml^{-1} of each ion. The standard was buffered on-line as in the experimental section, and loaded for abut

90 seconds. The channels were then washed with buffer and water, and subsequently the microdevice was transferred to the ICP laboratory where it was implemented into an FI manifold coupled with ICP-MS for on-line elution and monitoring. A typical elution profile is shown in Figure 4.6. The elution peaks are depicted by the ICP-MS counts response *vs.* time. The peaks have basic Gaussian shapes with slight tailing. The half width of elution peaks for the studied metals are 1.8, 2.3 and 2.7 seconds, which are equivalent to 63, 80.5 and 98 nl for Cd, Co and Ni respectively. The figure also shows an interesting similarity among the peaks related to the same metal eluted from three identical microchannels. At the tested concentration, 5 ng ml^{-1}, between-channel relative standard deviations for Cd, Cd and Ni were 4.7, 6.4 and 6.8 % in that order.

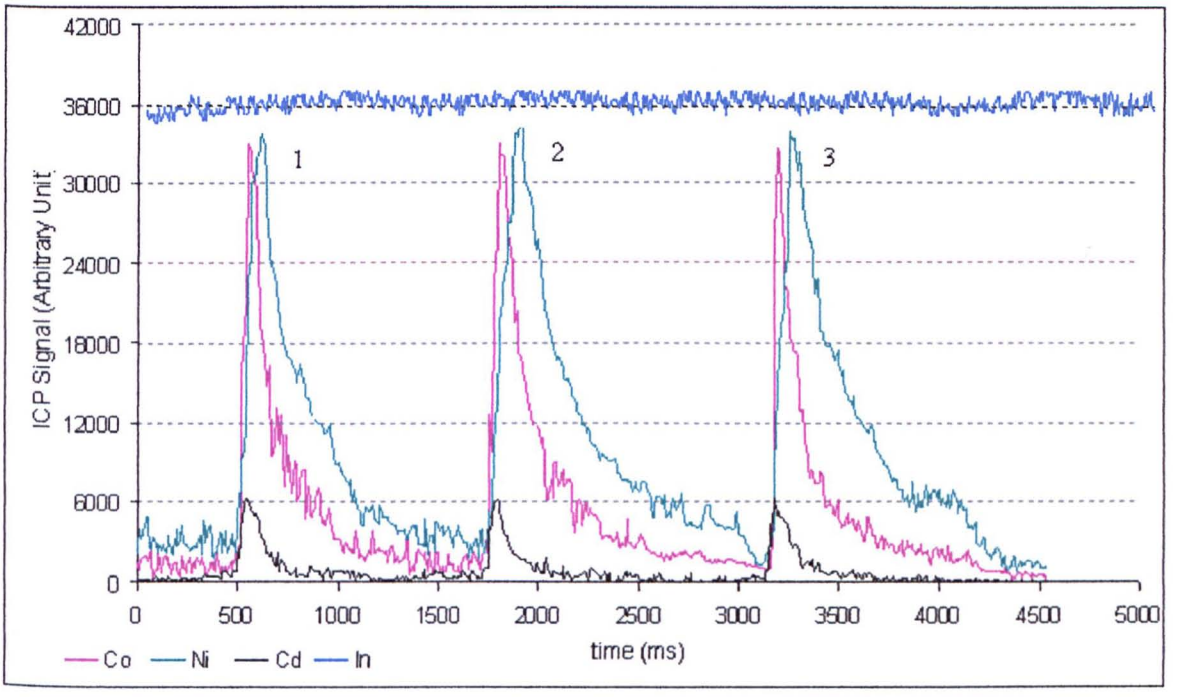


Figure 4.6 Multielement scans showing the elution profiles for studied metals in standard solutions containing 5 ng ml^{-1} loaded for 90sec. Also shown is the stable baseline signal from 50 ng ml^{-1} internal standard, In.

4.3.4 Breakthrough of packed microchannel

The breakthrough values of the packed channels for the studied elements were investigated. The test was performed using a single channel as they are identical. A standard solutions containing 20 ng ml^{-1} of each metal individually loaded for different periods of time at fixed flow rate i.e., 20 µl min^{-1}. As can be seen in Figure 4.7, the ICP-MS signals for each metal increases linearly with increased loading time for all metal ions, before reaching a plateau around 120 seconds. This is a useful procedure to estimate the saturation values for the packed microchannels, since it is not easy to measure the mass of the packed resin precisely. In addition, it gives an essential idea about the possible dynamic range in which these miniaturised devices can be applied.

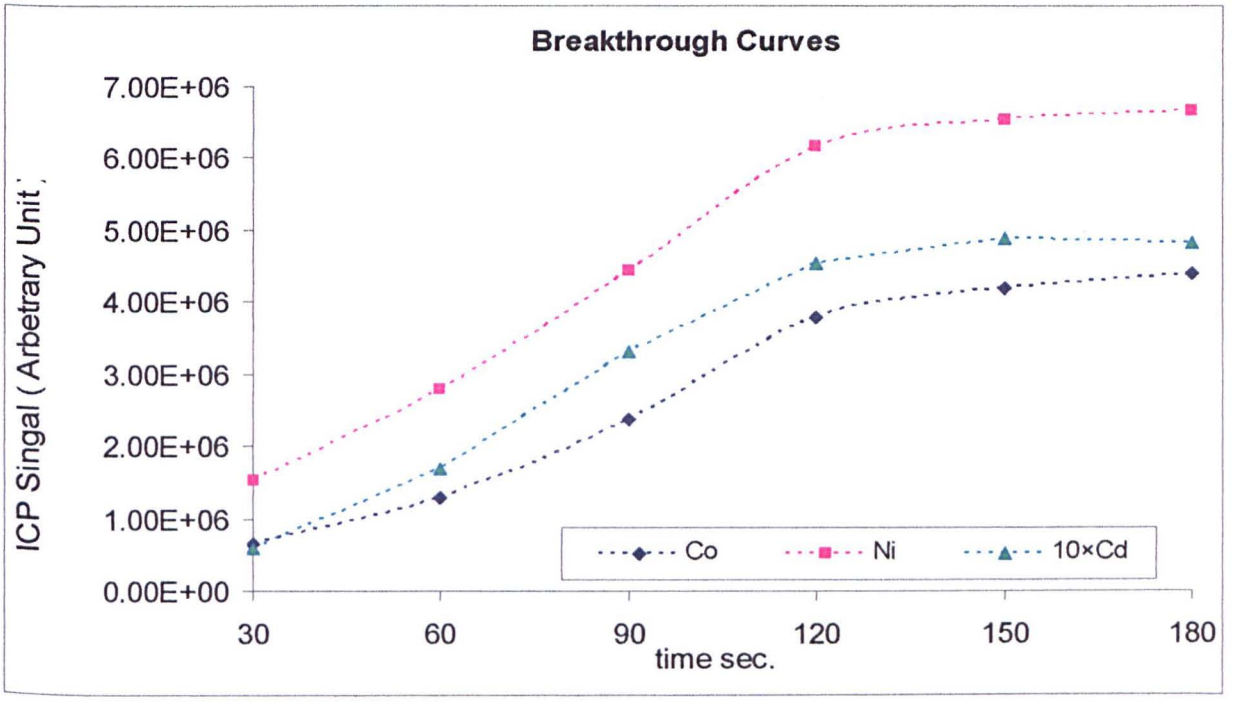


Figure 4.7 Breakthrough of the packed microchannel obtained using standard solutions of 20 ng ml^{-1} of Cd, Co and Ni loaded at 20 µl min^{-1}.

4.3.5 Calibration and analysis of CRMs

The feasibility of the constructed microdivice to function as an efficient sample preparation (eliminate matrices) devices, was examined by analysing selected trace metal ions in seawater. Calibration curves of four points across the concentration range of 0-0.5 ng ml^{-1} for Cd and Co, and 0.1-1.0 ng ml^{-1} for Ni, were generated. The calibrations were obtained by loading a series of standards prepared in acidified pure water (0.1% nitric acid), using the flow manifold, where samples are buffered on-line prior to passing through the microchannels. Then the loaded standard was eluted to the ICP-MS for quantification. The applicability of calibrations curves obtained from standards to compute ions' concentration in seawater was confirmed in previous studies by Greenway and co-workers. Their investigations proved that simple pure water calibration solutions can be used to determine analytes in more complex matrices.[84,287] The calibration parameters obtained from acidified standards are presented in Table 4.2.

Table 4.2 Calibration data for packed microchannels.

	Cd	Co	Ni
RSD at 0.25 ng ml^{-1} (%n=3)	2.47	2.09	3.03
Calibration Coefficient R^2	0.997	0.999	0.996
LOD/ ng ml^{-1}	0.008	0.006	0.009

Real samples CASS-2 and SLEW-1 were treated in a similar way. The determined values alongside with the certified values are tabulated in Table 4.3.As can be seen in this table, the measured concentration using this method agrees with the certified values. Apparently, the measured values for Cd in the two samples are bellow the

quantification limit (calculated based on LOQ = 3.33×LOD). This may explans the high recovery values (which may result from the uncertainty associated with the qunantification). However, the overall results prove the feasibility of this device as an efficient miniaturised sample preparation tool.

Table 4.3 Analysis results for reference materials CASS-2 and SLEW-1. Concentration in ng ml^{-1} (*at 95 % confident limit, n=3*).

Metal	CASS-2		SLEW-1	
	Measured (Recovery %)	Certified	Measured (Recovery %)	Certified
Cd	0.021±0.003 (110%)	0.019±0.004	0.020±0.004 (111%)	0.018±0.003
Co	0.025±0.008 (100%)	0.025±0.006	0.045±0.006 (97.8%)	0.046±0.007
Ni	0.297±0.043 (99%)	0.2987±0.036	0.749±0.026 (100.8%)	0.743±0.078

4.3.6 Stability of the microdevice

One of the proposed applications of these microchips is to be utilised for temporary sample storage. Therefore a stability study was conducted to investigate this feasibility. In this assessment, standard solutions containing 5 ng ml^{-1} of the studied ions were loaded into the packed channels, and then the microchips were stored in the fridge at 4 °C for up to 7 days. As can be seen in Figure 4.8, all metal ions were recovered quantitatively in the first four days; afterward the recovered amounts started to decline steadily to values below 50% after 7 days. Similar findings, however, have been reported by other worker for the packed minicolumns.[280] The diminution in the recovery as a function of storing time may be ascribed to the leaching out of the

retained ions with the water remaining in the PTFE tubes connected to the devices or might be due to beads drying during storing for an extended period because the low amount of water within the dead volume. However, this miniaturised device offers potential advantages to be used as an efficient tool to perform sampling and sample preparation simultaneously (could be in the field) and for temporary storage i.e., it is not necessary to take and /or process the samples and analyse them within the shortest possible time. Furthermore, as sample manipulation steps are minimised using these devices, they would be attractive tools in speciation studies to obtain a truer representation of the actual species in the original samples than with conventionally taken samples, which are subject to possible pH and temperature changes before analysis. In this respect, the use of highly selective reagents that could bond certain species is much preferred. For instance, the recently reported silica-immobilised purpurolgallin,[288] which showed high selectivity for Fe(III) in presence of other ions, can be employed as packing materials in the miniaturised devices to preserve Fe (III) from water samples.

