CRANFIELD UNIVERSITY

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ELECTROCHEMICAL METHOD FOR THE RAPID *IN SITU* SCREENING OF HEAVY METALS IN SOIL AND WATER SAMPLES

Institute of Bioscience and Technology

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ABSTRACT

The aim of this work has been to develop a field-based electrochemical sensor for the detection of cadmium (Cd), lead (Pb), copper (Cu), zinc (Zn), arsenic (As) and mercury (Hg) in soil and water samples. *In situ* analysis, using rapid sample screening tools, can facilitate monitoring and remediation processes by minimising delays and substantially reducing costs.

Disposable screen-printed electrodes (SPEs) have been fabricated using low-cost thick film technology. Carbon SPEs, coupled with anodic stripping voltammetry (ASV), provided a rapid, inexpensive *in situ* electrochemical screening sensor capable of simultaneously detecting Cd, Pb and Cu in soil and water samples in the microgram per liter range ($\mu g\ l^{-1}$).

A simplified soil extraction procedure, using 1 mol l^{-1} aqua regia and a three minute ultrasonic sample agitation, was developed for use with the sensor, addressing the lack of existing field-based soil extraction protocol. Extraction efficiency was evaluated using a soil certified reference material (CRM). Recoveries of 64%, 52% and 57% for Cd, Pb and Cu respectively were obtained, with a relative standard deviation (RSD) of <8% for all analytes (n=10). 82 soil samples were tested using the combined extraction + ASV sensor and compared against standard ICP-MS analysis. Correlation coefficients of 0.9782, 0.9728 and 0.9869 for Cd, Pb and Cu, respectively, indicated a good linear relationship between the two methods.

A novel gold (Au) SPE, fabricated using a curable Au ink, was next evaluated for the detection of As and Hg. This is an improvement in the current state-of the-art as no surface modification or subsequent electrode adulteration to facilitate metal analyte depositioning is required. Furthermore, the Au ink can be cured at a low temperature (130°C), permitting electrode fabrication using disposable, cheap, plastic substrates. A patent has been filed.

A limit of detection (LOD) of 2.7 $\mu g\ l^{-1}$ and 1 $\mu g\ l^{-1}$ for As^{3+} and Hg^{2+}, respectively, was obtained following a 30 s depositioning. Speciation measurements for As were performed by reducing As^{5+} effectively over a short (10 min) reduction time using cysteine. An LOD of 42 $\mu g\ l^{-1}$ following a 30 s depositioning was obtained. Initial studies also indicated the possibility of using the Au SPEs for measuring Cd, Pb and Cu, providing an alterative Hg-free procedure.

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ΔE_p	Anodic to cathodic peak separation
A	Ampere
Ag/AgCl	Silver/silver chloride
ASV	Anodic Stripping Voltammetry
BCR	Community Bureau of Reference
CME	Chemically modified electrode
CRM	Certified reference material
CV	Cyclic Voltammetry
DME	Dropping mercury electrode
DPASV	Differential Pulse Anodic Stripping Voltammetry
DPASV	Differential Pulse Anodic Stripping Voltammetry
E_{dep}	Deposition potential
FAAS	Flame Atomic Absorption Spectroscopy
FASA	Fuzzy Areal Site Assessment
GFAAS	Graphite Furnace Atomic Absorption Spectrometry
HMDE	Hanging mercury drop electrode
HMDE	Hanging mercury drop electrode
HPLC	High Performance Liquid Chromatography
ICP-AES	Inductively Coupled Plasma Emission Spectroscopy
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
I_p	Peak current
LOD	Limit of detection
MFE	Mercury film electrode
$mg\ kg^{-1}$	Milligrams per kilogram
$mg\ l^{-1}$	Milligrams per litre
min	Minute
$mol\ l^{-1}$	Mole per litre
mV	Millivolts
N	Number of electrons or number of data points
NAA	Neutron Activation Analysis
$ng\ l^{-1}$	Nanograms per litre

nm	Nanometers
PLS	Partial Least Squares
ppb	Parts per billion
ppm	Parts per million
PSA	Potentiometric Stripping Analysis
RSD	Relative standard deviation
S	Second
SD	Standard deviation
SPE	Screen-printed electrode
t_{dep}	Deposition time
US-EPA	United States Environmental Protection Agency
μA	Microampere
μC	Microcoulomb
$\mu g\ l^{-1}$	Microgram per litre
μl	Microlitre
V	Voltage
V	Scan rate
v/v	Volume/volume
XRF	X-ray Fluorescence

CHAPTER 1

GENERAL INTRODUCTION

1.1 OUTLINE

In 1990, the member states of the European Union were estimated to have generated 330 million tonnes of industrial waste, a figure that is growing annually as these countries increase their industrial output (European Commission, 1998). This has led to increasing concern regarding the impact of anthropogenic activities on both human health and the environment. As a consequence, European Union (EU) legislation for the assessment, remediation and control of contaminated land and waters has become increasingly stringent. These aim at maintaining minimum environmentally safe levels, based on recommendations from the World Health Organisation (WHO).

In order to comply with legislative requirements, samples from sites suspected of being contaminated have to be sampled appropriately and analysed to determine the nature of the contaminants and the concentrations involved. A particular concern is the number of sites contaminated with heavy metals.

Traditionally, the analysis of samples contaminated with heavy metals is performed in centralised laboratories using techniques such as Flame Atomic Absorption Spectroscopy (FAAS), and Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES). Although these specific and sensitive analytical techniques are routinely used, the analysis is costly and time-consuming. Errors associated with storage and transportation of samples can also occur.

This has led to the need for rapid and cost-effective field-based methods of analysis for a range of contaminants. Heavy metals, such as cadmium (Cd), lead (Pb), copper (Cu), zinc (Zn) arsenic (As) and mercury (Hg) are of particular concern as they are non-biodegradable, persistent and, even at extremely low concentrations, can produce undesirable effects to human health and the environment.

Consequently, the aim of this project was to develop an alternative field-based electrochemical sensor for the detection of Cd, Pb, Cu, Zn, As and Hg in soil and water samples. The ideal sensor would be capable of providing end-users with an on-site screening tool to allow rapid *in situ* site assessments, and monitoring of remediation processes. Such an approach offers immediate decision-making potential usually hindered by central laboratory sample analysis, whilst reducing the cost/amount of samples ultimately being sent to a central laboratory for a final detailed analysis.

Differential Pulse Anodic Stripping Voltammetry (DPASV) coupled with screen-printed electrodes (SPE) offers a convenient technique for such on-site analysis. This approach is favoured for reasons of cost, simplicity, speed (<4 min) and sensitivity ($\mu g\ l^{-1}$). The use of single-use disposable sensors also negates the need for solution stirring, de-aeration and lengthy electrode cleaning procedures.

To employ the electrochemical sensor to *in situ* analysis of heavy metals in soils, an appropriate extraction method is required. Standard laboratory based methods including EPA SW-846 series, acid digestion of soils, BS 7755-3.9:1995, extraction of trace elements soluble in aqua regia and sequential extraction procedures, such as the scheme devised by Tessier *et al.* (1979), have been investigated. Although these methods are routinely employed in central laboratories, the lengthy analysis times, using several extractants, render them unsuitable for on-site applications. As a consequence, a simple field-based acid extraction procedure was also investigated.

The specific objectives of this project can therefore be defined as:

(i) Fabrication of SPEs suitable for the detection of Cd, Pb, Cu, Zn, As and Hg in soil and water samples.

(ii) Assessment of a new gold (Au) ink for the measurement of Hg and As using disposable SPEs.

(iii) Development of a rapid field-based electrochemical assay capable of the simultaneous analysis of the selected analytes utilising DPASV and SPEs.

(iv) Development of an appropriate analyte extraction method to enable rapid field-based analysis of soils.

(v) Assessment of the precision and accuracy of the developed extraction method using a soil certified reference material (CRM).

(vi) Evaluation of the effectiveness of the complete field-based measurement solution (extraction + analysis) by comparison with data obtained using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) for 82 soil samples varying in analyte concentration and soil type.

(vii) Demonstration of sensor performance under field conditions.

The introductory chapter provides the basic scientific background required to view the development steps undertaken during this project in a meaningful context and covers a discussion on environmental monitoring, current environmental legislation, heavy metals in the environment, including uses and toxicity and sample preparation and handling issues, in particular requirements for soil analysis. Conventional techniques as well as more recently developed methods for their detection are reviewed. Lastly, electroanalytical techniques, focusing on DPASV and the use of SPEs will be discussed. Specific topics related to the detection of Cd Pb Cu and Zn using carbon SPE and As and Hg using Au SPE, and wet digestion soil sample preparation techniques will be discussed in detail in the chapters dealing specifically with these issues.

1.2 ENVIRONMENTAL MONITORING

All anthropogenic activity gives rise to some form of pollution and in developed countries where land has been used and re-used, sometimes over centuries, the problems as acute. However, over the last 200 years, due to the "industrial revolution", accompanied by major population growth, the situation has deteriorated (Fifield and Haines, 1995). The mass production of "modern day" commodities, the discovery and use of new materials and chemicals, together with energy and mineral consumption are the main cause of pollution in the biosphere.

Although emissions of heavy metals to the environment have generally decreased in Europe over the last twenty years, the levels of heavy metal pollution are still unacceptably high in large areas (Lindqvist, 1995). Many contaminated sites of former polluting industries and landfill dumps are forgotten until contamination re-emerges to pollute water supplies or redevelopment surveys reveals the hazard (Stanners and Bourdeau, 1995). Although many of these sites are the legacy of past activities, present industrial, agricultural and urban activities also drastically modify the relative distribution of metals in soils, leading to accumulation and modification of their chemical forms (Guardia and Garrigues, 1998). The concentrations of certain heavy metals such as Hg, Cd and Pb have also increased in top soils in large areas during the 20th century (Lindqvist, 1995).

Over recent years, concern has increased regarding the impacts of elevated levels of heavy metals in the environment. Increasing emphasis is placed on environmental issues such as waste reduction, contamination of natural water systems, improving industrial processes through monitoring and minimisation of pollutant release and alternative 'clean technologies'. Environmental monitoring is driven primarily by legislation drafted as a result of continuing health concerns, reviewed in Section 1.3.

As a result of stringent legislation, the number of environmental samples submitted for analysis in the frame of routine monitoring or risk and sustainability assessment studies is continuously growing (Sastre *et al.,* 2002). An ever increasing number of compounds, in complex sample matrices, including drinking water, food, ground water, surface waters and soils have placed growing demands on the field of environmental analysis.

The first step in environmental monitoring with respect to chemical analysis is to determine whether the total metal content of the sample is within the range of background levels or over the concentration limits according to national legislation. In the latter case, more detailed analysis is performed to assess the mobility rate of the heavy metals in the soil-plant system for a better evaluation of the environmental impact of the contamination event (Sastre *et al.*, 2002).

Following sample collection, chemical analysis is performed off-site, by transferring samples to a central laboratory, or *in situ*, at the sampling location. Centralised testing involves highly trained personnel, elaborate sample preparation methods and sophisticated and expensive instrumentation. This type of analysis allows high recovery rates and accuracy, low limits of detection and detailed sample analyses.

However, this approach is costly and time-consuming. Furthermore, errors can occur due to sample preservation and handling, owing to losses by adsorption on and/or contamination by laboratory wares and sampling bottles. Speciation information can also be lost due to chemical and physical changes in the sample prior to measurement (Tercier and Buffle, 1993).

In situ analysis, using rapid field-based sample screening tools, can facilitate monitoring and remediation processes by minimising delays and substantially reducing costs. Errors associated with delays between sampling and analysis are also negated, thus reducing uncertainty in assessment of environmental health and human exposure.

Sample screening tools refer to responsive analyte systems that allow samples containing specific analytes at concentrations above a pre-set value to be 'filtered out'. The primary objectives of a sample screening system is to provide a expeditious reliable response, to reduce preliminary operations and minimise the use of conventional costly analysis, which should only be used to derive additional analytical information (Valcarcel *et al.,* 1999).

The term environmental monitoring implies that pollution is detected ideally as soon as it occurs. For example, monitoring performance of waste treatment equipment to detect either malfunction or end-of-pipe analysis to ensure legislative compliance is best served using immediate decision making tools. For this reason, the development of field-based methods for rapid *in situ* measurement is of increasing importance. *In situ* sensors have demonstrated their advantages as analytical tools for decentralised monitoring (e.g. monitoring of blood glucose) (Section 1.7.4). Utilising the technology in environmental monitoring would provide a simpler and more cost-effective approach for both industry and regulators.

1.3 LEGISLATION

Soil

Over the last 30 years, concerns regarding contaminated land and soils have resulted in extensive UK and EC legislation. Much of this stemmed from the 1972 European Soil Charter. This recognised for the first time that 'any biological, physical or chemical degradation of soil should be of primary concern and that appropriate measures to protect soils should be implemented without delay'.