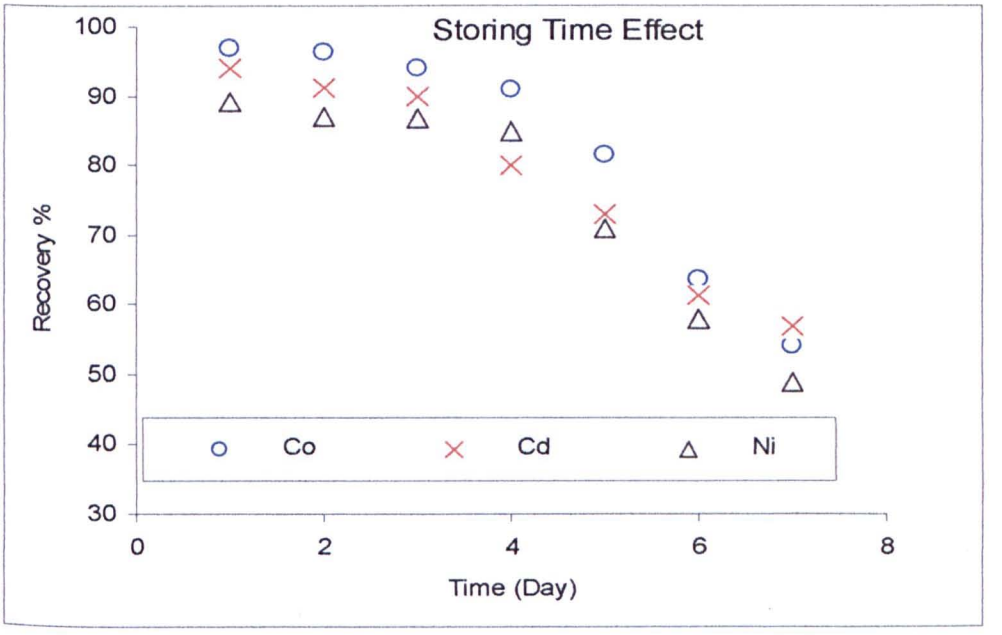


Figure 4.8 Effect of storing time on metals recovery.

4.4 Conclusion

The work in this chapter demonstrates a microfabrication process based on lab on chip technology to construct miniaturised microfluidic devices incorporating multi channels having the status of sample preparation tools. Glass microchip containing three etched microchannels, each of which incorporates a short section packed with resin, which can function as an integrated sample preparation for trace metals, was fabricated using lithography and wet etching. A technical approach to interface these devices with the real world e.g., FI system executing sample and reagents manipulation, and to effect its coupling with an ICP-MS instrument, has been described. The system demonstrated excellent performance as a miniaturised sample preparation apparatus for trace elements in seawater (i.e. Co, Cd and Ni) prior to ICP-MS monitoring. The developed procedure has been validated using seawater standard reference materials in laboratory, however, the device is ideal for simultaneous sampling and sample preparation in field based work.

Chapter 5

On chip solid phase extraction and amperometric monitoring for the analysis of transation metals

Chapter 5 – On chip solid phase extraction coupled with an amperometric detector for the analysis of transation metals

5.0 Aims

The work reported in this chapter aims to develop miniaturised electrochemical detection systems and establish appropriate approaches to accomplish their integration with microfabricated devices, accommodating integrated solid phase extraction (SPE) segments amenable for sample preparation in the analysis of trace metals. The miniaturised devices with integrated detection will allow the measurement of numerous analytes (in addition to trace metals) to be moved from the central laboratory to the field, and performed rapidly, inexpensively and reliably.

5.1 Introduction

Despite the increasing applications of microfloudic devices in many areas in analytical science since the early 1990s, their applications in the field of inorganic analysis are nevertheless limited. To date, most of the published works have concentrated on the area of biochemical analysis (*e.g.* DNA analysis), clinical diagnosis, medicinal chemistry and industrial chemistry.[289]

Over the past 10 years, there have been many published reports on the development of miniaturised systems, based on the concept of lab on a chip, for the analysis of metal ions, most frequently as separation apparatus making use of electrophoresis principles. In these miniaturised systems, different detection methods including

optical spectroscopy[290] (*e.g.*, UV-visible detection coupled via fibre optics), laser-induced fluorescence (LIF)[291], thermal lens microscope (TLM)[292] and chemiluminescence[293] have been reported.

Detection schemes based on LIF systems usually use similar designs to those utilised for conventional capillary electrophoresis (CE) instruments. Generally speaking, the combination of CE and LIF on a microfludic device is favoured because of their high performance for fluid control, separation and detection sensitivity. Thus, despite the high degree of miniaturization accomplished for the CE platform as a consequence of the microfabrication approach, the entire CE instrument may not able to reach its full potential as a portable analytical tool. Optical based detection can be problematic, as the Beer–Lambert Law predicts a proportional reduction in sensitivity as the path length is scaled down. [294]

Electrochemical detection is attracting increased interest for detection with microfluidic devices. [289, 295,296,297] A number of reviews have recently appeared on the subject.[298,299] This is in part because it scales better upon miniaturization than absorbance or fluorescence detection, since the output signal is dependent on electrode surface area rather than on available detection volume.[300] As a result, limits of detection (in concentration terms) do not degrade as rapidly for electrochemical detection as they would for optical techniques. Another advantage is that chip materials, such as polymers[301, 302, 303] which may or may not be compatible with optical detection methods, likewise can be used. The construction of electrochemical lab-on-a-chip devices also benefits from the fact that the required on-chip electrodes can be manufactured employing conventional microfabrication methods based on sputtering or evaporation of mainly gold or platinum.[304, 305]

Electrochemical monitoring based on amperometric mode has been proven to be adequate for chip-based microdevices in a broad range of applications;[299] however, most of the reported work in the area of inorganic analysis has been conducted making use of conductivity detection.[289] This is because of the nature of conductivity as a universal detection technique that can be applied to screen electroinactive species, and also due to the relatively simple experimental set up to carry out conductivity measurement.

As mentioned in chapter one, amperometry, in which the potential of a working electrode is fixed at a certain value (within the working window of the employed electrode) where the species of interest are oxidised or reduced as they pass through the electrochemical cell, is a very popular detection mode in hydrodynamic systems such as flow injection and liquid chromatography. The choice of the working electrode material is an important factor in amperometric detection. This stems from the required electron transfer between the electrode and the analyte. Carbon based electrode materials, such as glassy carbon or porous graphite, perform well for the monitoring of trace metals. Because this method relies on the application of potential to the working electrode, selectivity can be accomplished by careful selection of the applied potential and/or the choice of electrode material.[306] Moreover, in flow-through voltammetric (amperometric) techniques, the S/N ratio is increased thus the detection limit is improved through the increased mass transfer rates. This is called the *hydrodynamic approach* for improving the S/N ratio. This observation is due to the fact that surface concentrations, in conventional electrochemical cells, often deviate from the concentrations in the bulk of the sample solution due to minor ionic fluxes

from or into the sensing membrane. In flowing solutions, these concentration differences are minimized and the theoretical detection limits are approached.[307] Apparently, the detection limit of ampermetric detection is higher than the level of trace metal ions in many environments. Thus to make use of this straightforward monitoring techniques, it is essential to employ a proper means for preconcentration. In this the chapter it was proposed that integrating SPE for sample preparation and amperometric detector onto a single microfluidic will result in a very sophisticated miniaturized analytical instrument. The SPE will enrich the targeted ions to the working dynamic range of the detector and clean analytes from many interfering electroactive species present in the matrix.

5.2 Experimental work

5.2.1 Chemicals and reagents

All solutions used throughout the experimental work in this chapter were prepared in distilled, deionised water from Elgstat system (receptivity $\geq$ 18 MΩ). Cu solutions were prepared by successive dilution of 1000 μgml^{-1} (SpectrosoL, Merck, Poole, Dorset, UK) standard in 2% aqueous solution of high purity nitric acid. The buffer was prepared from ammonium acetate and pH adjusted using acetic acid and ammonia solution (20% ammonium hydroxide) (Romil Ltd., Cambridge, UK). Chelating agent solutions, oxalic acid and ethylenediaminetetraaceticacid (EDTA) (Sigma Chemical Company, Poole, Dorset, UK) and pyridine-2, 6 dicarboxylic acid (PDCA) (Fluka Chemicals, Buchs, Switzerland) were used for metal elution. The microfluidic devices incorporating three dimensional microchannels were fabricated

from Poly(dimethylsiloxane) (PDMS) (Sylgard 184 resin, ISL, Stourbridge, West Midland).

5.2.2 Instrumentations

The flow injection manifolds for reagent and sample delivery were similar to the previous systems described in chapter 4; the low pulse Gilson's Minipuls-3 peristaltic pump(Gilson, Inc., Middleton, USA) and identical PTFE tubing were used. The voltammetric system used for the studies was an EG & G Princeton Applied Research 264A potentiostat with model 303A electrode assembly (NJ, USA) and X-Y chart recorder (BBC Servogoerz Metrawatt SE790, Australia) to record the hydrodynamic voltammogram or Kipp & Zonen type BD111 chart recorder to record real time signals when systems operate in continuous mode; interfaced with flow injection manifolds.

5.2.1 Fabrication of micofluidic devices

Throughout the work in this chapter, two approaches were investigated to integrate microfluidic devices incorporating an SPE section (packed channel), with an electrochemical detector. Initially, the microfluidic devices were fabricated from glass; later, improved devices were fabricated from cheaper raw materials, PDMS, making use of a simple casting and moulding process.

5.2.3.1 Fabrication of glass microdevices

The microfluidic devices used in the initial stage of this work, were fabricated from glass making use of lithography, wet etching and thermal bonding. The microfabrication process, the procedure of resin packing into the wide microchannel

and the devices' interfacing with flow injection manifold, were accomplished following a similar procedure to that previously described in chapter four.