Another landmark was the implementation of the EC Directive of 27 June 1985, 85/337/EEC. This placed an obligation on each member state to ensure that, projects likely to have significant effects on the environment are made subject to assessment and monitoring. It provided a framework and a list of objectives, but left a great deal of detail and the means of implementation to individual member states.

Since then, a series of directives (Pollution Handbook, 2003) have been issued by the EC, for the control of incident risks and pollution discharges and for the establishment of soil quality standards. These provide guidance for specific legislation enacted by each member state, with the directives implemented as a variety of legislative instruments (frameworks) in member countries of the EU and are also reflected in the legislation of many non-member countries.

Key principles of EU environment policies are 'sustainable development' and 'polluter pays'. 'Sustainable development' was first introduced in the 1987 report of the World Commission on Environment and Development, *Our Common Future* (the Brundtland Report); this defined the term as 'meeting the needs of the current generation without compromising the ability of future generations to meet their own needs' (Pollution Handbook, 2003). In 2001, Member States agreed the Commissions proposals for a sustainable development strategy. COM (2001) 264 *A Sustainable Europe for a better world:A European Union Strategy for sustainable development* aims to ensure that the general principle becomes an integral part of all EU policy areas (Pollution handbook, 2003).

The polluter pays principle (PPP) states that the cost of preventing pollution or of minimising environmental damage due to pollution should be borne by the polluter. This originates from the proceedings of the UN Conference of the Human Environment, Stockholm 1972. In the European Community PPP was first mentioned in the First Action Programme on the Environment (1973-77) (Pollution handbook, 2003).

Environmental Impact Assessment (EIA) and Strategic Environmental Appraisal (SEA) are also integral to EU environment policies. EIA is a tool for systematically examining and assessing the impacts and effects of development on the environment. The 1985 *Directive on the assessment of the effects of certain public and private projects on the environment* (85/337/EEC); amended by Directive (97/11/EC) (1997), requires authorities to request an EIA for development projects likely to have significant effect on the environment.

SEA relates to the assessment of plans, policies and programmes. In June 2001, the *Directive on the assessment of the effects of certain plans and programmes on the environment* (2001/42/EC) was adopted to ensure a uniform and high standard of assessment throughout all Member States (Pollution handbook, 2003).

Only a limited number of countries, such as Holland and the UK, have elaborated comprehensive legislation with respect to the registering and clean up of polluted sites. Reference is made to general rules of law or with other provisions relating to the removal of abandoned waste, or pollution of groundwater. Decisions on sites are often taken according to national guidelines that do not necessarily contain quantitative limits (European Commission, 1998).

However, in 1983 Holland pioneered the setting of regulatory standards in its Soil Cleanup (Interim) Act. This sets limits using an 'ABC' approach; A-background, B-when further investigations are necessary, C-when cleanup is required (Stanners and Bourdeau, 1995). In 1994 new rules were integrated into Dutch legislation, with the objective of controlling pollution and if clean-up is necessary, to retrieve the multifunctional quality of the soil (i.e. Improvement to a standard suitable for any possible use.) Thus whenever the multifunctionality of soils is endangered, soil clean up is required.

The 'ABC' standards have since been reconsidered, abolishing 'B' values, and replacing 'A' and 'C' levels with "optimum target levels" and "action levels" respectively. The limits set for the selected analytes are presented in Table 1.1. Whilst the principle of multifunctionality is still the basic approach, the rule is not applied inflexibly and in practice the levels for remediation will vary on public health, environmental protection and site specific criteria, such as current and future use, and soil characteristics (Tromans and Turrall-Clarke, 1994).

In the UK, attempts were made to determine threshold values for pollutants when some form of action is required for two proposed end uses, domestic gardens and parks, playing fields and open spaces. In 1987, the Interdepartmental Committee of the Redevelopment of Contaminated Land (ICRCL) set limits based on trigger and action guideline concentrations (Table 1.2). The trigger value indicates when further investigation is necessary and the action level indicates when some form of remedial action is likely for the proposed end use of the site. No action guidelines were set for the selected analytes.

Table 1.1 The New Dutchlist - soil quality standards.

Contaminant	**Soil/sediment (mg kg^{-1}) dry weight**	
	Optimum	Action
Cadmium	0.8	12
Lead	85	530
Copper	36	190
Zinc	140	720
Arsenic	29	55
Mercury	0.3.	10

Source: Layla Resources Ltd, 2000.

The UK system differs from that of the Dutch by defining contamination in relation to the end use of the site (i.e. contaminated land should be restored according to the planned usage.) In part this is a function of the geology of the UK which permits a more pragmatic approach as glacial clay capping tends to lock the potential pollutants in place. The Netherlands geology includes granular sands and soils with a high water-table permitting easy groundwater movement.

Table 1.2 ICRCL 59/83 Trigger concentrations for selected analytes in soil.

Planned use	**Threshold Values (mg kg^{-1} air-dried soil)**					
	Cd$^{(a)}$	Pb$^{(a)}$	Cu$^{(b)}$	Zn$^{(b)}$	As$^{(a)}$	Hg$^{(a)}$
Domestic gardens, allotments	3	500	-	-	10	1
Parks, playing fields, open space	15	2000	-	-	40	20
Any uses where plants are grown	-	-	130	300	-	-

(a): Group A contaminants which may pose significant hazards to health, (b): Group B contaminants are phytotoxic but not normally hazardous to health. Source: Layla Resources Ltd, 2000.

However, the UK Department of Environment (DoE) (now Department for Environment, Food and Rural Affairs -DEFRA) adopted a strategy similar to the Dutch, with new site-specific, risk-based criteria replacing the ICRCL trigger concentrations. In 1990, the Environmental Protection Act (EPA) was passed. This criminalised the act of pollution for the first time and introduced the concept of "duty of care".

It also introduced Integrated Pollution Control (IPC), with the aim of protecting land from contamination as well as remediating already contaminated land. However, Section 143 placed a duty on local authorities to maintain a register of contaminated land. This provoked outcry from professional organisations with vested interests in real estate (Syms, 1996) and was repealed and replaced by a new policy, contained in section 57 of the Environment Act 1995 (Clarke, 1996). This *Framework of Contaminated Land* was made legal in 1998 and provided a national framework for remedial and regulatory action for the control of contaminated land, emphasising the Government's commitment to the two fundamental principles: "sustainable development" and "polluter pays".

The contaminated land (England) regulations 2000 (SI 227) (contained in Part IIA of the 1990 Environmental Protection Act) together with the DETR Circular 2000/02 containing Statutory Guidance came into force on 1 April 2000. This included elaborated guidance on the definition, identification and remediation of contaminated land, and recovery of costs and liability for remediation.

EIA features increasingly as an explicit requirement of environmental planning in UK legislation. Guidance was first published in 1995 in the then department of environments '*Guide to risk assessment and risk management for environmental protection*'. Revised departmental '*guidelines on environmental risk assessment and management*' for England and Wales were issued in 2000 with the Department for Environment, Food and Rural Affairs (DEFRA) re-endorsing their use in 2002.

The United States of America adopt the approach of multifunctionality, similar to the Dutch, with contaminated land controlled through the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA) enacted by Congress in 1980. This law created a tax on chemical and petroleum industries and provided broad Federal authority to respond directly to releases or threatened releases of hazardous substances which may endanger public health and the environment. Over 5 years $1.6

billion was collected and used to set up a fund for the remediation of abandoned and uncontrolled hazardous sites (www.epa.gov/superfund). CERCLA was amended by the Superfund Amendment and Reauthorisation Act (SARA) in 1986.

In Australia the National Environmental Protection Council produced The *National Environment Protection Measure* (NEPM) for contaminated land management, with the latest *contaminated land guidance* issued in 11th October 1999 (Layla Resources Ltd, 2000).

Water

The reduction and control of water pollution and contamination has always been a key priority in EU environmental policy. Directive 2000/60 established a framework for Community Action in the field of Water Policy. The overall purpose of the Directive was to establish a framework for the protection of surface fresh water, estuaries, coastal waters and groundwater.

A Dangerous Substances in Water Framework Directive (76/464) listed 129 substances considered to be toxic, persistent or bio-accumulative in the environment and that priority should be given to eliminating them (Pollution Handbook, 2003). List I, the 'black list' contains substances which require strict limitation and are considered to be toxic (Cd and Hg). List II, the 'Grey list' includes those substances considered less harmful when discharged to water (Pb, As, Cu, Zn).

In 1980 the Drinking Water Directive (80/778) *relating to the quality of water intended for human consumption*, set 3 types of quality standards for 60 substances; Guide levels (GL); Maximum Admissible Concentrations (MAC); and Minimum Required Concentrations (MRC). This was repealed in 1998 with Directive (98/83/EC) which covers all water for domestic use (whether from tap, bottle or container) and water used by the food industry where this affects the final product.

In the UK legislation to control water pollution has been developed over a number of centuries, with the earliest recorded legislation prohibiting the pollution of water dating from 1388. The Water Act of 1989 established the National Rivers Authority (NRA) to take over responsibilities of water authorities in England and Wales in relation to water pollution, water resources management, flood defence and fisheries.

Much of this was repealed by and re-enacted in the *Water Industry Act 1991* and *Water Resources Act* 1991. The *Environment Act* 1995 established the Environment Agency to take over the functions and responsibilities of the NRA which was abolished. An Inspectorate within the DoE was established specifically to deal with drinking water. Its principal aim is to ensure that water undertakers in the UK are fulfilling their statutory requirements for the supply of wholesome water. The inspectorate monitors compliance with the standards set in the *Water supply (water quality) Regulations 1989 and 2000*, which implements the 1998 EC Drinking Water Directive (98/83/EC).

The World Health Organisation (WHO) is the international reference point for the setting of drinking water standards and guidance on implementing safe water activities. MCL's set by WHO, UK, EU, USA and Australia are presented in Table 1.3.

Table 1.3 Drinking water maximum contaminant levels in mg l^{-1}.

Contaminant	UK	EU	WHO	US-EPA	Australia
Cadmium	0.005	0.005	0.003	0.005	0.002
Lead	0.025 0.01 from 2013	0.05	0.01	0.015	0.01
Copper	2	0.5	-	1.3	2
Zinc	-	1	-	-	-
Mercury	0.001	0.001	0.001	0.002	0.001
Arsenic	0.01	0.05	0.01	0.05 0.01 from 2006	0.007

Sourced: (Pollution Handbook, 2003); (www.who.int/water_sanitation_health/dwq/guidelines), (www.epa.gov/safewater/mcl.html), (www.npi.gov.au/database/substance-info/profiles/11.html).

With environmental monitoring and assessment driven primarily by legislation the need for rapid field-based analytical methods for quantification is crucial. This would support legislation with respect to rapid site characterisation and assessment, monitoring of remediation processes and for the enforcement of regulations on heavy metal discharge by many industrial, agricultural and domestic sources.

1.4 HEAVY METALS IN THE ENVIRONMENT

The term 'heavy metal' is widely recognised and used, commonly adopted as a group name for the metals and metalloids which are associated with pollution and toxicity, although also includes some elements which are essential to living organisms at low concentrations (Alloway, 1990). These metals have a high atomic density over 5 g/cm^3, for example, Pb-11.3 g/cm^3, Cu-8.9 g/cm^3, Cd-8.7 g/cm^3, Hg-13.5 g/cm^3 (Tercier, 1998).

Much focus has been directed at Pb, Cu, Zn, and Cd, As and Hg as they are among the commonest heavy metals found as contaminants in the environment, are widely used in industrial processes and present a significant threat to human health and ecosystems. These metals also have a tendency to form sulphide minerals, often occurring together in ore deposits (Lambert, 1997). This leads to Pb-Zn-Cu-Cd-As-Hg contamination being spatially associated, particularly in areas surrounding mining and smelter activities.

Being at the interface between the atmosphere and the earth's crust as well as the substrate for natural and agricultural ecosystems, the soil acts as a ‘sink’, open to input of heavy metals from many sources (Alloway, 1990). Heavy metals occur naturally in soils and surface waters at low concentrations, as a result of weathering and other pedogenic processes acting on the rock fragments on which the soils develop. Heavy metals are sometimes referred to as trace metals as they occur in concentrations of less than 1% (Harrison, 1999).

However, Man's input to the trace element composition of the biosphere has been dramatic, resulting in widespread changes in the environment (Alloway, 1990). These metals are continuously emitted into the environment from agricultural, industrial, and urban activities, such as fossil fuel burning, mining, metal and electroplating works, sewage sludge application and waste incineration, via the atmosphere or released directly to soils and wastewater. Table 1.4 contains expected levels of the metals on a world wide basis in unpolluted soils (depends on soil type/parent material) and estimated 'worst-case' scenarios of polluted soils.