5.2.3.2 Fabrication of PDMS micodevices

In the developed model, the microdevices were fabricated in house from PDMS according to a prototype casting and moulding process, allowing the construction of three-dimensional (3-D) cylindrical channels. The channel network was constructed using nichrome wires of 100 μm diameter or copper wires of 50 μm diameter as templates. Straight pieces of the wire were held in place via small holes predrilled in the side walls of 50×40×30 mm Perspex mould. These wires, in contact with each other, created a microchannel manifold. One end of each wire was inserted inside a short piece of PTFE tube of 1.6 mm o.d., which fitted fit exactly the hole in the mould. The tubes were extended inside the mould to a distance of 5 mm to make a large channel when removed, and this large channel was used later to connect the device with the real world via PTFE tubes or needles instead of the reservoirs. The PDMS mixture was prepared following the manufacturers instructions (10:1), poured to fill the mould to the desired level and placed into the oven to dry at 60 °C for 1 hour. The mould was then removed from the oven and carefully disassembled to remove the PDMS device and all wires were pulled off leaving microchannels. Afterward the PDMS block containing the three dimensional microchannels was returned to the oven and heated at 120 °C for 3 hours to harden the device. The template of the wide section was made from a piece of copper tube 0.5 mm o.d. and 3-5 mm length, which was removed by dissolving it using concentrated HNO_3 leaving a wide section that was packed with the SPE materials. The fabrication stages of the PDMS device are schematically represented in Figure 5.1. This simple fabrication

procedure was proved to be appropriate for the production of microfluidic devices with basic cylindrical microchannels architecture with three dimension using low cost materials. The most obvious advantages are that the time consuming and troublesome surface micromachining usually used to produce the master template containing the channel architecture, and the technical difficulties associated with the bonding stage are eliminated. At this stage the application of this process is limited to the design of straight channels; however, it could be developed further to be adequate for the fabrication of more complex structure manifolds.

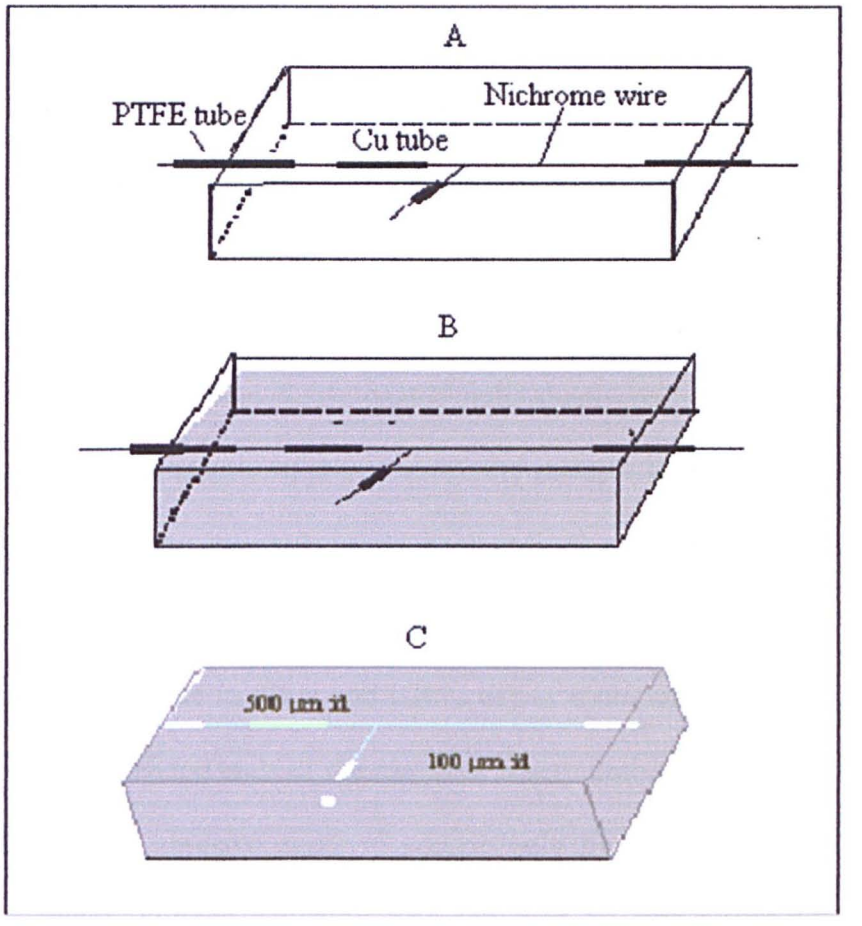


Figure 5.1 Microfabrication of 3 D PDMS microfluidic devices; **A** mould assembly; **B** filling the mould with PDMS; **C** PDMS microdevice removed from mould.

5.2.4 Construction of electrochemical detectors

Two simple models have been examined to construct miniaturised electrochemical cells from low cost materials; subsequently the feasible technical approaches for the integration of these electrochemical cells with microfluidic devices were described.

In the first model, which was developed for microdevices fabricated from glass, the electrochemical cell was constructed by assembling the microelectrodes (in microdisc fashion) in a block made of Perspex and epoxy resin. Once fabricated the block was coupled with the microdevice in a configuration that allowed the working electrode to be mounted with exit channel to facilitate off channel monitoring. This prototype was constructed according to the following method:

Three micro electrodes were fabricated from 2 cm length and 1 mm diameter glassy carbon rod as the working electrode; 2 cm pieces of 0.3 mm diameter Ag and 0.4 mm diameter Pt wirers were used as reference and counter electrodes respectively. The three pieces (electrodes) were firstly coated individually with a thin layer of epoxy resin for electrical insulation and placed vertically inside a hole drilled into a slice of Perspex which was then assembled with another 4 slices of similar size to form the base of rectangular box. The box was then filled with epoxy resin (Bostik, Leicester, UK) to hold the electrode in place and left to dry at room temperature for 24 hours. Upon drying, the extended ends of the electrodes were polished into flat surface discs using silicon carbide abrasive paper in successively finer grades (3M™ Wetordry, 400 &1000 grade). Further polishing was achieved by first polishing with 1 μm Dialap paste, and then by two polishing procedures using graded alumina (3 and 0.05 μm, respectively) in water-based slurry. The other end of each electrode was connected to copper wire making use of silver glue (for carbon) or soldering (for Ag and Pt). Heat shrink sleeves were used for complete insulation and strengthen. The

polished silver disc was coated with a thin layer of AgCl via electroplating, to form a pseudo reference electrode. This was carried out by immersing the silver disc into a saturated solution of KCl containing 0.1 M HCl. A positive voltage, from a constant power supply, was applied at the silver electrode with respect to a platinum electrode to anodise it for about 15 minutes. The current density was kept below 5 mA/cm^2. The deposited AgCl layer was found to be permanent unless mechanically removed (e.g., during polishing the working electrode). The Perspex block containing the three electrodes and the microfluidic device were assembled together using a house made holder engineered from PVC (see figure 5.2).

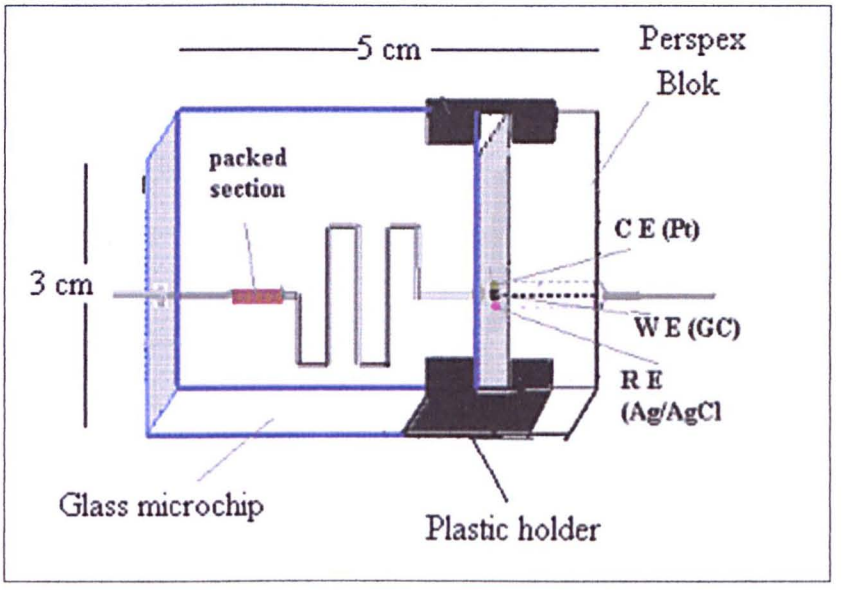


Figure 5.2 The alignment of the electrochemical detector with the glass microdevice using plastic holder; R E, reference electrode; W E , working electrode; GC, Glassy Carbon ; CE, counter electrode.

The reference electrode fabricated according to the previous process was considered as a pseudo reference electrode. It exhibited a realistically stable potential, however, it required recoating with AgCl layer occasionally. Therefore, in a later stage, it was attempted to construct a real miniaturised Ag/AgCl reference electrode. This

miniaturised electrode was fabricated successfully from a glass tube furnished with a porous silica frit. A piece of 4 mm od glass tube was heated in a flame and pulled slightly to fashion a conical geometrical shape of which the od of the narrow end was about 0.50 mm. About 3 mm of the narrow end was filled with colloidal sol-gel, prepared by the hydrolysis of potassium silicate solution, as described in chapter three, and allowed to polymerised *in situ* to form a porous plug which functioned as a liquid diffusion junction that allowed ionic conduction, but prevented contamination of the analyte solution by the reference electrode electrolyte (or vice versa). The electrolyte was filled with a saturated solution of KCl (3 M). An Ag/AgCl wire prepared as stated in the above paragraph was placed inside the electrode and attached to an insulated copper wire to the external circuit (i.e., potentiostat). The cap was made out of Teflon or plastic cap of small sample tubing; thus it was easily removable for replenishing the filling solution.

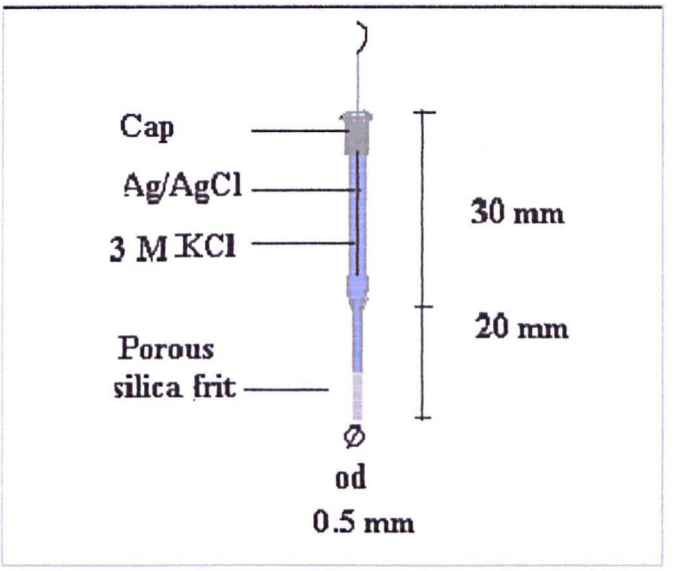


Figure 5.3 Schematic diagrams detailing the miniaturised Ag/AgCl reference electrode

The potential difference between the reference electrodes and the commercial Ag/AgCl reference electrode (Radiometer Analytical, UK) at room temperature

measured in a saturated KCl was around 0 ± 0.03 mV. The reference electrode also showed a reproducible and stable potential which was not influenced significantly by changes in PDCA or KCl concentrations and buffer pH in the range 3–9. When not in use, the reference electrode was stored with the frit immersed in a solution that was identical in composition and concentration to the reference electrode solution (e.g., 3 M KCl).

In the second approach, in which the PDMS microfluidic device was used, the elastic characteristics of PDMS made it simple to mechanically insert the electrodes inside the channel, thus the monitoring was performed in channel. The electrodes were connected to copper wires for circuit connection and insulated with a thin layer except their tips, which were then polished to form mirror like discs.

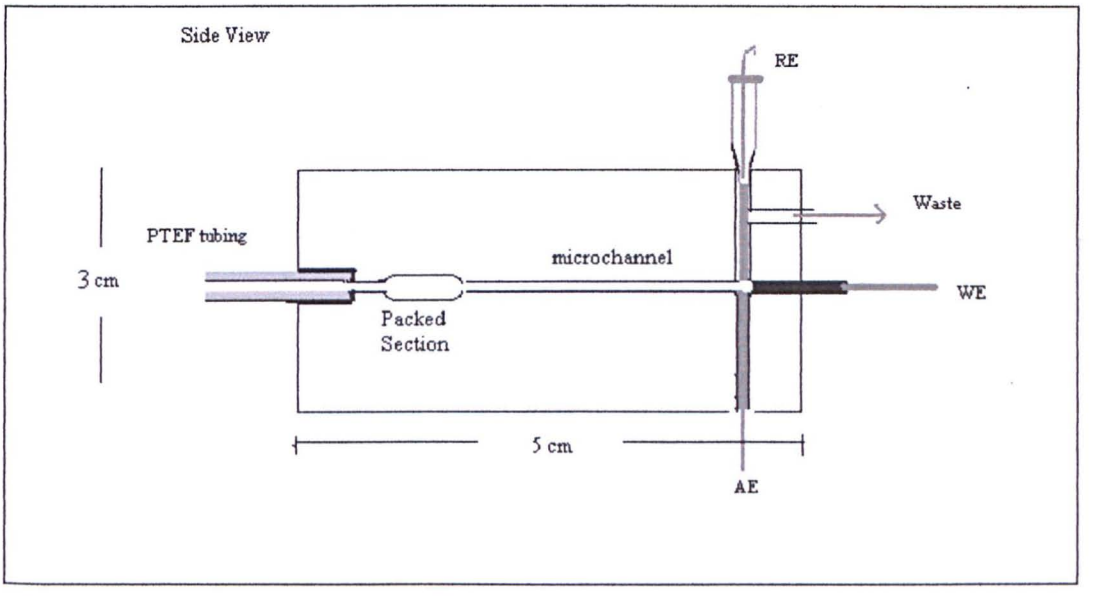


Figure 5.4 Side view of the PDMS microdevice shows the placement of the electrodes inside the monitoring channel; R E, reference electrode; W E, working electrode; CE, counter electrode.

5.2.5 Interfacing micofluidic devices with real world

During the operation the microfluidic devices were coupled with flow injection systems to execute sample and reagent delivery similar to that previously utilised in

chapter four. The flow injection manifold allows on-line buffering to be effected immediately prior to the microdevice, and enables the operator to deliver sample and eleunt reagent alternatively to the microfludic device with reasonable precision, even manually. The stream splitting principle was employed to make the reagent flow rate compatible with the microdevice. This was accomplished via a 3-way valve that enabled the main stream to be segregated into 1:10 ratio with excellent accuracy. Figure 5.5 shows the manifold alongside the detailed interface. Since the ampermetric measurement of ions is conducted in the reduction mode, it is essential to place the device inside a closed nitrogen purged box to prevent (or lessen) the chance for oxygen to interfere.

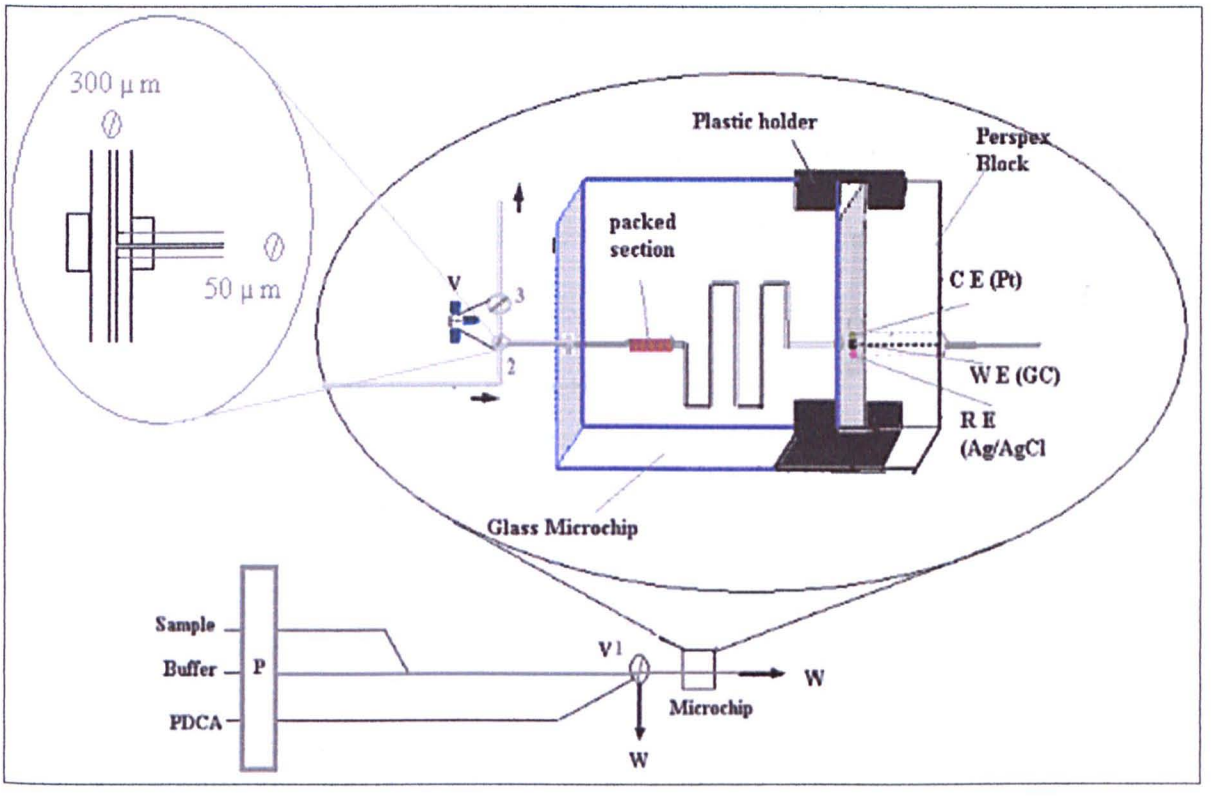


Figure 5.5 Flow injection manifold for sample and reagents manipulation showing The splitting valve and alignment of the electrochemical detector with the glass microdevice using plastic holder; R E, reference electrode; W E , working electrode; GC, Glassy Carbon ; CE, counter electrode; V, selection valve; W, waste.

5.3 Results and discussion

The core objective of this chapter was to develop a prototype of analytical system by integrating a miniaturized solid-phase extraction (SPE) with an electrochemical detector, into a single miniaturised device. Thus sample preparation and monitoring could be achieved in an on-line manner. The main purpose of ion accumulation in the small packed section (SPE compartment) preceding the detector is to improve detection limit. It is apparent that, if this accumulation is preferential, because of selective interactions between the ions and the SPE material (i.e., chelating resin) then, it can serve additionally as a separation step, thus improving the selectivity. As stated in the experimental part (section 5.2), two approaches have been attempted making use of two designs for the miniaturised electrochemical cell. In the initial stage of the study, microfluidic devices fabricated from glass were coupled with a miniaturised electrochemical cell, accommodating three electrodes, in an experimental set up that allowed off-channel measurement. Later, the microfluidic devices were microfabricated from PDMS. The inherent elastic property of PDMS enabled the electrodes to be placed inside a detection channel, thus the measurement was performed in channel.

5.3.1 Glass microfluidic device integrated with miniaturised electrochemical cell

To use the miniaturised electrochemical cell fabricated in section 5.2.4 as an efficient integrated detector for the microfluidic devices, it is essential to align the working electrode opposite to the channel outlet to ensure that the solution coming out of the channel strikes the surface of the working electrode directly. Of equal importance, the electrodes should be located close enough to ensure that there is an adequate solution

contact. In this design, the electrochemical cell was aligned with the microfluidic device making use of a plastic holder machinated in-house. A set of four screws were used to control the electrode position flexibly.

For the evaluation of electrochemical detector applicability, initial experiments were carried out to observe detector response for copper ions in different solutions resembling ones employed in the loading and elution steps during the SPE process. Standard solutions of Cu ion were prepared in the range 1-20μgml^{-1}, in 0.5 M acetate buffer of pH 4.8, 0.2 M nitric acid or hydrochloric acid. The solutions were pumped individually through the channel and the electrochemical cell potential was scanned in the range 0.0 -1.1 V. In acidic media, however, the background current was extremely high and therefore no current related to the reduction of Cu ions was monitored. This high background current originates from the reduction of excessive concentration of acidic proton, H^+, in this potential window.[308] It was proposed that neutralising acidic solutions prior to reaching the electrode would resolve this limitation. Therefore it was attempted to mix acidic effluent solution with buffer or alkaline solutions that pumped from a side channel. However, no significant change in the signal was observed. It seemed that the channel length in this designed manifold is not sufficient for complete mixing which is required to attain neutralisation. Thus, acids could not be used to elute metal ions and hence complexing agents were proposed as an alternative.