Table 1.4 Typical levels of metals in un/polluted soils (mg kg^{-1}).

Metal	**Un-polluted top soils**	**Polluted soils**
Cd	0.06 - 1.1	750
Pb	1.5 – 80	13000+
Cu	6 – 60	4500+
Zn	17 – 125	4500+
As	<0.1 – 93	2500
Hg	<0.4	50

Source: Harrison (1999); Kabata- Pendias (2001).

Groundwater may also be affected through discharge of pollutants into aquifers, or by percolation through the overlying ground (Tromans and Turrall-Clarke, 1994). This usually occurs at a much slower rate than in surface waters or soils, as a result of the low rate of movement of groundwater itself. However, due to this, even when the source of contamination has been removed, any contamination will take a correspondingly long period to flush out from the system (Fifield and Haines, 1995).

In many cases soils can act as natural buffers, controlling the transport of heavy metal contaminants. For example, phosphorous, with a high average soil content of 600 mg kg^{-1} , can act as a key control, as the metals form insoluble heavy metal phosphates, which can substantially decrease their mobility (Alloway, 1990). The pH and organic matter content of soils also play a major role in the complexing and subsequent dispersion and bioavailability of elemental ions. As such, the persistence of contaminants in soil is much longer than in other components of the biosphere.

The addition of these metals to soils normally results in accumulation in the upper horizon, which is rich in humic substances and organic acids. These have a high affinity to heavy metal ions, extracting them from the water that passes through the soil by the process of ion exchange. The heavy metal M^{2+} ion replaces two H^+ ions in complexes (Baird, 1999). These metals can also be retained in soil by adsorption onto the surface of mineral particles and organic matter and by precipitation reactions. In general, different soil types, their physical and chemical properties, pH, water and organic matter content, plant species and soil organisms associated with the soil will influence the degree of trace element contamination of soils and surrounding surface and ground waters.

The toxic effects of elevated levels of metals to the environment and human health is determined by the chemical form of the metal, that it, upon its speciation, rather than on the concentration (Baird, 1999). Christie (1997) gives a definition of speciation as 'The different physio-chemical forms of an element which together comprise its total concentration in a given sample'. The individual physiochemical forms may include particulate and dissolved forms, such as simple inorganic species, organic complexes, and metal ions adsorbed onto a variety of particles (Tercier, 1998).

For example, the toxicity of metallic lead and lead in the ionic form Pb^{2+} differs substantially. Forms, which are almost totally insoluble, pass through the human body without doing much harm. However, under most conditions, the predominant soluble species in soils polluted from industrial sources is the free ion M^{2+}, with neutral species present in increasing amounts where the pH is greater than 6.5. The M^{2+} ion is the labile form of these metals in soils, available for uptake by organisms (Baird, 1999).

The proportion of these different forms may vary continuously with space and time due to concurrently occurring physical, chemical and biological processes. These include pH, the chemical nature of the metal species, the stability of the metal complexes, the binding power of the functional groups and the ionic strength of solutions and competing ions (Alloway, 1990).

Several organisms have also shown the ability to detoxify metals by depositing them in an innocuous form. In the hepatopancreas of the snail, spherical, concentrically layered deposits are found which contain various metals such as Zn, Cu and Pb (Hughes and Poole, 1989). In certain fungi, organic substances are produced that reduce metal toxicity. Citric acid, for example, is produced by many fungi and chelates Cu and other metals ions. Plants can also detoxify metals by producing metal-binding polypeptides (phytochelatins) to sequester metals and render them unavailable for transport within the plant (Ross and Kaye, 1994).

These processes combined, control the concentrations of metal ions and complexes in the soil solution and thus exert a major influence on their uptake by plant roots, toxicity and mobility. Therefore, the measurement of total metal concentration alone is insufficient for assessing toxicity issues. As discussed in Section 1.7.1, voltammetric techniques are capable of distinguishing between the different forms present, adding to its desirability in environmental analysis.

A short account on the individual metals, including toxicity is given below. However, specific toxic effects in humans vary according to the metal type, multi-ion combinations, tissue concentrations, length and content of exposure and the age of the individual. For detailed accounts on heavy metals in the environment refer to Donard and Caruso (1998); Sauve *et al.* (1997); Alloway (1990); Harrison (1999); Baird (1999); and Frie and Hutzinger, (1975).

1.4.1 Cadmium

Cadmium (Cd) is a relatively rare metal, being 67th in order of elemental abundance (Alloway, 1990). Cadmium pollution has been increasing rapidly in recent decades, with more than half of all the Cd used in industry produced in the last 30 years (Alloway, 1990). Cadmium is obtained as a by-product from the mining and smelting of Zn, Cu, Pb and other iron ores. Its principal uses are; as protective plating on steel; in various alloys; in pigments for plastics, enamels and glazes; as a stabiliser for plastics; and in Ni-Cd dry-cell batteries (Alloway, 1990).

The main sources of Cd pollution include the mining and smelting of Cd-containing sulphide ores, which can contain up to 5% Cd; atmospheric pollution from metallurgical industries; the disposal of wastes containing Cd such as the incineration of plastic containers and batteries; the application of Cd-containing phosphate fertilisers; sewage sludge application to land; and the burning of fossil fuels.

Cadmium is closely associated with Zn in its geochemistry; both elements have similar ionic structures and electronegativities, however Cd seems to have a stronger affinity for sulphur (S) than Zn and also exhibits a higher mobility in acid environments (Kabata-Pendias, 2001). During weathering processes, Cd goes readily into solution and although Cd^{2+} is the dominant form, it may also form complex ions such as $CdCl^{+}$, $CdOH^{+}$, $CdHCO_3^{+}$. Cd reacts and complexes readily with chlorides and hydroxyl groups and tends to be more mobile in soils than other metals (Alloway, 1990). The main factors controlling Cd mobility are pH and oxidation potential. Under strong oxidising conditions, Cd is likely to form minerals (CdO, $CdCO_3$) and is also likely to accumulate in phosphate deposits (Kabata-Pendias, 2001).

Cadmium has no biological function and is highly toxic to plants and animals. The major hazard to human health is its chronic accumulation in the kidneys where it can cause dysfunction if the concentration in the kidney cortex exceeds 200 mg kg^{-1} fresh weight (Alloway, 1990). Cd can also cause; calcium displacement from bones, causing them to become brittle; anaemia, hearing loss, high blood pressure. The nervous system is also susceptible to Cd, with effects varying from damage to the brain to peripheral nerves, causing uncoordinated muscular control and poor eyesight. (Stoica, 1999).

Food is the main route by which Cd enters the human body; one third of Cd dietary intake is attributed to the ingestion of animal products, while plant products provide the remaining two thirds (Nasreddine and Parent-Massin, 2002), but tobacco smoking and occupational exposures to CdO fumes are also important sources.

1.4.2 Lead

Lead is a widespread contaminant in all areas except those remote from populous regions or where human settlement is more recent. It enters the soil from many different sources and this ubiquity mirrors the widespread use of the metal. Metallic lead is used for the production of batteries, shields for x-rays and various metal products such as pipes, ammunition, and alloys with other metals. Lead compounds are used in pigments, dyes, paints, insecticides and epoxy curing agents (National Pollutant Inventory Substance Profile, 2002).

The major sources of Pb are from mining and smelting activities, sewage sludge usage, burning of fossil fuels and lead-containing paints. Lead compounds (tetra-alkyl leads) were also used as a petrol additive (leaded petrol), however this was phased out in the early 1990s, as 60% of lead emission arose from the automobile exhaust (Palchetti *et al.,* 2000).

Lead has highly chalcophilic properties and concentrates in S phases in rocks and the major ore mineral is the sulphide galena, PbS (Davies, 1990). Environmental chemistry is dominated by the plumbous ion Pb^{2+}. During weathering, Pb sulfides slowly oxidise and have the ability to form carbonates and also to be incorporated in clay minerals, Fe and Mn oxides and organic matter (Kabata-Pendias, 2001). Pb has a long residence time compared to most other pollutants. As a result Pb and its compounds tend to accumulate in soils where, due to their low solubility and relative freedom from microbial degradation, they will remain accessible to the food chain (Davies, 1990). Increasing pH may increase the Pb solubility, but this mobilization is usually slower than the accumulation in the organic-rich layers of soils (Kabata-Pendias, 2001).

There is no significant evidence that Pb plays any essential role in metabolism. Fortunately its solubility and mobility and hence bioavailability are low. Nonetheless, in many environments concentrations are sufficiently high as to pose a potential risk to health, especially near major lead using industries and in major cities (Davies, 1990). The main exposure routes are the food-chain, soil dust inhalation or for children, hand-to-mouth activity of contaminated soil and Pb-containing paints, termed 'pica'. Mild lead poisoning causes anaemia, headaches, sore muscles, and fatigue. Acute Pb poisoning may cause severe dysfunction of the kidney, reproductive system, liver, brain, and central nervous system, leading to sickness or death (National Pollutant Inventory Substance Profile, 2002). Lead poisoning is especially dangerous to the health of unborn and young children. Young children can experience learning difficulties, slow growth and defective hearing. Lead exposure during pregnancy can contribute to premature birth, low birth weight, and damage to the developing brain and nervous systems.

1.4.3 Copper

Copper ranks 26th in abundance in the lithosphere (Baker, 1990). The principal uses of Cu are in the production of wire, and of it alloys, brass and bronze. Cu compounds are used in inorganic dyes, feed additives, foliage sprays, agrochemicals, wood preservatives, and pest control (National Pollutant Inventory Substance Profile, 2002). The main sources of Cu pollution are agricultural applications of agrochemicals and sewage sludge, mining and smelting processes and the manufacturing of products containing Cu.

In nature Cu forms sulphides, sulphates, sulphosalts, carbonates and other compounds and also occurs under reducing environments as the native metal (Baker, 1990). These minerals are easily soluble in weathering processes and release Cu ions, especially in acid environments. Therefore, Cu is considered one of the most mobile heavy metals (Kabata-Pendias, 2001). However, Cu is specifically adsorbed or fixed in soils and higher concentrations in the surface horizons of a soil indicate bioaccumulation or soil additions from anthropogenic sources (Baker, 1990). Cu has three oxidation states; solid metal (Cu^0), cuprous ion (Cu^{1+}) and cupric ion (Cu^{2+}). The common form in most soils is the Cu^{2+} ion, adsorbed at mineral surfaces (Kabata-Pendias, 2001). All soil minerals and organic soil constituents are capable of binding Cu ions from solution and these properties depend on the surface charge carried by the adsorbents (Kabata-Pendias, 2001). The surface charge is strongly dependent on pH, therefore the adsorption of Cu ion species is a function of pH. Chelation and complexing are key reactions governing Cu behaviour in soils. If the pH is not maintained at a desirable level (6-7 for many crops) the availability of Cu is more likely to be a problem (Baker, 1990).

Cu is one of the essential micronutrients and is also necessary for a wide range of metabolic processes, with at least 30 Cu-containing enzymes known, all of which function as redox catalysts or dioxygen carriers (Flemming and Trevors, 1989). Cu is relatively non toxic to mammals and is used as a food additive to promote growth in pigs (Flemming and Trevors, 1989). However, at elevated levels Cu can cause phytotoxic effects and hereditary toxicosis in humans (Alloway, 1990). This was first described in 1912, and was named Wilson's disease. For individuals suffering this, the Cu-binding ligands involved in Cu homeostasis are deficient and toxic levels accumulate in several tissues. Drinking water that contains elevated levels of Cu can lead to vomiting, diarrhoea, stomach cramps and nausea (National Pollutant Inventory Substance Profile, 2002).

1.4.4 Zinc

Zn and Zn compounds have many uses in industry. Zn is mainly used as a protective coating for iron and steel, the production of zinc alloys (brass), rolled Zn (pigment) and Zn dust (reducing agent). Zn oxide is used in rubber, chemicals, paints, plastics, pharmaceuticals and lubricants and Zn cyanide in insecticides (National Pollutant Inventory Substance Profile, 2002). The main pollutant sources of Zn are mining and smelting activities, burning of fossil fuels and the application of sewage sludge and agrochemicals such as fertilisers and pesticides to soils.

Zinc is present in soils as free Zn^{2+}, organo-zinc complexes in the soil solution or adsorbed onto clay particles and humic compounds (Kiekens, 1990). Zn also forms complexes with chloride, phosphate, nitrate, sulphate. Below pH 7.7 Zn^{2+} is the predominant species, while $ZnOH^+$ is more prevalent above this pH (Kiekens, 1990).