Complexing agents including EDTA, PDCA and oxalic acids have been tested. The feasibility of these reagents to elute Cu ions was examined using a mini column (packed with the resin) incorporated into a flow injection manifold coupled with

UV/Vis, similar to that used in chapter three. All reagents were found to recover Cu ions efficiently. For the purpose of this study, it was essential that the metal complex should exhibit an evident reduction peak in the potential widow of glassy carbon. Therefore, standard solutions of Cu-complexes of the proposed chelating reagents were scanned in the potential range stated above. Interestingly, no reduction peak for Cu was observed in the examined potential window with EDTA. This could be due to the fact that the reduction potentials of Cu complexes with this reagent are shifted to more negative values (outside the potential range of glassy carbon electrode). In a previous study, Omanivic *et. al.* showed that the reduction peak of Cu-EDTA on glassy carbon appeared at -0.9 V.[309] In the case of oxalic acid, the background current was relatively high, although lower than that observed with mineral acids. On the other hand, the reduction peaks of Cu-PDCA complex appear similar to the reduction peak obtained in acetate buffer. Figure 5.4 shows the voltammogram scans for series of Cu standards prepared in buffered PDCA solution obtained from flowing solution at flow rate (20 µl min^{-1}) and recorded at scan rate of 200 $mVsec^{-1}$.

$E_{1/2}$ values measured from the voltammogramic waves were approximately -0.50 V. This value was used in the subsequent studies in which the device was utilised for on-line monitoring when coupled with the flowing system.

The use of PDCA prepared in acetate buffer to elute metal ions in the SPE process was suggested to eliminate the drawback associated with background current (base line) that resulted due to the change in pH and solution composition during sample

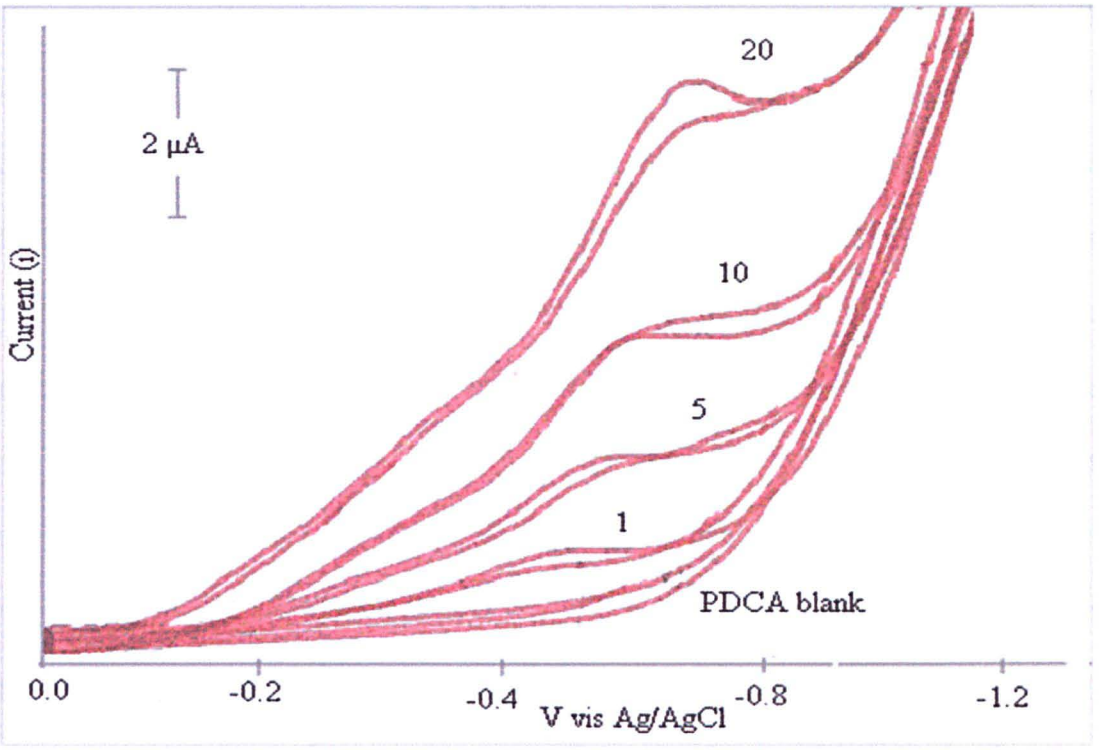


Figure 5.4 Voltammogram scans for series of copper standards in PDCA at 200 mV/sec; numbers above signals are in units μgml^{-1}.

loading and elution. This was confirmed by coupling the electrochemical cell to a simple single channel FI manifold allowing solution of 50 nM PDCA in buffer and unmixed buffer to pass over the electrochemical cell in an alternating manner while the current signal was monitored continuously. The recorded signal (not shown) indicated that there was no significant change in the baseline signal due to solution alternation. Therefore, PDCA in buffer can be used to elute metal ions in this simple system with no need to use a make up solution to correct baseline signal.

A further experiment was conducted to monitor the change in the current during sample loading and the subsequent elution; real time signal. For the purpose of this test, a minicolumn (2.5 cm length × 3 mm i.d, Omnifit) packed with the chelating resin was implemented into the FI manifold described previously and the outlet of the

flow system was allowed to pass through the microfluidic device, to be screened with the miniaturised electrochemical cell aligned to the channel exit. A minicolumn was employed in this experiment to retain a large amount of ions, and therefore an intensive signal would be monitored on the elution step. 5 $\mu g\ ml^{-1}$ Cu standards in acetate buffer was loaded into the column at a flow rate of 0.5 ml min^{-1} for 60 seconds then eluted with 50 nM PDCA in buffer. The real time output signal recorded with the chart recorder pre-set at high speed is shown in figure 5.5. The figure demonstrates that the miniaturised electrochemical detector is sufficiently sensitive to monitor Cu ions eluted using buffered 50 nM PDCA. The electrochemical cell also shows reproducible response as demonstrated by the patterns during two successive runs. In addition, the record confirms the previous finding; that the change in background current during the loading and elution process is negligible.

Once the feasibility of buffered PDCA was confirmed as a preferable eluting reagent, a further experiment was conducted to study the linear response of the detector for change in concentration. In this experiment, the electrochemical cell was integrated with the microfluidic device and interfaced with the FI manifold as depicted in figure 5.5. Standard solutions of Cu ion prepared in buffered PDCA in the range 0.10-5 μgml^{-1} were allowed to pass through the microdevice and monitored as they reached the detector without preconcentration. Figure 5.6 shows examples of the recorded electrochemical cell response (in nA) for several concentrations. The relationship between concentration and signal intensity is linear down to 0.5 μgml^{-1} with a correlation equation, $I(nA) = 15.88C(\mu gml^{-1}) - 6.74$. The correlation coefficient over the concentration range 0.5-5 μgml^{-1} is $R^2 = 0.998$ with measurement precision, RSD, at 2 μgml^{-1} (n=4) is 1.97%. The detection limit is about 0.31μgml^{-1}.

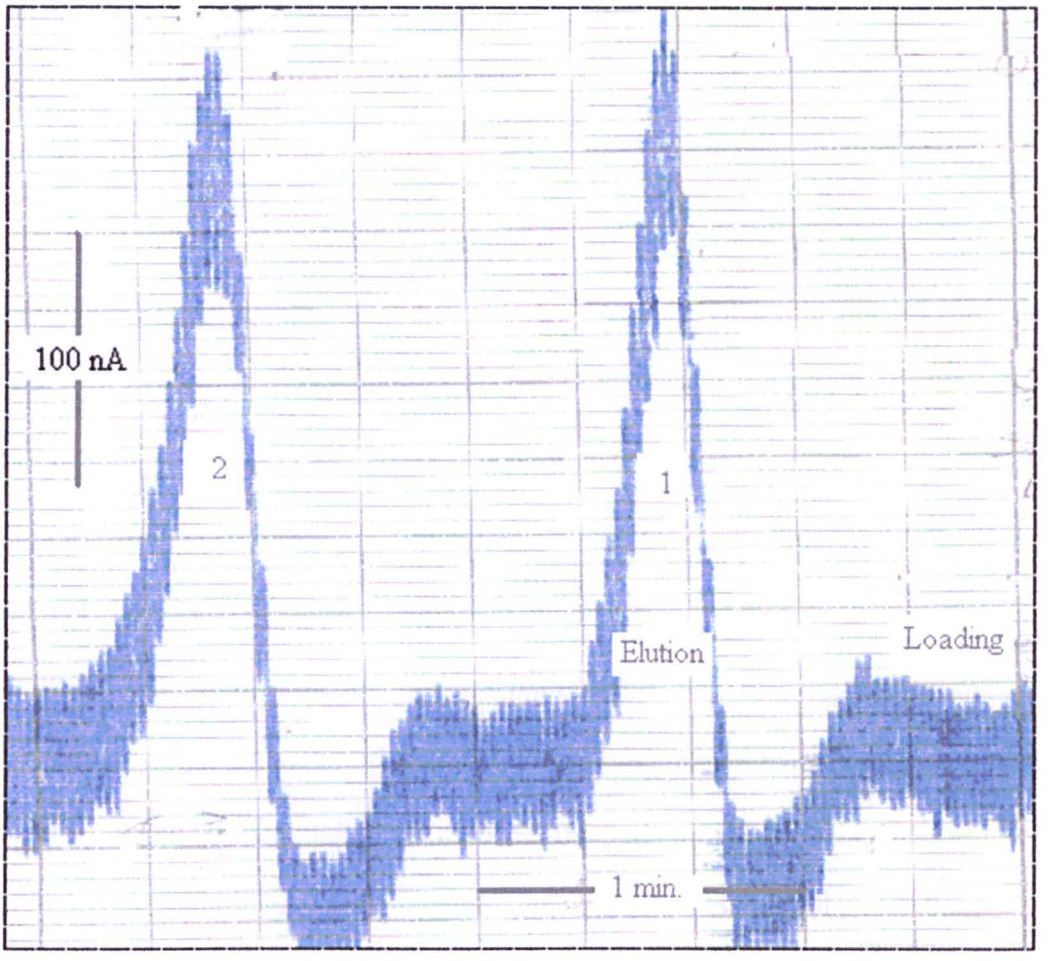


Figure 5.5 The response of ampermetric detector for 5 μg ml^{-1} Cu loaded for 60 sec and eluted with 50 nM PDCA; 1 and 2 show two replicates.