Weathering processes produce the mobile Zn^{2+}, especially in acid, oxidising conditions. Zn is, however, also easily adsorbed by mineral and organic components and thus in most soil types its accumulation in the surface horizons is observed (Kabata-Pendias, 2001). The adsorption of Zn^{2+} can be reduced at lower pH (<7) by competing cations, resulting in easy mobilisation and leaching of Zn. Zinc is considered readily soluble relative to other heavy metals in soil and is most readily available and mobile in acid light minerals soils (Kabata-Pendias, 2001). Soluble Zn-complexes that occur, particularly in sewage sludge, are very mobile in soils and therefore are easily available to plants (Kabata-Pendias, 2001).

Zn, in trace quantities, is an essential element for humans, animals and higher plants; the most significant metabolic role in plants is its activity as a component of a variety of enzymes (Kabata-Pendias, 2001). Concern about excessive Zn in soils relates to its possible uptake by crops and consequent adverse affects on the crop, livestock and human diets (Kiekens, 1990). Excessive Zn in the human diet can cause vomiting and stomach cramps (short term exposure) to anaemia and pancreas damage (long term exposure). Ingesting high Zn levels may also interfere with the body's ability to absorb and use other essential minerals such as Cu and Fe (National Pollutant Inventory Substance Profile, 2002). Zn compounds may be very harmful and have the same health effects as the constituents that make up the compounds (i.e. Zn cyanide.)

1.4.5 Arsenic

Arsenic is the 12th most common element in the earth's crust (Hindmarsh, 2000). It is found in rocks, soil, water and air. Traces amounts are found in plants and animals. Natural As presents no threat to humans, provided it is left undisturbed (Hindmarsh, 2000). It is obtained as a by-product from the mining and smelting of Cu, Pb, Au, and Ag. Arsenic was extensively used as a pesticide and fungicide; however this role is now largely filled by chlorinated hydrocarbons. The major present day usages of As compounds are as pesticides, wood preservatives, as growth promoters for poultry and pigs, in the glass industry as a clarifier, and in the production of semiconductors (O'Neill, 1990).

The main sources of As pollution are sewage sludge application, coal combustion and industrial processes such as smelting of other metals (Feeney and Kounaves, 2000). In contrast, the source of As in places as diverse as Bangladesh and New Hampshire has been attributed to geochemical mechanisms involving weathering bedrock or from the oxidation of arsenic in the aquifer sediment upon contact with oxygenated waters (Feeney and Kounaves, 2000).

Over 200 As-containing minerals have been identified with approximately 60% being arsenates, 20% sulphides and sulphosalts, and 20% being arsenides, arsenites, oxides and elemental As (O'Neill, 1990). The most common of the As minerals is a sulphide - arsenopyrite (FeAsS). Arsenite (As^{3+}), arsenate (As^{5+}), monomethylarsonic acid (MMAA) and dimethylarsine acid (DMAA) are the most encountered forms and also the most studied in soils owing to their ability to dissolve in water under environmental conditions. Generally, the speciation of As is restricted to the determination of As^{3+} and As^{5+} (Montperrus *et al.*, 2002). The normal oxidising conditions of the earth's crust favour the formation of the As^{5+} oxidation state; present as the $H_2AsO_4^-$ species, and As^{3+}; present as the $H_3AsO_3^0$, are characteristic of reducing environments (Kabata-Pendias, 2000). Microbial activity can cause methylation, demethylation and/or change in oxidation state and the presence of S species may, if the redox potential is low enough, favour the formation of As sulphide minerals (O'Neill, 1990).

Arsenic toxicity is strictly related to its chemical forms, inorganic arsenic being the most toxic, methylated species present intermediate toxicity and larger biomolecules are non-toxic (Montperrus *et al.,* 2002). This is in contrast to many of the common heavy metals in that the majority of the organo As compounds are less toxic than inorganic As compounds.

Persons at risk include those working and living downwind from smelters and those drinking water from contaminated wells (Hindmarsh, 2000). Places where well water is contaminated include Taiwan, South America, India, Mexico, Bangladesh, China, Alaska and some parts of the south-western United States (Hindmarsh, 2000).

Inorganic As in drinking water is considered a human carcinogen with multiple sites of attack. Some adverse effects such as skin and internal cancers or cardiovascular and neurological effects have been attributed to chronic exposure primarily from drinking water (Ferreira and Barros, 2002). Large doses of As can cause death. Lower levels of As may cause nausea, vomiting, abnormal heart rhythm, damage to blood vessels and decreased production of blood cells.

For detailed reviews, recent papers include; As speciation in natural waters (Hasegawa *et al.*, 1999); mobilisation of As from contaminated sediments (Pongratz, 1998); and its clinical and environmental significance (Hindmarsh, 2000).

1.4.6 Mercury

Since Hg contents of rocks not containing organic matter is very low, most environmental Hg originates from anthropogenic sources (Steinnes, 1990). The use of Hg is widespread; laboratory chemicals, batteries, thermometers, as a catalyst in the chemical manufacturing industry, dental amalgams, photography and formerly fungicides and pharmaceuticals products. The principal sources of Hg pollution are mining and smelting works, burning of fossil fuels, industrial production processes, in particular the Hg cell chloralkali process for production of Cl and caustic soda, the application of Hg containing sewage sludge and P fertilisers to agricultural soils and consumption-related discharges including waste incineration (Steinnes, 1990).

Hg occurs in several minerals but the only frequent one is cinnabar HgS, which is not easily altered. Depending on the redox conditions, Hg occurs in three different valency states; Hg^0 Hg_2^{2+} and Hg^{2+} of which Hg^0 and Hg^{2+} are the states normally encountered in soils (Steinnes, 1990). Due to its strong ability to form complexes, Hg^{2+} rarely occurs in the free ionic form under natural conditions and is retained in soils mainly as slightly mobile organocomplexes. However, the presence of Cl^- ions in soils appears to decrease the sorption of Hg^+ onto both mineral particles and organic matter because the highly stable Hg-Cl complexes are rather poorly sorbed (Kabata-Pendris, 2000).

The accumulation of Hg in soil is controlled mainly by organic complex formation and precipitation and migration processes are limited. Therefore the Hg content of surface soils is slowly built up even under low inputs of Hg (Kabata-Pendris, 2000). The strong affinity of Hg to organic matter increases with increasing soil acidity. Even acid soils or the addition of acid rain does not increase its solubility and the Hg leaching from soil may be stimulated by factors increasing the leaching of organic matter (Kabata-Pendris, 2000). Thus Hg is leached out from the soil profiles in an acid environment in a form bound to organic compounds and in neutral and slightly alkaline soils, it is leached out in an inorganic active fraction. In contrast to other trace metals, the amount of mobilised Hg decreases at $pH < 3$ and at $pH > 12$ due to the extremely high buffering capacity of humics, both in acidic and alkaline states (Kabata-Pendris, 2000).

Microorganisms, such as bacteria and fungi, are capable of biomethylating mercuric compounds to form organic species of Hg including methyl, ethyl, and phenyl types. The mechanism of methylation is important as methylated mercury is readily mobile and easily taken up by living organisms. The potential for microbial methylation of mercury by bacteria and fungi exists under both aerobic and anaerobic soil conditions. In this way, methylmercury, the most toxic of the organic forms, is taken up by fish species, providing a route to enter the food-chain and thus the human diet. Fish at the top of the food-chain can have methylmercury concentrations up to 130,000 times more than the surrounding water (Richter *et al.,* 2002). In January 2001, the US Food and Drug Administration issued an advisory counselling pregnant woman to avoid consuming specified long-lived predatory fish and to limit ingestion of all other fish (Oken *et al.,* 2003).

Although soil contamination with Hg itself is usually considered not to be a serious problem, the main concern is that methyl-Hg will result. Nevertheless, even simple Hg salts or metallic Hg creates a hazard from the toxic nature of Hg vapour, which travels long distance causing local and global pollution (Kabata-Pendias, 2000).

Hg has no essential biological function and is among the most toxic elements to man and higher animals. Mercury salts show a high acute toxicity. Some organomercurials, in particular low-molecular-weight alkyl mercury compounds, are considered even more hazardous to humans because of their high chronic toxicity with respect to various largely irreversible defects of the nervous system (Steinnes, 1990). Indeed, the nervous system is more sensitive to Hg toxicity than any other system in the body. Overall, Hg and its compounds show strong teratogenic effects and carcinogenic and mutagenic activity. For a detailed review of environmental exposure and toxicity of Hg refer to (Tchounwou *et al.,* 2003).

1.5 THE ANALYTICAL PROCEDURE - SAMPLE COLLECTION AND PREPARATION

For metal determinations, the matrix of concern may be responsible for the level of assay problems encountered as it may impose relatively pronounced effects during the preparation steps or interferences during measurements. Samples collected in the environment can be categorised on the basis of difficulty of the analysis as follows: water, atmospheric aerosols, soils and sediments, animal tissues, plant samples, solid wastes (Hoenig, 1998).

Water

For water samples, sampling is carried out using clean bottles, preferably made from polyethylene for inorganic analysis. The sample is then preserved by acidification with nitric acid (<2 pH) (Radojevic and Bashkin, 1999). This greatly reduces bacterial activity. This treatment also prevents adsorption of metal ions onto the vessel or suspended particles, but can mobilise metals associated with suspended matter. In this case, the sample can be filtered using a membrane filter of 0.45μm porosity and subsequently acidified (Hoenig, 2001). Unless pre-concentration procedures are necessary, the sample can then be analysed. Reference should be made to the British Standard BS EN ISO 5667-3:1996 Water quality – sampling – guidance on the preservation and handling of samples for detailed optimal sample handling and storage conditions.

Soil

For soil samples the procedure is rather more complex and involves a number of steps; drying; homogenisation; dissolution and mineralization. Sample collection is generally performed using an auger or corer to a uniform depth of approximately 7-20 cm. The soil sample is placed in a polyethylene air tight container. Drying is performed either by air or oven drying, normally close to 100°C (Anderson, 1998). Homogenisation is carried out by crushing, grinding, milling, then sieving to obtain <2mm fraction. Dissolution and mineralisation serves to convert the sample to a form compatible with the measurement technique utilised (generally solution), simplify the sample matrix and extract the analytes into solution. British Standard BS EN ISO 1038-6:1993 Soil sampling – guidance on the preservation and handling of samples, details optimal sample handling and storage conditions.

In environmental studies the main mineralisation and dissolution techniques (which often take place simultaneously) involve drying ashing or wet digestion procedures. In drying ashing the organic content of the sample is decomposed (mineralised) using high temperatures; in a furnace at *ca.* 450°C over several hours; and the ash obtained is dissolved in an appropriate acid.

The advantages of dry ashing are; a large number of samples can be prepared simultaneously; minimal amounts of acids are used; a total destruction of organic matter (the associated elements are generally transformed to carbonate or oxide forms) and the resulting solutions are usually clear, colourless and odourless (Hoenig, 2001). The drawbacks are loss of some analytes (i.e. Hg, As, Se through volatilisation, and retention/loss of analytes into ceramic or silica ashing vessels). This method would also be difficult to adopt for rapid field based analysis.

In most modern wet digestion procedures various combinations and proportions of strong acids with hydrogen peroxide ensure the mineralization and dissolution of the sample. Compared to dry ashing, wet ashing presents a wide range of options that concern the choice of reagents and their mixtures as well as devices used for the procedure (Hoenig, 2001). The advantages over dry ashing are use of lower temperatures, so reducing loss through volatilisation and retention losses of elements on the walls of the digestion vessel are less frequent. Perhaps the most important attribute of wet digestion techniques is that, using specific schemes of partial and/or selective dissolutions, it is possible to define the analyte distribution in various constituents of the sample. A detailed review of wet digestion techniques is given in Chapter Three.

Throughout the analytical procedure; from sample collection, to the analyte determination itself; a number of errors can be introduced, leading to inaccurate data. These include; non-representative sampling of the system under investigation; storage of samples can lead to physical, chemical and biological changes; contamination from apparatus (i.e.milling equipment and reagents; and matrix interference during the analyte determination). These errors result either in analyte loss (incomplete recoveries) or in its contamination (excessive recoveries). Analyte losses may be provoked by volatilisation, adsorption, transformation, precipitation or co-precipitation resulting from storage/treatments utilised during sample preparation steps (Hoenig, 2001). With increasing demands for trace and ultra trace determinations, using highly sensitive instrumentation, these become important issues.

To reduce these errors samples should be stored in a refrigerator at 4 °C or for longer times in a freezer, until analysis can be performed. The length of time for which samples can be stored varies, depending on the analyte and matrix. Recommendations include use of appropriate materials to avoid sample pollution and limited mass of sample and size of vessel to be used. This latter factor minimises the contact area with the solution. Further practices include use of high purity reagents, clean vessels, and simplified handling, hence avoidance of unnecessary filtrations and solution transfers. Contamination risks increase with temperature, pressure, long-term contact of solutions with vessels and with decreasing analyte concentration.

Matrix interference errors are associated with the measurement technique itself resulting from differences between compositions of calibration standards and samples. The interaction between the analyte with the matrix and the possibility of co-extraction of interfering substances can often lead to signal depression (loss of analyte. The composition of samples, particularly soils is very variable leading to a large number of possible interferents, the exact nature of which is difficult to predict since soils are complex systems with physical and chemical and biological characteristics that vary over time and space. Major components including silicon, aluminium (Al) and iron (Fe) along with oxygen and organic substances are responsible for most metal ion-soil matrix interactions (Marin *et al.,* 2001). Other major components include but are not confined to Manganese (Mn) magnesium (Mg), potassium (K) calcium (Ca) and titanium (Ti) (Marin *et al.,* 2001). How much they will interfere with the analysis depends on the nature of the analyte, the extraction method chosen, the nature of the sample (each sample is a unique mixture) and the detection mode. Matrix interferences are discussed in Chapter Three.

A large number of factors associated with the analysis of environmental samples can influence the accuracy of data obtained and the analyst has to be aware of the inherent difficulties. Simplification or minimising the steps involved would reduce errors, costs and analysis time. For example, *in situ* analysis would reduce errors related to transport and storage of samples (no sample degradation issues) allowing more accurate toxicological assessments to be made, while reducing costs and analysis times.

1.6 ANALYTICAL TECHNIQUES FOR THE DETECTION FOR HEAVY METALS

A number of analytical techniques are available for the analysis of metals in soils and water samples, listed below. The choice will depend on the analyte of interest, detection limits, instrumentation cost, analysis time, and sample preparation or selectivity.

☐ Atomic Absorption Spectrometry (AAS)
☐ Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) and Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

☐ X-Ray Fluorescence (XRF)
☐ Neutron Activation Analysis (NAA)
☐ Visible Spectrophotometry
☐ Existing field-based instrumentation/technologies
☐ Electroanalytical Techniques

An overview of the techniques is given here; electroanalytical techniques are discussed in detail in Section 1.7.

1.6.1 Atomic Spectrometry

The area of heavy metal analysis is currently dominated by techniques which can be grouped together under the general title of atomic spectrometry. The individual techniques are:

☐ Flame atomic absorption spectrometry (FAAS)
☐ Graphite furnace atomic absorption spectrometry (GFAAS)
☐ Inductively coupled plasma emission spectrometry (ICP-AES)
☐ Inductively coupled plasma mass spectrometry (ICP-MS)

Atomic absorption spectrometry

Atomic absorption spectrometry is the process that occurs when a ground state atom absorbs energy in the form of light of a specific wavelength and is elevated to an excited state (Thomas, 2003). The amount of light energy absorbed at this wavelength will increase if the number of atoms of the selected element in the light path increases. The relationship between the amount of light absorbed and the concentration of analyte present in known standards can be used to determine unknown concentrations by measuring the amount of light they absorb (Thomas, 2003).

Flame atomic absorption spectrometry (FAAS)

Flame atomic absorption spectrometry is one of the most widely used techniques for the determination of heavy metals and is the choice for smaller laboratories (Bersier *et* *al.,* 1994). This technique involves the sample being transported to a spray chamber by compressed air, where it forms an aerosol before being introduced into the flame. Light of a characteristic wavelength, specific to the analyte, passes through the flame. The amount of light absorbed, due to the analyte, is directly proportional to the concentration of the analyte in the flame and is derived from Beer's Law:

$$\mathbf{A = \varepsilon bC} \qquad (1)$$

Where A is absorbance; ε is the molar absorptivity; b is the path length of the light through the absorbing solution and C is the concentration of absorbing substance (Skoog *et al.,* 2000).

Although FAAS can be used for multi-element determinations, providing a good sample through-put; when coupled with an auto-sampler and an on-line dilution device; it requires specific light sources and optical parameters for each element to be determined and may require different flames (Thomas, 2003). The light source is usually a hollow cathode discharge lamp whose cathode is composed of the element of interest (Alloway, 1990). For multi-element determinations, all samples are analysed for one element, then the system adjusted for the next element and so on. The analytical working range generally covers two or three orders of magnitude, with detection limits in the range of 0.05 – 1 mg l^{-1} and a precision of 2-5% relative standard deviation (RSD) for most metals (Alloway, 1990). It is simple and rapid to use, with few well documented interferences. Its main drawback is its speed in relation to multi-element techniques such as ICP-AES and ICP-MS. Where direct aspiration FAAS does not provide adequate sensitivity; resulting from the inefficiency of sample introduction by pneumatic nebulisation (<10% efficient) and the large dilution introduced by the expanding flame gases (Alloway, 1990), reference is made to specialised techniques such as GFAAS, hydride generation atomic absorption spectrometry (HGAAS) and cold-vapour atomic absorption spectrometry (CV-AAS).

Graphite furnace atomic absorption spectrometry (GFAAS)

In GFAAS an electrically heated graphite tube replaces the flame. A small volume of sample (10-100μl) is introduced directly into the tube, which is then heated through a series of steps to remove the solvent and major matrix components, and then to atomise the remaining sample (Thomas, 2003). At the atomisation stage, an atomic absorption measurement is made through the tube. Because the whole sample is atomised and the atomic vapour produced is partly confined within the graphite tube, sensitivity and detection limits are significantly improved. In terms of concentration, the detection limits are 2-3 orders of magnitude better than those of FAAS and ICP-AES (Bersier *et al.,* 1994). The enhanced sensitivity and its ability to analyse very small samples significantly expands the capabilities of AAS.

The main drawbacks of GFAAS are higher operator skills, lower sample throughput, longer analysis times and increased chemical interference and background absorption (Thomas, 2003). Interferences are now largely controlled by a combination of high quality graphite material, platform technology and Zeeman background correction with matrix matching and standard additions (Bersier *et al.,* 1994).

Hydride generation atomic absorption spectrometry (HGAAS) and (CV-AAS) can be applied to significantly increase sensitivity for selected elements. HGAAS is for hydride forming elements such as arsenic (As), bismuth (Bi), selenium (Se) and Antimony (Sb). The analyte is converted to its hydride by sodium borohydride reagent and transported into a suitable atomising device in the AAS (Thomas, 2003). CV-AAS is used for the analysis of Hg and is based on the unique properties of Hg. Elemental Hg has an appreciable vapour pressure at ambient temperature and the vapour is stable and monatomic and can easily be reduced to metal form from its compounds (Thomas, 2003). The Hg vapour may be introduced into a stream of inert gas and measured by AAS without the need of atomiser devices.

Atomic emission spectrometry

Atomic emission is a process in which the light emitted by atoms or ions is measured. The emission occurs when sufficient thermal or electrical energy is available to excite a free atom to an unstable energy state (Thomas, 2003). Light is emitted when the atom or ion returns to a more stable configuration or ground state. The wavelengths of light emitted are specific to the elements present in the sample, with the intensities of the emitted lines a function of concentration of the atoms in the exciting source and hence of the solution content (Thomas, 2003).

One of the most critical components for this technique is the atomisation source as it must also provide sufficient energy to excite the atoms as well as atomise them (Alloway, 1990). Inductively coupled plasma, in principle, the emission from the flame-like plasma formed on a quartz torch by coupling a radiofrequency (RF) electromagnetic field to the electrons in an ionised Ar plasma. The plasma is heated by the passage through the plasma of current, which, because of the high frequency, is concentrated in the outer surface of the plasma (Alloway, 1990). As viewed from the top, the plasma has a circular shape. This characteristic of the source confines the sample to a narrow region and provides an optically thin emission source. The sample aerosol; produced by pneumatic nebulisers fed from a peristaltic pump; is directed into the central hole of the plasma, where temperatures are typically 6500 K. At these high temperatures, atomisation is virtually complete for most elements and strong atomic and ionic emission can occur (Alloway, 1990).

ICP has similar sensitivity as FAAS, however is much faster than AAS, capable of determining 60 or more elements at once by monitoring at preset wavelengths. Chemical interferences are minimal due to the very high temperatures involved and can be overcome by increasing plasma power. Spectral interferences, due to direct line coincidence, occur when the dispersion device is not capable of separating the analyte emission line from the matrix emission line. However, recent instruments offer high resolution, unlimited selection of analyte wavelengths, and various correction techniques incorporated into the software (Thomas, 2003). Due to its widely acknowledged attributes, including low detection limits, a wide linear dynamic range and high precision, ICP-AES is becoming the dominant technique for rapid spectroscopic multi-element analysis (Bersier *et al.,* 1994).

A more recent development is to use the ICP as an ion source for a mass spectrometer (MS). A basic ICP-MS is the synergistic combination of ICP with a quadrupole mass spectrometer, utilising the ability of the ICP to efficiently generate singly charged ions from the elemental species within the sample (Thomas, 2003). The charged ions are directed through the MS and separated according to their mass/charge ratios. Ions of the selected mass/charge are directed to a detector, which quantitates the number of ions present.

ICP-MS possesses the multi-element capabilities and broad linear range of ICP-AES, as well as detection limits; 0.01-500 ng l^{-1} range in the final sample digest; superior to that obtainable by GFAAS (Thomas, 2003). All elements can be determined using ICP-MS. Interferences are a complex issue, however are well documented. The analyst should be experienced in the recognition and correction of spectral, chemical and physical interferences (Thomas, 2003).

1.6.2 X-ray Fluorescence Spectrometry (XRF)

X-ray fluorescence spectrometry is used for elemental analysis based on the detection of emitted x-ray radiation from excited atoms. X-rays are short wavelength electromagnetic radiation, a conventional x-ray spectrometer generally utilises the region of 0.1-11 nm (X-ray fluorescence spectrometry, 2002). This technique is a two-step process that begins with the removal of an inner shell electron of an atom. The resulting vacancy is filled by an outer shell electron. The second step is the transition from the outer shell electron orbital. This rearrangement of electrons results in emission of x-rays characteristic of the given element, with the intensity related to the concentration of the element (EPA method #6200).

X-ray fluorescence spectrometry is a simultaneous multi-element technique that is capable of detecting most elements (with the exception of those with an atomic number below 8, for which special equipment is necessary) (Alloway, 1990). It is especially suited to analysis of solids but there are difficulties obtaining quantitative data, due to resolution, relatively low sensitivity (parts per million (ppm)) and matrix interferences.

1.6.3 Neutron Activation Analysis (NAA)

Neutron Activation Analysis is a multi-element, solid sample technique for the analysis of soils, plants and biological materials, that makes use of the γ-radiation (gamma rays) induced by neutron irradiation (exposure) of the sample (Alloway, 1990). The principle involved in NNA consists of first irradiating the sample with neutrons in a nuclear reactor to produce specific radionuclides. Following irradiation, the characteristic γ-rays emitted by the decaying radionuclides are quantitatively measured by a suitable semiconductor radiation detector, and the γ-rays detected at a particular energy are usually indicative of a specific radionuclide's presence (website: Oregon State University).

It is possible to measure 35 elements simultaneously in sample volumes of 5–10 mg, with detection limits for heavy metals are in the range 1 – 0.01 mg kg^{-1} depending on the analyte (Alloway, 1990). However, its considerable potential for heavy metal and other elemental analysis is restricted by the requirement for access to a neutron source, usually a nuclear reactor. While non-reactor sources have been used, generally the lower sensitivity resulting from their lower neutron flux, and their non-isotropic irradiation field, compared to reactor sources, have limited their application (Alloway, 1990). Thus, the use of NNA is limited to national laboratories or those with access to a local reactor centre, sophisticated instrumentation and computer facilities.

1.6.4 Visible Spectrophotometry

The study and analysis of chemical species in solution by the use of light (electromagnetic radiation) is called spectrophotometry (website: Theory (Exp. 3 – spectrophotometry). When light from the visible region (380-750 nm) passes through a solution containing a material that absorbs specific wavelengths of that light, the unabsorbed wavelengths are transmitted through the solution and produce a characteristic spectral response. The observed colour is said to be the complement of the colour absorbed by the species in the solution. The amount of radiation absorbed at an appropriate wavelength is proportional to the concentration of the light-absorbing chemicals in the sample (Csuros, 1997). The instrument used to determine those wavelengths that the sample absorbs and the intensity of that absorption is called a spectrophotometer.

Selectivity in the analysis is achieved in two different ways; solvent extraction with precise control of pH and the use of masking agents; and colour-forming complexing agents, which are sufficiently sensitive and selective for use in the aqueous sample without extraction being necessary.