Finally, the functionality of the miniaturised system to perform on-line SPE and monitoring was tested. However, no signal was observed upon the elution of metal ions that were expected to be sequestered by the packed resin; the amount of metal ions eluted was below the detection limit. This could have resulted because the quantity of the packed resin was not sufficient to preconcentrate to the dynamic range of the detector. This is supported by the reported breakthrough values for the packed microchannels, as in chapter four. Equally important, as apparent from Figure 5.6, the relatively high background noise would prevent the detection of lower concentration. Moreover, the reproducibility of microelectrode placement at the same distance with

respect to channel exit, when the system was disassembled (e.g., for electrode cleaning) and reassembled, was poor.

In order to resolve this significant shortcoming, two methods were attempted. These were, fabricating devices with larger microchannels to allow the packing of more resin and to minimise noise by placing the electrode inside the channel. Therefore microfluidic devices with PDMS top, housing the electrodes, were fabricated. Although initial experiments demonstrated that the noise was significantly reduced and the detection limit also improved as the electrodes placed over the channel, however, non-controllable leakage was experienced with this option, and hence it was considered to be not practical and finally rejected. At later stage of the work in this chapter, it was proposed that microfludic devices with 3 D microchannels could be fabricated by the use of a simple casting and moulding procedure.

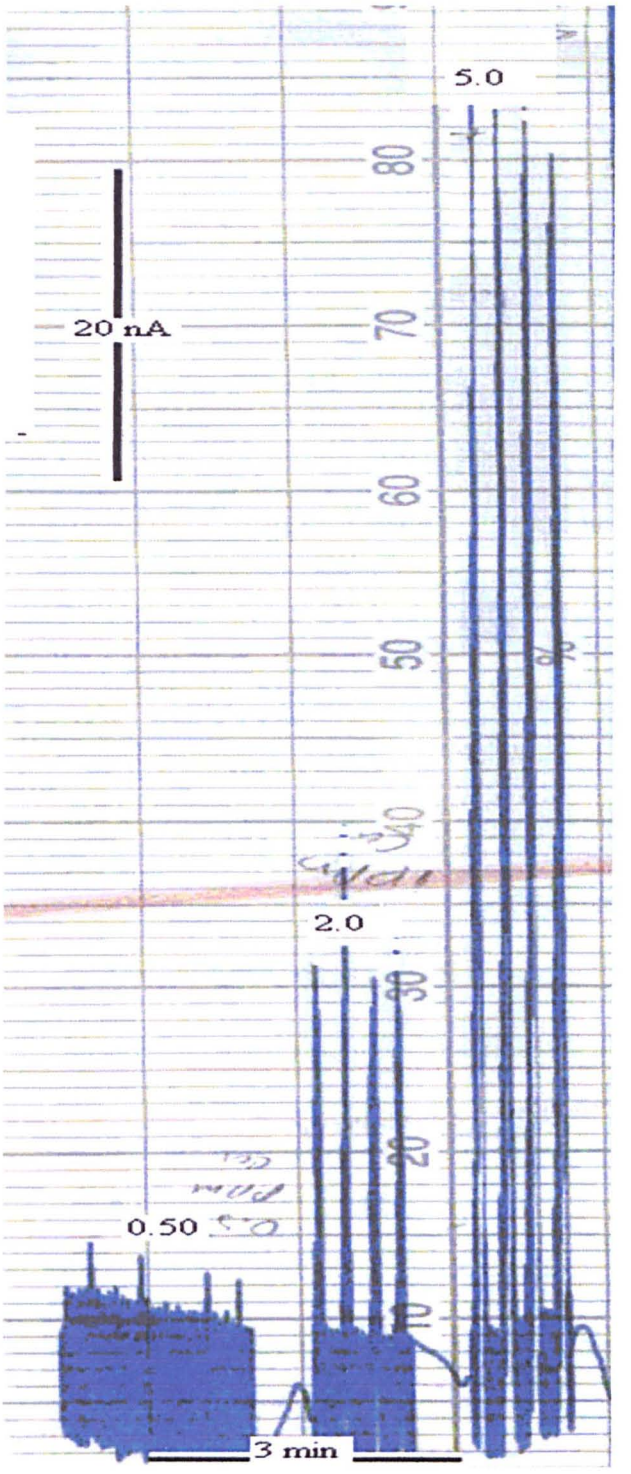


Figure 5.6 The response of end-channel electrochemical detector at -0.50 V for series of Cu standards in 50 nM buffered PDCA of pH 5.4.Sample were injected with 2 µl loop implemented in the flow injection manifold prior the splitting valve and monitored direct without preconcentration.

5.3.2 PDMS microfluidic device integrated with miniaturised electrochemical cell

PDMS microchips are commonly fabricated using a master as a mould and the result is then an open structure that often needs to be sealed with unstructured or structured lids by utilising different bonding and alignment procedures. Despite the extensive development of these procedures, the production can still be time-consuming and requires specialised facilities (e.g., for master production). The technology described in this work allows the fabrication of three-dimensional (3D) microfluidic networks entirely made of PDMS, making use of a different approach. A similar method has recently been reported to construct microchannels in PDMS substrates.[310,311,312] As demonstrated in section 5.2.4, premixed PDMS was cast to cover metal wires of 100 or 50 μm diameters, which define the channel network when removed in a subsequent step. In this procedure, the problems associated with alignment and bonding were avoided, and the fabricated device exhibited higher mechanical strength and was thus more appropriate for high-pressure applications. The metal wires also provide cylindrical channels, which facilitate the fluidic connection to be accomplished making use of fused silica capillaries. However, in this work PTFE tubes were employed as templates to cast the connection points. Since PDMS is an elastic material, tubes, capillaries or electrodes can be inserted into channels with 50% smaller diameters, resulting in minimal dead volumes.[311] This provides advantages when it comes to interfacing the microdevice with the real world, such as when it is coupled with a system facilitating sample/reagent introduction. In addition the integration of miniaturised electrodes for monitoring could be easily accomplished. Therefore, the poor reproducibility associated with electrode alignment encountered with glass microchip, could be eliminated. The other problem associated with the

limited capacity of the packed section in glass microdevices was resolved by casting a wider channel in which it was possible to pack more beads.

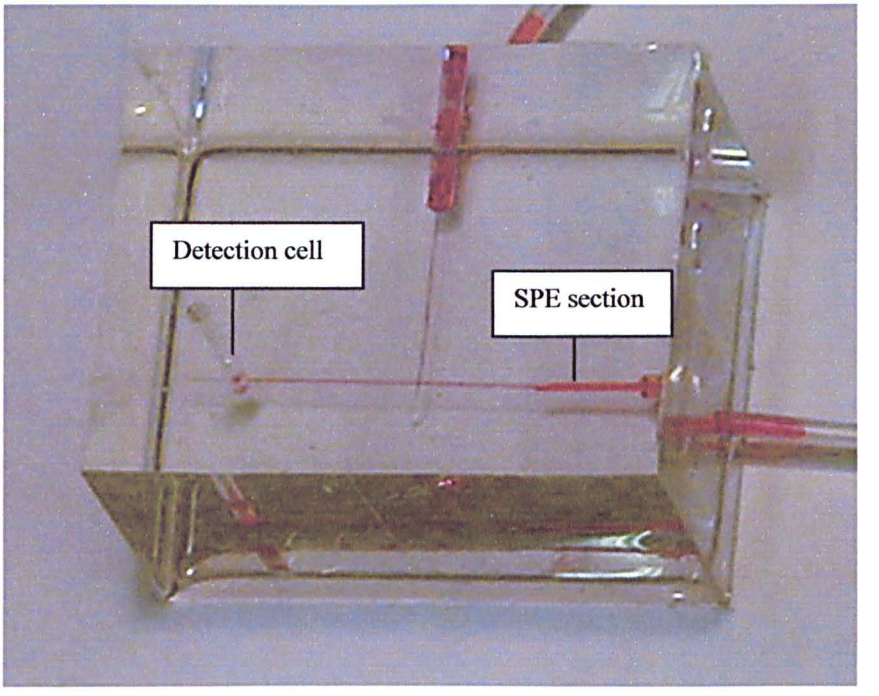


Figure 5.7 Photograph of a PDMS device showing the 3 D microchannels.

Figure 5.7 shows an example photograph of the produced PDMS microfluidic device. For the purpose of this study, the electrodes were implemented into a specific detection channel as represented in figure 5.4. As described with the glass-based microdevice, the response of the developed electrochemical detector to the increment in concentration of Cu ions was studied. A series of Cu standards prepared in buffered PDCA in the range 0.1-5 μgml^{-1} were loaded, using the FI system, to the microfluidic device and monitored at a fixed potential of -0.50 V vs AgAgCl. Figure 5.8 shows examples of the recorded electrochemical cell response (in nA) for various