Until the widespread use of atomic spectrometric techniques, visible spectrophotometry (also known as colorimetry) was a commonly used technique for heavy metal analysis. Detection limits can reach nanograms per gram level, depending on the analyte (Kebbekus and Mitra, 1998). Interferences from other elements present in the sample matrix often affect the analysis.

1.6.5 Existing field-based instrumentation/technologies

The demand for rapid field-based analytical systems has resulted in a range of portable instrumentation being developed, based on a variety of analytical techniques. Electroanalytical field-based methods are discussed in Section 2.1.

Laser-induced breakdown spectroscopy (LIBS)

Laser-induced breakdown spectroscopy entails a laser beam being focused onto a target material and transient and highly energetic plasma is formed when the laser irradiance exceeds the breakdown threshold of the material. The emitted radiation from the plasma can be spectrally resolved and the intensity of the spectral lines measured for quantitative and qualitative purposes respectively (Hou and Jones, 2000).

The use of fibre-optics and portable devices has allowed the development of battery powered instruments. These consist of a sampling probe connected to the main analytical unit by electric and optical cabling. The hand-held probe contains a small laser to generate laser sparks on a surface and a fibre optic cable to collect the spark light. The collected light is spectrally resolved and detected using a compact spectrograph/CCD detector system.

The advantages of these instruments are that no sample preparation is required, and the analysis of both conducting and non-conducting liquid, solid and gaseous samples is possible. Disadvantages include interferences from sample components, availability of suitable standards, especially solids; leading to semi-quantitative data, sensitivity is poorer than conventional atomic spectrometric techniques; and the use of the laser increases the complexity and cost of the instrumental system (Hou and Jones, 2000).

X-ray fluorescence spectrometry (XRF)

Portable field screening XRF is a recognised method; USEPA Method #6200; and is the most widely used method for the determination of Pb in paint (Hou and Jones, 2000). Limitations include higher limits of detection (ppm range) and lower precision than lab-based XRF systems and the need for handling precautions due to the radioactive source.

Immunoassays

Immunoassays provide an alternate approach for the determination and speciation of metal ions, with the advantages of fast analysis, low cost, simple operation, portability, and high sensitivity and selectivity. However, immunoassays can lack robustness, including short shelf life and reproducibility. Theoretically, this technique is applicable to the measurement of any species for which a suitable antibody can be generated (Hou and Jones, 2000). Immunoassay uses the very specific interactions between the analyte and antibodies. Each antibody is specific to the target substance, frequently termed an antigen. Antibodies may be monoclonal, which means that they are of single antigen specificity or polyclonal in which a mixture of target-specific antibodies are present. To monitor the reaction between the sample antigen and the reagent antibody, the antibody or the antigen is usually tagged with a chemical or enzyme label, which allows easy detection. Labels exhibiting electroactivity can be coupled to, for example, electrochemical transducers, thereby creating electrochemical immunosensors (Wang and Tain, 1998a). A detailed study on antibody-based sensors for heavy metal ions was reported by Blake *et al.* (2001), and Gerlach *et al.* (2001) demonstrated the detection of Hg using a commercially available immunoassay kit BiMelyze (BioNebraska, NE, USA).

Photometric measurement

Dasgupta *et al.* (2002) reported on a Light-Emitting Diode (LED)-based photometric measurement for As, with the formation of arsenomolybdate from As(V) and ammonium molybdate as the detection chemistry. However, further work was envisaged to limit interferences from phosphate present in samples, further improve the LOD and reduce the use of consumables and overall capital/operating costs.

For the analysis of As in water, the most widely used field kit is based on the Gutzheit test (Huang and Dasgupta, 1999). Several hundred millilitres of a water sample is taken, rendered strongly acid with HCl and Zn dust added. Nascent hydrogen reduces all As to arsine, which is purged out by the evolving H_2. After passage through a plug soaked in lead acetate to remove H_2S, the liberated gas passes through a mercuric bromide ($HgBr_2$)-impregnated filter that turns yellow upon exposure to arsine. The concentration of As is determined by comparison with a colour chart.

A systematic study using reference materials and comparison with AAS indicated the $HgBr_2$ test does not provide meaningful quantitative data below 150 $\mu g\ l^{-1}$ (Huang and Dasgupta, 1999). Simple inexpensive light emitting diode-based spectrometers, making use of the same methods, have been designed for field use and have provided greater sensitivity. The main disadvantages are the hazards to the operator associated with production of toxic gas and the cost of large reagent use.

Hussam *et al.* (1999) evaluated the Merck field kit (Arsenic Quant Test Strips, Alfa-Aesar, USA) for semi-quantitative determination of As. The kit contained the reagents (HCl and Zn) with the ambient AsH_3 gas concentration being measured by a commercial single-point arsine monitor (SPM, Hydride Chemcassette detection

System, Zellweger Analytics, Inc., IL, USA). The instrument measures the change in colour of a proprietary reagent on a paper tape by reflectance photometry. It was concluded 'The kit is not only inadequate to screen water samples containing less than 100 ppb of As, it also produces toxic arsine gas that may be a health hazard'.

The drive to move analytical chemistry into the field, primarily as low cost, rapid screening tools, has resulted in new technologies being researched and developed. However, achieving the main goal of obtaining meaningful data which can alert the analyst when legal threshold values have been exceeded ($\mu g\ l^{-1}$ levels in drinking water), using inexpensive and simple instrumentation, is a difficult task. More often than not current field techniques which offer the required sensitivity are expensive and require skilled personnel, while inexpensive methods are often cumbersome (large reagent use) and do not provide adequate sensitivity for trace analysis. As discussed in the following sections electroanalytical techniques can offer rapid and sensitive (ppb) measurements utilising inexpensive instrumentation.

Field based screening assays do not compete with laboratory techniques in terms of repeatability and at times sensitivity. However, field screening can provide an attractive alternative to the end-user in terms of initial financial outlay, cost per analysis and huge cost savings related to the number of samples being sent to a centralised laboratory for detailed analysis. As discussed in Section 1.2, the rapidity of data collection also aids environmental monitoring and pollution control.

1.7 ELECTROANALYTICAL DETECTION METHODS FOR HEAVY METALS

Electroanalytical techniques are concerned with the interplay between electricity and chemistry, namely the measurements of electrical quantities, such as current, potential, and charge, and their relationship to chemical parameters (Wang, 1994a). The connection between chemistry and electricity is a very old one, going back to Allesandro Volta's discovery, in 1793, that electricity could be produced by placing two dissimilar metals on opposite sides of a moistened paper (Lower, 1994). In the twentieth century, Michael Faraday showed there is a quantitative relationship between the amount of electrical charge and the quantity of electrolysis product (Lower, 1994). This involves a heterogeneous process, where an oxidation/reduction (redox) reaction involves the transfer of electrons from one reactant to another (Skoog *et al.*,1996):

$$\text{Oxidised form (O)} + \text{ne}^- \Leftrightarrow \text{reduced form (R)} \qquad (2)$$

This reaction takes place at the working electrode, where the applied potential is the controlled parameter that causes the analyte in solution to be oxidised or reduced at the electrode surface. For a given (electrochemically active) molecule at an electrode surface, shifting the electrode potential in a negative direction will favour reduction, whereas shifting it in a positive direction will favour oxidation.

The Nernst equation, developed by Walter Nernst (1864-1941) relates the potential E which is developed at an electrode to the standard electrode potential E^o and to the activities of the species O and R at the electrode surface:

$$E = E^o - \frac{RT}{nF} \ \text{In} \ \frac{aR}{aO} \qquad (3)$$

Where E is the actual electrode potential produced by the chemistry at the electrode surface when the reactants O and product R are at the concentrations designated in the equation; E^o is the standard electrode potential of the couple *vs.* a standard hydrogen reference electrode (SHE), which is characteristic for each half-reaction and is the hypothetical potential that would be generated if all reactants had an activity of 1 at standard temperature and pressure. R = gas constant (8.31441 J K^{-1} mol^{-1}); T = temperature (K); n = number of electrons; F = Faraday constant (96,485 C); In = natural logarithm (2.303 log); aR and aO are the products of the chemical activities of all the species which occur on the reduced and oxidised sides of the electrode equation.

By substituting numerical values for the constants, converting to base 10 logarithms and specifying 25°C for the temperature, the Nernst equation is as follows:

$$\mathrm{E} = \mathrm{E}^{\mathrm{o}} - \frac{0.0592}{n} \log \frac{aR}{aO} \tag{4}$$

This relationship predicts that a cell potential will change by 59.2 mV per 10-fold change in the concentration of a substance involved in a one-electron oxidation or reduction; for two-electron process, the variation will be 29.6 mV per decade concentration change (Lower, 1994). These predictions are only fulfilled at low concentrations of all ionic species and for those systems for which the redox reaction is rapid in both directions. The Nernst equation is true at a potentiometric electrode (i.e. where no current is allowed to flow) such as a reference electrode; in voltammetry the working electrode has an applied potential, which is relative to the reference electrode and the circuit is completed with a counter electrode, which allows current to flow. The reference electrode does not have a potential applied and achieves equilibrium in solution. The potential developed by the reference is monitored by the potentiostat, allowing the working electrode to be poised at the correct potential (relative to the reference).

A typical electrochemical cell used in controlled-potential experiments is shown in Figure 1.1. The working electrode is where the reaction of interest takes place, the reference electrode provides a stable potential against which the potential of the working electrode is compared, and the counter electrode carries the cell current, completing the circuit.

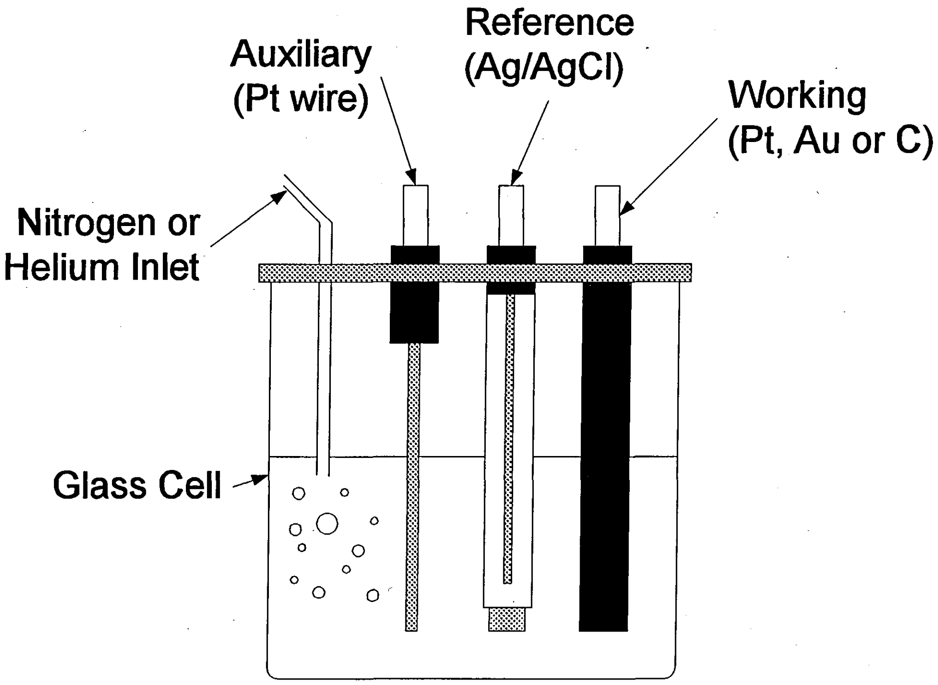


Figure 1.1 A typical electrochemical cell.

The reference electrode must exhibit a constant composition of both forms of its redox couple (Wang, 1994a). This essentially means that the concentration of any ionic species involved in the reaction must be held at a fixed value. One of the most common choices of reference electrode in voltammetric studies is the silver/silver chloride system (Ag/AgCl):

$$\mathrm{Ag} \mid \mathrm{AgCl}_{(s)} \mid \mathrm{Cl}^-_{(aq)} \qquad (5)$$

The half-cell electrode reaction is:

$$\mathrm{AgCl}_{(s)} + e^- \Leftrightarrow \mathrm{Ag}_{(s)} + \mathrm{Cl}^-_{(aq)} \qquad (6)$$

Applying the Nernst equation to this reaction we have:

$$E = E^o - 0.0592 \,.\, \log\,[\mathrm{Cl}^-] \qquad (7)$$

The reason for this derivation is that, by convention, solid reactants are given an activity number of one and solute activities are given by their molar concentration.

The electrode potential is thus governed by the chloride ion concentration of the solution. The E° for the half reaction is +0.222 mV (using a chloridised silver wire in 1 M KCl at 25°C and 1 atmospheric pressure). As the chloride ion concentration changes, the electrode potential will change, as predicted by the Nernst equation:

[Cl⁻] concentration (mol l⁻¹)	**Log [Cl⁻]**	**E (mV)**
1	0	222 – 0 = 222
3	0.477	222 – 28.2 = 193.8
6	0.778	222 – 46 = 176
0.1	-1	222 + 59.2 = 281.2
0.01	-2	222 +118.4 = 340.4
0.001	-3	222 +177.6 = 399.6
0.0001	-4	222 +237 = 459

These figures reveal that the reference potential deviates most significantly from the standard potential at low chloride concentrations (<0.1 mol l^{-1}) and indicate the importance of maintaining a substantial concentration of chloride ions in the sample when using a screen printed Ag/AgCl reference electrode.

An alternative and commonly used reference electrode is the saturated calomel electrode (SCE) (Monk, 2001). Relative to the standard hydrogen electrode (SHE) at 25°C, this has a potential of 0.2444 V (Monk, 2001).

$$Hg_{(aq)} \mid Hg_2Cl_{2(s)} \mid KCl \qquad (8)$$

The half-cell electrode reaction of the SCE is as follows:

$$Hg_2Cl_{2(s)} + 2\,e^- \leftrightarrow 2\,Cl^- + 2\,Hg_{(l)} \qquad (9)$$

As discussed in Section 1.7.3, the working electrode can either be a dropping mercury electrode (DME), used in polarography, solid electrodes such as gold (Au), platinum (Pt), other noble metals, and carbon (C), including screen-printed carbon inks and glassy carbon, hanging mercury drop electrode (HMDE) and mercury film electrodes (MFE).

The counter electrode should be an inert conducting material, such as platinum wire or graphite rod (Wang, 1994a).

The three electrodes are immersed into a solution, containing a supporting electrolyte, which is added to the solution, and the analyte species. The cell container can be an ordinary laboratory glass beaker, covered, with suitably sized holes for each of the electrodes and a tube for deoxygenating. Sample solutions range from between 5 and 50 ml, but smaller volumes as low as a few microlitres can be used with micro-cells (Dewald, 1996). The exact cell design and the material used for its construction are selected according to the experiment at hand and the nature of the sample (Wang, 1994b).

Since the work of Faraday, a variety of electroanalysis techniques have been reported. These include oxidation/reduction titrimetry, potentiometry, coulometry, electrogravimetry and voltammetry. Detailed accounts of these methods can be found in Wang (1994a); Skoog *et al.* (1996); Brainina and Neyman (1993); Brett (1999); Ekland and Bond (1999); and Juttner *et al.* (2000). Voltammetric methods are discussed here, focusing on anodic stripping analysis

1.7.1 Voltammetry

Voltammetry is a class of electroanalytical techniques involving the monitoring of current when a time-dependent potential is applied to an electrochemical cell (Ekland and Bond, 1999). In a voltammetric measurement, the potential is varied in a systematic manner. If an electroactive species is present, a current will be recorded when the applied potential becomes sufficiently negative or positive for that species to be electrolysed. This current, due to a change in the oxidation state of an electroactive species, is termed the Faradaic current (Wang, 1994a). The resulting voltammogram shows the variation in current as the potential is changed. The current (Amps) *vs.* the potential (Volts) can be interpreted to give both qualitative and quantitative information. The Faradaic current is proportional to the concentration of the electroactive species and the potential at the current peak, which is related to the standard electrode potential of the redox species, provides qualitative information.

In addition to the faradaic current, a background, non-faradaic current, unrelated to the redox reaction of interest, passes through the circuit. This is due to processes occurring at the interfacial region of the working electrode, namely the "electrical double layer" (Wang, 1985). This current determines detection limits, depending on the relative magnitude of the non-faradaic current to the magnitude of the faradaic current.

Polarography was the first type of voltammetry to be used, described by Heyrovsky in the 1920's. It introduced the elements of renewed mercury electrodes and selectivity through control of electrode potential (Wang, 1994a). It differs from other voltammetric techniques in that it uses a dropping mercury electrode (DME). It also differs as the limiting currents are controlled by diffusion alone rather than by both diffusion and convection (Skoog *et al.*, 1996). Because convection is absent, polarographic limiting currents are generally one or more orders of magnitude smaller than hydrodynamic limiting currents.

The DME consists of 12 – 20 cm long glass capillary tubing, connected by a flexible tube to an elevated reservoir of Hg (Wang, 1994a). Electrical contact is made through a wire inserted into the Hg reservoir. Mercury flows by gravity through the capillary at a steady rate, emerging from its tip as continuously growing drops. Adjusting the height of the mercury column controls the drop time - typically 2-6 seconds. For a measurement, the excitation signal used is of the linear-scan type, in which the potential of the working electrode is increased or decreased at a typical rate of 2-5 mV/s. The potential is scanned slowly with time so that a potential is nearly constant during one drop life. Thus each successive drop measures the current at a slightly different potential and a polarogram is generated (Wang, 1994a).

Although classical Polarography was an important tool used by chemists for the determination of inorganic ions and certain organic species during the 1940's, several limiting factors ensured that by the 1950's and early 1960's it was largely supplanted by various spectroscopic methods, which had superior sensitivity and specificity (Skoog *et al.*, 1996). The main limitations were problems associated with the use of mercury, namely the ease with which mercury is oxidised; total range of Hg in aqueous solution ca. +0.3 V to -0.2 V vs. Ag/AgCl; limiting the number of applications, and the contribution of the non-faradaic current, which limited sensitivity to concentrations of approximately 10^{-5} M. The DME is also cumbersome to use and tends to malfunction as a result of clogging.

However, since the introduction of Polarography, a wide range of instrumental modifications and control systems have been developed. These have been aimed at improving sensitivity, selectivity, and scope. These include pulse voltammetry, stripping analysis, and improvements in cell design. More progressive trends involve techniques such as sensor surface modification; Brett and Fungaro (2000); Stulik (1999); Degefa *et al.* (1999); application of microelectrodes and their arrays; Kovacs *et al.* (1995); Seddon *et al.* (1997); Sabon and Darling (1999); creation of miniature total analysis systems; Stulik (1999); Brett (1999); Wang (1997); and micro-fabrication technologies; Suzuki (2000); Feeney and Kounaves (2000).

Development of these techniques, coupled with momentous changes in electronics, computers, and computation, has made possible the commercial development of relatively inexpensive instrumentation. Fleet and Gunasingham (1992) reported "During the last decade, in the area of trace inorganic analysis, voltammetric techniques, especially differential pulse and stripping analysis, have provided strong competition for spectroscopic techniques".

1.7.2 Stripping analysis

Stripping analysis is a combination of electrochemical procedures and consists of two main steps. During the first step, the analyte is concentrated at the surface of an electrode under controlled conditions ('depositioning'). This serves to extract the analyte from the sample solution, providing a degree of selectivity, and deposit it on the electrode. Thus the concentration of the analyte at the surface of the electrode is far greater than it is in the bulk solution, offering major advantages over direct voltammetric measurements. The deposition is performed either by physical adsorption on the electrode surface, by electroplating onto a solid electrode, or by electrolytic deposition into a liquid mercuric film, forming an amalgam or insoluble Hg salt (Dewald, 1996). The concentrated analyte is then electrolytically or chemically re-dissolved (stripped) from the electrode.

Versions of stripping analysis include anodic stripping voltammetry (ASV), cathodic stripping voltammetry (CSV), potentiometric stripping analysis (PSA) and adsorptive stripping voltammetry (AdSV). Detailed accounts of these methods can be found in Esteban and Casassas (1994) and Economou and Fielden (1997).

Anodic Stripping Voltammetry

Anodic Stripping Voltammetry (ASV) is the most popular stripping method for the determination of trace metals. The metal ions of interest are pre-concentrated by electrodeposition on the working electrode. The pre-concentration is carried out by cathodic deposition at a controlled time and negative potential. The metal ions reach the working electrode by diffusion and convection, where they are reduced and in the case of Hg electrodes, are concentrated as amalgams, with their resulting concentration in the amalgam being higher than in the bulk solution (Wang, 1994a), as illustrated for the cationic metal M^{n+} below:

$$M^{n+} + e^- + Hg \leftrightarrow M(Hg) \qquad (10)$$

Pre-concentration is followed by the stripping or measurement step, in which the metal is oxidised electrolytically from the working electrode. The analytical response is obtained by applying a positive (anodic) potential waveform scan. When the potential reaches the standard potential of the metal-ion couple, that particular metal is oxidised back into the solution, causing a current:

$$\mathrm{M\,(Hg)} \rightarrow \mathrm{M^{n+}} + \mathrm{e^-} + \mathrm{Hg} \qquad (11)$$

The technique is both quantitative and qualitative, with the magnitude of the current equating to metal ion concentration, whilst the potential identifies the particular species present. A typical anodic stripping voltammogram (Figure 1.2) will show one or more current peaks with increasing potential, each peak relating to the presence of stripped metal ions in solution.

Differential pulse anodic stripping voltammetry (DPASV) is a derivative form of ASV that uses a series of discrete potential steps (ΔE) rather than a linear potential ramp (Figure 1.2). The waveform applied to the working electrode is in effect a linear ramp with a superimposed square wave of given amplitude (E_A) and period (P). The differential pulse voltammogram is obtained by measuring the working electrode current immediately before the potential step is applied (i_1), and again immediately prior to cessation of the applied square wave pulse (i_2). The measured analytical current is the difference between these two measured currents ($i_1 - i_2$). Output is in the form of a current versus potential plot as shown in Figure 1.1. The current sampling regime used in DPASV is able to discriminate between the analytical and capacitive currents within a sample as it measures a *differential* current that is essentially free of capacative charging effects. The very high analytical sensitivity achievable with this technique is largely due to the same analyte species being repeatedly deposited and stripped by the fluctuating stripping potential.

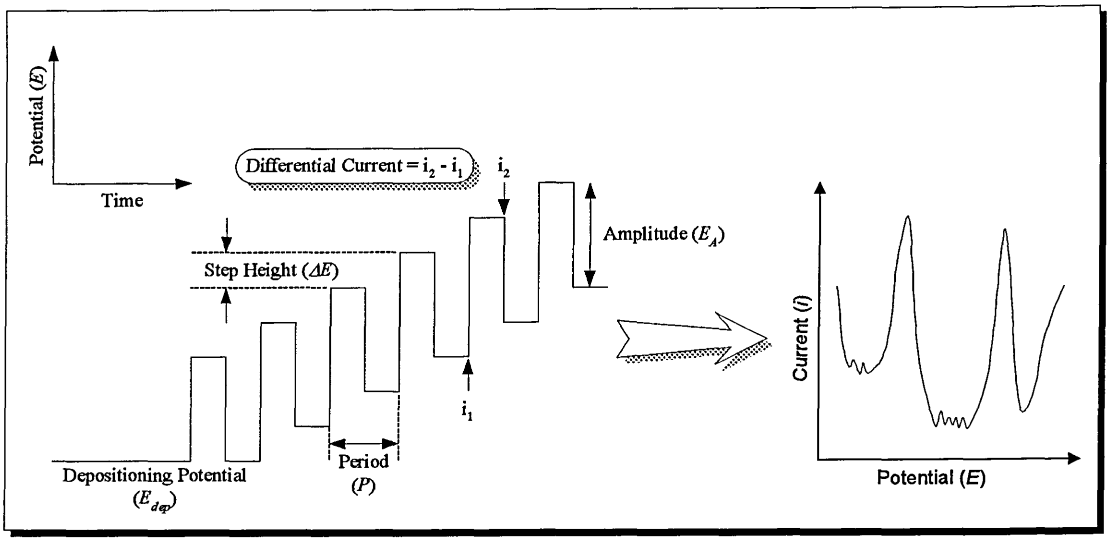


Figure 1.2 Example of the waveform applied in DPASV and the resultant current versus potential voltammogram obtained. The potential of the working electrode is increased in discrete potential steps (*ΔE*). The particular amplitude and frequency of the potential steps coupled to the current sampling regime act to reduce capacative charges, resulting in a reduced background current.

Overall, differential pulse voltammetry (DPV) can offer increased sensitivity over linear-scan voltammetry with up to a 1000-fold decrease in detection limits being possible. Honeychurch (2000) reported "DPV results in better sensitivity and the resolution between closely adjacent peaks is greatly improved". However, Wang (1985) reported "DP stripping peak currents are more susceptible to interference by surface active materials than are the corresponding linear scan peaks because of the sensitivity of DP measurements to small changes in the rate of the electrode reaction". Although additional procedures may be necessary to avoid possible interferences, the DP excitation waveform is widely used when detection limits at the ppb level and below are required.

Other potential-time waveforms, aimed at reducing relative contribution of non-faradiac currents to the measured current, have been developed and include staircase voltammetry (SCV), square wave voltammetry (SWV), and alternating current voltammetry (ACV). These methods are detailed in; Osteryoung (1993); Wang (1985); and Dewald (1997).