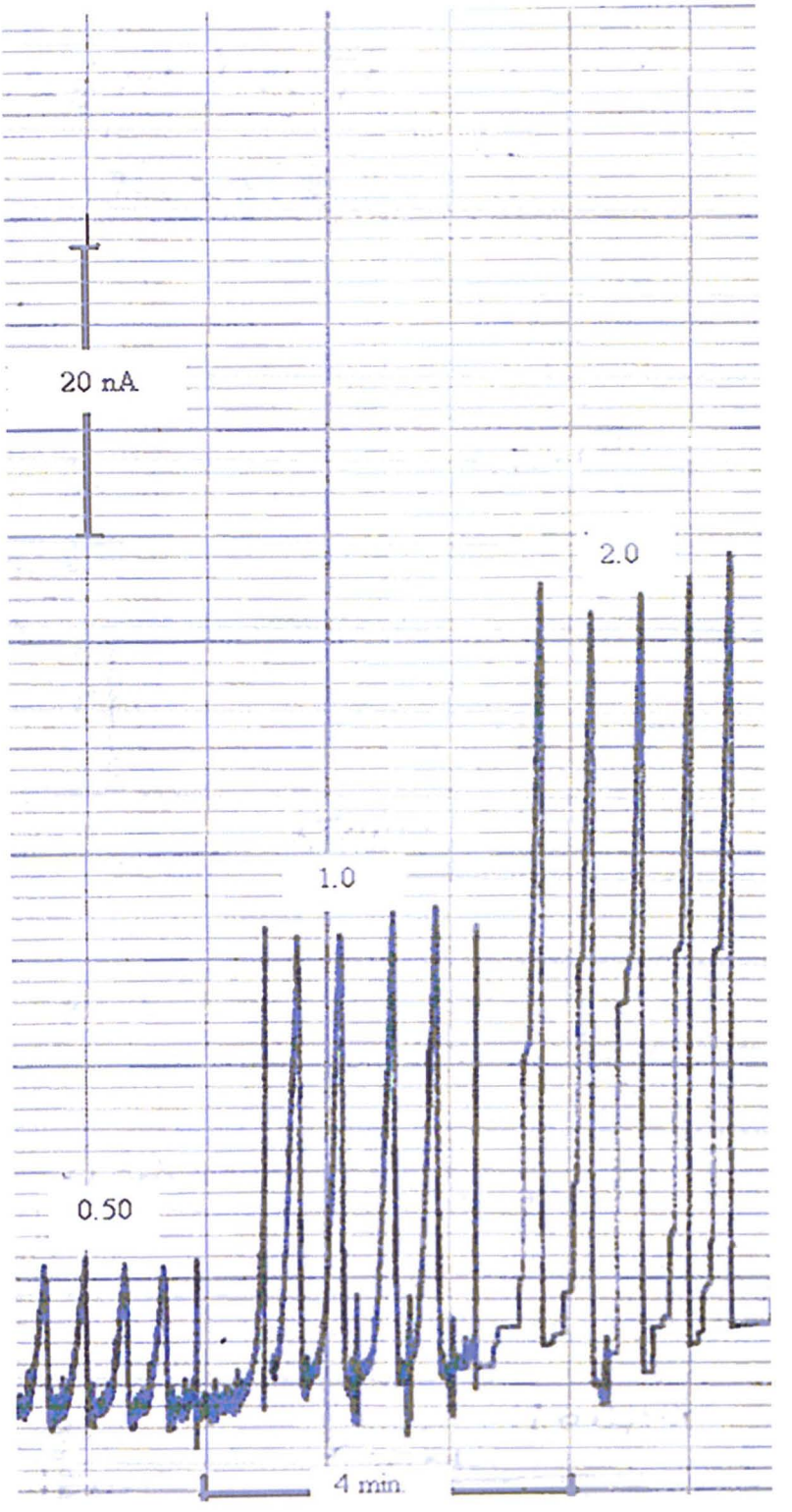


Figure 5.8 The response of in-channel electrochemical detector at -0.50 V for series of Cu standards in 50 nM buffered PDCA of pH 5.4.Samples were injected with 2 µl loop implemented in the flow injection manifold prior to the splitting valve and monitored directly without preconcentration. Numbers at the peaks assigned to concentration in $\mu g ml^{-1}$.

concentrations in the studied range. The relationship between concentration and signal intensity is linear down to 0.5 μgml^{-1} with a correlation equation $I(nA) = 26.31C(\mu gml^{-1}) + 2.97$. The correlation coefficient over the concentration range 0.5-2 μgml^{-1}, R^2, is 0.995 and the precision RSD% at 1 μgml^{-1} (n=4) is 1.13. The detection limit is about 0.22μgml^{-1}. Obviously, the sensitivity and detection limit have been significantly improved. Thus, the next step was to examine the applicability of this miniaturised device to operate as an integrated system capable of executing SPE and monitoring and an on-line mode. This has been accomplished by loading a series of standards of Cu ions in the range 50-1000 $ngml^{-1}$ through the microfluidic device, in which the SPE section was packed with newly prepared resin (8-HQ-CPG), at flow rate of 20 $\mu l\ min^{-1}$for about 3 minutes. Subsequently, the retained ions are eluted with a solution of 50 nM PDCA in acetate buffer of pH 5.2. The peaks assigned for the plug of the eluted ions were recorded in figure 5.9. As clear from the figure, the electrode responds linearly to the increment in concentration over the dynamic range 100 to 400 $ngml^{-1}$. A calibration graph generated by plotting signal intensity *vs.* concentration is depicted in figure 5.10. The detection limit is about 52 $ngml^{-1}$.

With this design, no separation was attempted, as this study at this stage, was aimed to investigate the feasibility of integrating miniaturised SPE, which would function as a sample preparation apparatus, with a miniaturised amperometric detection onto a single microfluidic device to facilitate sample preparation (by SPE) and on line monitoring. Therefore a simple design consisting of a single channel was considered to be sufficient to demonstrate the principle.

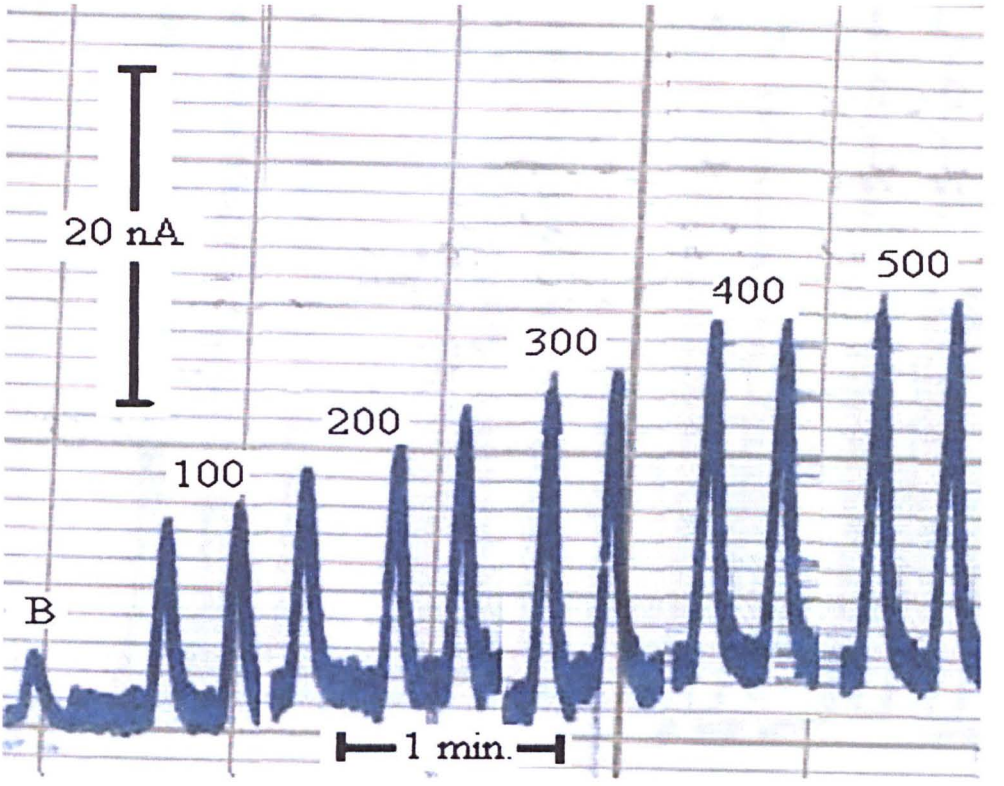


Figure 5.9 Calibration data obtained by loading a series of Cu ions in buffer at flow rate 20µl min^{-1} for 3 min and subsequent elution using 50 nM PDCA.

This miniaturised system could be improved further by incorporating a separation capability to make it useful for real samples where many ions can be screened. Such miniaturised systems will be adequate for field (on-site) monitoring.

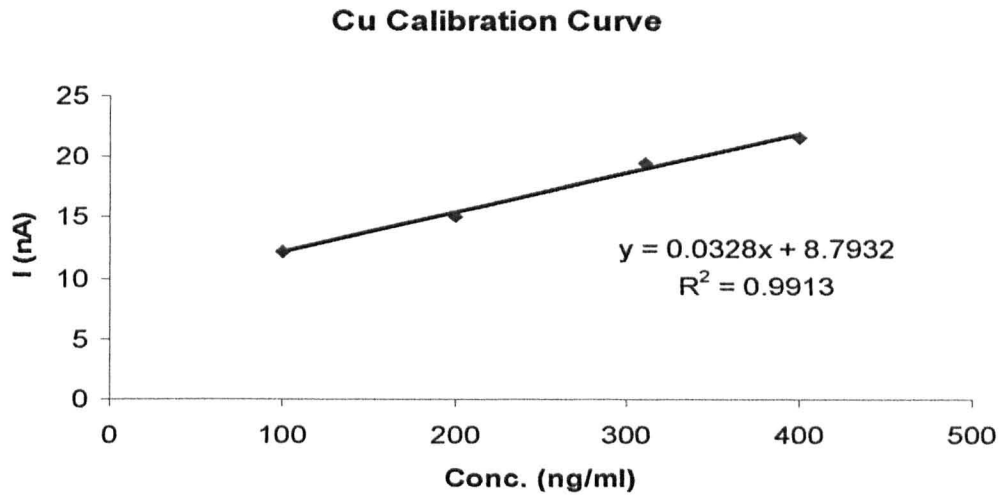


Figure 5.10 Linear portions of Cu calibration graph obtained from the date represented in figure 5.9.

5.4 Conclusion

The work reported in this chapter demonstrates the feasibility of integrating a microfluidic device, incorporating an SPE section that functions as an integrated sample preparation device for trace metals, with an electrochemical cell. The initial prototype, which was fabricated from glass chip, showed some weakness because of the alignment of the electrochemical cell and the limited amount of resins that can be packed. However, the improved manifold that was fabricated from PDMS proved to be more adequate due to the extensive flexibility for electrodes placement inside the channel and the ability to cast a larger section to accommodate more resin. The applicability of this manifold was demonstrated by monitoring the studied ion, Cu; in the range 100 - 400 $ngml^{-1}$. The work also describes a simple and low cost casting process to construct microfluidic devices with 3 D and cylindrical geometry from PDMS in a general laboratory with no need for specialised equipments.

Chapter 6

Conclusion and further work

Chapter 6 – Conclusion and further work

6.1 Overview

Solid phase extraction (SPE) has established itself as an excellent alternative technique to conventional methods for sample preparation in the analysis of trace metals from a wide range of matrices. SPE methods offer numerous advantages over traditional techniques, such as low reagent/sample consumption, less risk of sample contamination and loss, increased sampling frequency and throughput, and ease of automation.

The work in this thesis addressed three different subjects with reference to SPE procedure in view of the current research activity in this field:

1- development of a rapid chemical transformation for the covalent attachment of oxins on silica surfaces;

2- fabrication of porous monolithic silica materials making use of a simple gelation process, and demonstration of their convenience as solid supports for the production of miniaturised SPE columns;

3- use of the lab on chip concept and microfabrication techniques to downscale SPE apparatuses to be an integral part of microfluidic devices. In this regard, two approaches were investigated:

   a) construction of glass microfluidic devices incorporating multi-channels, packed with SPE beads, appropriate for sample preparation prior to ICP-MS monitoring.

b) fabrication of microfluidic devices from glass and PDMS accommodating a single SPE channel integrated with a house made electrochemical (amperometric) detector.

Although conclusions have been drawn at the end of each individual chapter, general conclusions in relation to these subjects will be drawn throughout the following sections. This will be followed by suggestions for further work and potential areas of exploration that are beyond the remit of this thesis.

6.2 Development of a rapid chemical transformation to immobilise 8-hydroxyquiniline on silica

This subject was approached throughout the demonstration of a rapid, cost effective and green chemistry compatible chemical transformation for the immobilisation of oxins onto silica surfaces (i.e., controlled porous glass). The developed method would be a practical process for rapid covalent chemical attachment of other chelating regents (i.e., regents amenable for diazo coupling), thus simplifying the manufacture of silica based solid phase extraction (SPE) materials. Immobilisation procedures that involve minimum synthesis steps are generally preferable as they significantly save time and generate low amounts of organic wastes. Of equal importance is the fact that short and simple immobilisation processes are more convenient for the attachment of reagent (i.e., 8-HQ) within the micro/meso-porous structure of monolithic micro columns, for the reason that the well-established protocol, which involves many prolonged steps, poses the risk of damaging the columns (e.g., dislodging the silica body from the capillary as pumping solutions for a long time may break the bonding of silica mass to the wall). There is also a significant risk that the porosity of materials

is downgraded as the reagents might create a multilayer, particularly when heating is applied, because of solvent evaporation.

The chelating resin produced according to the developed procedure (as reported in chapter two) maintains chemical configuration, including the spacer arm separating the reactive groups from silica matrix, identical to that immobilised following the traditional method, and revealed a satisfactory capacity exchange. In addition, it demonstrated an excellent capability for on line sample preparation of trace metals prior to ICP-OES monitoring. However, the application of the developed SPE procedure to analyse trace metal ions in sediment revealed a critical drawback. Unlike other environmental samples such as seawater and natural water, the high level of major transition metals (i.e., Al, Fe and Mn) in sediment samples would compete with analytes (which exist at trace level) by occupying all reactive functional groups on the resin. Unfortunately, there is no chelating resin able to discriminate between these ions and other transition metals. Nevertheless, the use of pyrophosphoric acid as a masking reagent was proved to be effective to eliminate the limitation arising from the presence of major metals.

6.3 Fabrication of miniaturised SPE columns from porous monolithic silica materials

The importance of this issue for the study stems from the fact that porous monolithic materials have replaced the conventional bead-based packed columns in a broad range of the recent applications in separation science. The large surface area and the possibility of casting these materials within the frontier of capillary, makes it possible to avoid many obstacles associated with the production of miniaturised columns using

the packed beads. Porous monolithic materials can be produced from polymers and silica using various synthetic approaches. However, silica based material was suggested to be more appropriate for the purposes of the study in this thesis, as it has many advantageous characteristics; more importantly, the simple production and the well established chemical transformation to realise surface modification. The work on this subject (as shown in chapter three), demonstrated a simple collide sol-gel process to synthesise porous monolithic materials by hydrolysing potassium silicate colloids in the presence of formamide and/or acetamide. The process enabled a straightforward fabrication of miniaturised columns by allowing the immediately mixed components to polymerise inside microcapillaries. The monolithic columns prepared using optimised ratio of the components showed excellent porosity with reasonable physical stability and exhibited relatively low flow resistance in hydrodynamic systems; therefore it was possible to implement them into flow injection systems operated by the use of peristaltic pumps. These characteristics suggest that the monolithic materials under investigation would be very useful solid supports for the immobilisation of chelating reagents, thus appropriate for manufacturing miniaturised SPE columns. Although there is a wide variety of chemical transformations that can be adapted to functionalise the porous structure within these monoliths with chelating reagents, rapid procedures that can be conducted making use of minimum steps, would be much preferable. The immobilisation procedures adopted in this study (chapter three) enabled the production of monolithic based chelating resins possessing comparable capacity exchange to those immobilised on conventional supports. Their applicability to function as efficient SPE materials was demonstrated by integrating them into miniaturised flow injection manifolds coupled with a UV-Vis spectrophotometer. The

developed systems revealed a very interesting performance in carrying out sample preparation and spectrometric detection in an on-line mode. However, the limited availability of specific selective chromogenic reagents utilised in the spectrophotometric monitoring, restricts the application of this system to a few ions in real environmental samples e.g., iron.

6.4 Miniaturised SPE integrated into microfluidic devices

In this part of the study, the newly emerged lab on a chip technology was employed to construct a true miniaturised microfludic device incorporating an integrated sample preparation section. The work on this subject (as in chapter four) revealed that using the microfabrication technology is a useful approach to construct miniaturised glass microfluidic devices with many identical microchannels which can be packed with resin and utilised as efficient portable devices for sample preparation of trace metals. This microfluidic device showed potential advantages on sample throughput and cost saving as demonstrated by their use to function as sample preparation tools for trace metal amenable to the integration/interfacing with ICP-MS. However, the high running cost of ICP-MS and its nature as a bulky and bench based instrument, limited the applications of microfluidic devices to laboratory based work. Thus, it is concluded that to make use of the potential advantage of the microfluidic device as a portable analytical instrument, it should be used with a portable detector; preferably an integrated one.

Electrochemistry could be utilised as an adequate method of detection in metal with the miniaturised analytical systems. Electrochemistry is attractive for miniaturised systems for many reasons; for example it is sensitive, compact, and the construction of miniaturised electrodes and their integration within the microdevices is accessible

by the use of low cost technology. Even though the selectivity of amperomeric methods for metal ions is poor, excellent results could be obtained by separating the species prior to their monitoring. The relatively high detection limit of the technique (10^{-6} M) can be compensated for by implementing a SPE device to preconcentrate the analyte metals prior to the detection part of the microfluidic device.

The work in chapter five described two designs for the integration of microfluidic devices, made of glass and PDMS, incorporating a SPE section for sample preparation, with electrochemical detection on a microchip platform. Yet the microfludic devices made of glass showed inadequate applicability to analyse metal ions at low concentrations, perhaps owing to the small amount of resin that can be packed into the microchannel and the technical difficulty associated with electrode alignment at channel outlet. On the other hand, microfluidic devices produced from PDMS by the use of simple casting and moulding techniques, allowing the fabrication of three dimensions microchannels, demonstrated reliable potential to preconcentrate and monitor copper ion from aqueous solution down to 50 ng ml^{-1}. The elastic property of PDMS allows simple integration for the electrodes and fluidic connection. Also the feasibility of the fabrication method developed in this connection (as described in chapter five) demonstrated by the fabrication of microfluidic devices having simple channel manifold (network), its simplicity and the low cost of raw materials, make it useful for mass production of microfluidic devices, particularly for researchers who have no direct access to sophisticated facilities (e.g. lithography).

6.5 Further work

As a result of the work described throughout the thesis, some further work can be identified to take forward what has been achieved. Essentially, three main directions are proposed for further research.

6.5.1 Production and application of the monolithic microcolumns

Further investigations, in this field, may be carried out to optimise materials' porosity. In this respect, other technologies based on sol gel synthesis could be examined. In this context the use of hydrophilic polymers such as poly(ethylene oxide), PEG, which are employed to control porosity in monoliths based on the hydrolysis of alkoxysilanes, would be investigated. It also would be extremely advantageous to investigate the possibility of adapting the experimental setting in order to find a proper technical approach to place the porous silica within the boundary of particular length of the capillary in order to allow silica phase to be generated *in situ* inside microchannel within microfluidic devices.

Sol gel technology such as that described in this thesis proved to be an inexpensive and affordable approach for in-house production of miniaturised monolithic materials possessing consistent porosity. However, the availability of monolithic materials, with various chemical functionality and geometrical sizes, on a commercial scale at affordable prices, could encourage further research in the synthesis of chelating resins based on monoliths.

In chapter three, the FIA incorporating the miniaturised columns has been designed to operate with laboratory standard hydrodynamic pumping instruments and a

conventional spectrophotometer; thus the final set up appeared as a bulky laboratory based system. However, the potential advantages of these columns as miniaturised SPE apparatus would be realised by their integration into FIA systems operated using miniaturised pumps and coupled with a microspectrometer (or proper light source) via fibre optics (e.g., Ocean Optics. Inc. S2000). This miniaturised system would be a very useful portable analytical instrument for field monitoring.

6.5.2 On chip SPE coupled with ICP-MS

The work in this thesis demonstrated that the on chip SPE approach is an appropriate for the production of miniaturised sample preparation for ICP-MS. In this study, the feasibility of this method was proved using a microdevice incorporating three identical integrated micchannels made of glass. However, microfluidic device accommodating large numbers of microchannels would be more efficient. It would also appropriate to investigate the option of constructing devices from low cost polymeric materials (e.g., PDMS), so that the device can be used for single use (disposable). Moreover, it is worth considering the possibility of integrating this device with adequate miniaturised pumping systems for fluidic dispensing (the same technology as used in an injected ink printer could be used) to make these miniaturised devices more compatible for remote applications.

6.5.3 On chip SPE coupled with amperometric detection

In the case of on chip SPE microdevices with integrated electrochemical detection it could be valuable to examine the option of modifying the design of the fabricated microdevice to make it possible to integrate a proper separation capability into the manifold, whereby, the eluted metal ions would be separated on their way to the detector. In this case electroosmotic pumping would be the preferred means for fluidic

manipulation, particularly, for the elution and separation, instead of hydrodynamic; however hybrid manipulation systems to drive solutions would be possible.

7.0 – Presentations and conferences

7.1 Poster Presentations

In the course of this study the followings are posters presented at attended conferences:

1. Awadh O AlSuhaimi and Tom McCreedy, *Evaluation of microporous silica monoliths and controlled pore glass as solid supports for the immolised L-cysteine for preconcenntration of some transition metals using on-line spectrophotometric detector*, Reaseach and Development Topics in Analytical Chemistry Meeting, Royal Society of Chemistry, University of East Angilia, June, 2001.

2. Awadh O AlSuhaimi and Tom McCreedy, *A rapid procedure for the immobilisation of 8-hydroxyquinoline onto silica surfaces for the on-line micro-solid phase extraction of transition metals*, Eleventh Biennial National Atomic Spectroscopy Symposium (11 BNASS), Royal Society of Chemistry Loughborough University, July 2002.

3. Awadh O AlSuhaimi and Tom McCreedy, *On-chip Solid Phase Micro Extraction for transition metals*, Reaseach and Development Topics in Analytical Chemistry Meeting, Royal Society of Chemistry, University of Kingston, July 2002.

7.2 Professional development programmes attended

1. *Micro Chemical Systems*, Dept. of Chemistry, the University of Hull, Hull, UK, 23-25 July 2001.
2. *Micro Total Analytical Systems*, Dept. of Chemistry, the University of Hull, Hull, UK,7-9 April 2003.
3. *Advances in Flow Analysis*, Dept. of Chemistry, the University of Hull, , Hull, UK, 27-29 July, 2004.

8.0 – References

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