DPASV is the most popular stripping method for the determination of trace metals. It has the ability to measure four to six metals simultaneously at sub-part per billion (sub ppb) concentration levels. No additional instrumentation or analysis time is required for multi-element determinations. Wang and Tain (1999) reported that "ASV has always been recognised as a powerful tool for measuring trace metals. Its remarkable sensitivity is attributed to the built-in pre-concentration step, during which the target metals are accumulated onto the working electrode. The portable instrumentation and low power demands satisfy many of the requirements for on-site measurements of trace metals".

DPASV can be a viable and competitive alternative to spectroscopic methods for the following reasons; the inherent sensitivity and low detection limits; suitability for the detection of inorganic, organometallic and organic compounds; low cost portable instrumentation suitable for field-based analysis; and simultaneous multi-analyte determinations. As discussed in Section 1.4, speciation measurements are essential to determine toxicity and predict the role and fate of trace metals in the environment.

Tercier and Buffle (1993) reported "amongst the tools available, voltammetric techniques for trace analysis are convenient as they are capable of discriminating by their very basic principle between mobile forms, such as free metal ions, and small labile complexes with sizes smaller than a few nanometers and particulate trace metal forms. The former can be obtained by direct measurements in raw material while the latter can be obtained by subtraction from the total metal concentrations measured in raw and filtered acidified samples".

DPASV is restricted to approximately thirty elements and is commonly used for the determination of metal ions at the trace level. The analysis of Pb and Cd are perhaps the most widespread applications (Dewald, 1996). DPASV has been used extensively for the analysis of clinical and environmental samples, foodstuffs, beverages, pharmaceutical tablets and in forensic chemistry for the determination of Pb in firearm discharge residues. The technique is applicable to a variety of matrices, including both liquids and solids from organic or inorganic origins (Wang, 1985). A selection of typical applications is presented in Table 1.5, whilst more extensive reviews can be found in Brainina and Neyman (1993) and Wang (1985).

Table 1.5 Some applications of ASV.

Analyte	Matrix	Reference
Cu	Human Hair	Pournaghi-Azar *et al.* (2000)
Zn	Plant material	Bond *et al.* (1997)
Zn, Pb, Cu	Sea/waste water, soils, air	Oi-Wah-Lau *et al.* (1998)
Cu, Pb, Zn	Rum	Barbeira *et al.* (1997)
Zn, Cd, Pb	Drinking water	Svancara *et al.* (1994)
Cu, Pb, Cd	Mussels, algae	Locatelli (1997)
Ag, Cu	Water, biological fluids	Labar and Lamberts (1997)
Cd, Pb, Cu	Drinking water, soils	Williams and D'Silva. (1994a)
Cd	Sewage sludge	Pacer *et al.* (1999)
Cd, Pb, Zn, Cu	Honey	Sanna *et al.* (2000)

Cathodic Stripping Voltammetry

Cathodic stripping voltammetry (CSV) is used to determine a variety of anions (chloride, bromide, sulphide) that form insoluble salts with the Hg(I) ion (Dewald, 1997). A relatively positive potential is applied to a Hg electrode where Hg is oxidised to Hg(I). In the presence of an anion, an insoluble film forms on the electrode surface. Stripping consists of an increasing negative potential scan which reduces the Hg salt to metallic Hg and the anion of interest. The stripping peak potential and height are characteristic of the type of anion and its concentration in solution (Dewald, 1997).

Potentiometric stripping analysis

In Potentiometric stripping analysis (PSA) the first step is performed in a manner similar to that of ASV; by means of a negative potential, metal ions are reduced and dissolved in a MFE. Metal ions which cannot be determined by amalgamation in Hg can be deposited on a solid electrode, such as Pt (Dewald, 1997). However, the stripping is achieved by chemical oxidation with an oxidising agent such as oxygen, Hg^{2+} or Cr^{6+} present in the solution (Esteban and Casassas, 1994). As the concentration of the metal in the amalgam is exhausted, the potential drops towards more positive values. The duration of each plateau (at the E^0 of the redox couple) represents the time required for oxidation of the metal, with the height of the potential step related to analyte concentration (Dewald, 1997). Alternatively, a small constant current (5-20 µA) can be applied to strip the metals from the amalgam, termed constant current stripping analysis (CCSA). PSA has been applied to the determination of heavy metals in a variety of matrices.

Adsorptive stripping voltammetry (AdSV)

Adsorptive stripping voltammetry (AdSV) entails a spontaneous adsorption process to preconcentrate analytes at the electrode surface. Accumulation of the analyte occurs by physical adsorption rather than by electrolytic deposition (Dewald, 1997). Hence, for a wide range of surface-active organic and inorganic species that cannot be pre-concentrated electrolytically, the adsorption approach serves as an effective alternative. The analyte is allowed to accumulate on the electrode surface for a specified length of time. The adsorbed materials are determined by applying an appropriate increasingly negative or positive potential scan, for reducible or oxidisable species respectively. Non-electroactive species are analysed from the tensammetric (adsorption/desorption) peaks produced during the stripping step (Dewald, 1997).

Adsorptive stripping voltammetry can also be applied to the determination of a variety of inorganic cations, including metals which do not form amalgams or which show non-reversible electrochemical redox behaviour (Esteban and Casassas, 1994). The formation of an appropriate surface-active complex of the metal; by adding a suitable chelator, precedes the pre-concentration step. In this way, metals such as Ni, Al, Se and Ti can be determined by AdSV following complexation with ligands such as dimethylglyoxime or catechol (Esteban and Casassas, 1994).

Possible interference in stripping analysis

The development and application of stripping analysis schemes are complicated by a number of issues such as organic interference from the sample matrix and overlapping stripping peaks. Chemical interference between individual elements and each sample constituent, and mutual interference's between the metal ions present can also create difficulties in the analysis (Staden and Matoetoe, 2000).

The presence of organic compounds, particularly surface-active substances, such as naturally occurring organic carbon, and industrial pollutants, for instance, long-chain amines and alcohols (Wang, 1985) can adsorb at the electrode surface. In most cases this leads to significantly reduced sensitivities. Chemically modified electrodes (CME); through chemical reaction, adsorption, or formation of a polymer film; can be used to improve selectivity and sensitivity of the electrochemical measurement. Matrix interferences are investigated and discussed in Section 3.3.2.

Overlapping stripping peaks result from the relatively narrow potential range over which most metals are oxidised, making it difficult to resolve the responses from metals that are electroactive in close proximity to one another (Wang, 1985). In addition to overlapping responses from two or more amalgam-forming species, solution-phase electroactive species present in the sample may produce a response at the same potential as the stripping peak of interest. The most common solution-phase interference is oxygen (Wang, 1985).

Chemical, mathematical and instrumentation approaches can be applied to solve this problem. Chemical approaches include the use of suitable complexing agents that shift the stripping peak potentials, chelation for selective masking of the interfering peak and the addition of a third element that forms a stable intermetallic compound with the interfering metal ion. Instrumental methods include shifting the deposition potential until the metal ion with the more negative potential is not deposited (Brainina and Neyman, 1993).

Chemometric treatment of data is a promising approach without the need for additional analytical procedures. This field includes linear regression, multivariate analysis, including pattern recognition, and artificial neural networks (ANN) (Cladera *et al.*, 1997). The use of chemometrics to obtain quantitative data is explored in Section 5.3.

The formation of inter-metallic compounds between metals deposited in the Hg electrode, or between the electrode material and a deposited metal result in severely depressed stripping responses. Various approaches have been suggested for minimising or eliminating these errors and are discussed in Section 2.3.5.

1.7.3 Working electrodes

The performance of stripping techniques is strongly influenced by the working electrode (Wang, 1994b). The selection of the working electrode depends primarily on the redox behaviour of the target analyte and the background current over the potential region required for the measurement. In general, the working electrode should possess electrochemical inertness over a broad potential window; including interference from water or oxygen; low background current, absence of pores and pronounced roughness of the surface, low ohmic resistance, and the possibility of simple surface regeneration (Brainina, 1995). Electrodes used in voltammetry are the static drop mercury electrode (SDME), solid electrodes such as gold (Au), platinum (Pt), glassy carbon, and carbon paste, and either solid or rotating Hg electrodes; the hanging mercury drop electrode (HMDE), the rotating disc electrode (RDE) and the Hg film electrode (MFE).

Mercury electrodes are often the preferred electrodes for electroanalytical investigations at more negative potentials (Desmond, 1996). This is due to Hg having a large accessible negative potential window, and can provide a highly reproducible, smooth and readily renewable surface (Wang, 1994a). However, it has a limited anodic range, due to the oxidation of mercury itself, and the electroactive analyte must be soluble in a conducting solvent.

The HMDE and RDE are popular choices for many applications. However these electrodes are bulky and expensive, with large mercury reservoirs and heavy motors (Wang, 1994b). They are generally combined with large cells of 20-100ml solutions, the operation of which requires careful cleaning, prolonged oxygen removal, solution stirring during the deposition, standard additions and replacement with new solutions. These electrodes also lack robustness, and require regular maintenance. As such they are unsuitable for on-site analysis. In contrast, MFEs can be applied to on-site analysis simply and cost effectively.

De Vries and Van Dalen published the theory of ASV with a MFE in 1963 and 1964. The MFE consists of a very thin (10-100μm) layer of mercury covering a conducting and inert support (Wang, 1994a). There are two general schemes for coating an electrode with a mercury film. Pre-plating involves an initial coating step in which the Hg film is formed on the electrode surface and *in situ* plating (co-deposition) in which the Hg film formation and the analyte accumulation are carried out simultaneously.

Pre-plated MFEs are commonly prepared by electrochemical cathodic deposition of Hg^{2+} ions from a mercuric nitrate solution onto the solid substrate (Desmond, 1998):

$$Hg^{2+} + 2e^{-} \rightarrow Hg_{electrode} \qquad (12)$$

In situ plating was originally proposed by Florence (1970) and involves co-depositing liquid mercury during the preconcentration phase of the stripping analysis. This is achieved by adding 1-5 x 10^{-5} mol l^{-1} mercuric nitrate directly to the analyte solution, prior to the analysis (Schiewe, 1997).

The choice of Hg film formation will depend on the particular application. However, Wang and Tian (1992) suggested "there is no practical advantage in incorporating an additional step in the analysis of pre-plating the mercury film". Frenzel (1993) found that "it is difficult to prepare pre-plated films of consistent and uniform thickness and even more difficult to preserve them in an active state for any length of time". Fischer *et al.* (1999) also suggests that "greatest sensitivity is obtained using *in situ* deposition of Hg, giving a thinner Hg film with a higher internal metal concentration, and which is less affected than the pre-plated film by interference by surfactants".

MFEs are stable, robust, and do not require any electronic ancillaries or mercury reservoir (Economou and Fielden, 1997). They offer a greater mechanical stability and large surface area to volume ratio, resulting in greater sensitivity and resolution between neighbouring peaks than those obtained by the HDME or SDME (Agra-Gutierrez *et al.,* 1999). The MFE is at least 1 order of magnitude more sensitive than the HDME and is therefore preferable for trace analysis (Schiewe, 1997). However, MFE are more susceptible to problems associated with the formation of inter-metallic compounds than the HMDE. This results from the higher concentration of the metals in the MFE, as compared to the HMDE (Wang, 1985).

The ideal substrate for the MFE must be electrically conductive, chemically inert to the Hg and analyte solution, and electrochemically inert in the potential range of interest (Dewald, 1996). Various metals and forms of carbon have been used as substrates. Noble metals, for example, Pt, Au, and Ag, although highly conductive, have the disadvantages of low hydrogen overpotential, solubility in Hg, surface oxide films, and formation of intermetallic compounds between the substrate and the deposited metal (Wang, 1985). This limits their utility in many analyses. Carbon materials, that have been employed, include unpolished carbon, pyrolytic graphite, wax and epoxy carbon, sprayed graphite layers, carbon fibres and glassy carbon (Svancara *et al.*, 1994).

Glassy carbon is a common choice due to its good electrical conductivity, chemical and electrochemical stability, low porosity, relatively high hydrogen overpotential, and wide availability (Economou and Fielden, 1997). Frenzel (1993) suggests that “glassy carbon has found widespread application and appears to be the ultimate choice for trace and ultratrace determinations of heavy metals”. However, weak adhesion to Hg often results in poor mechanical properties. The surface consists of sites of varying activity for the Hg plating (Wang, 1985). The resulting film is not shiny and uniform but consists of finely divided droplets of mercury. The size and distribution of these droplets depend on the deposition potential.

Tercier and Buffle (1993) suggest that "although glassy carbon substrates have been successfully used for laboratory based measurements, they are not suited to on-site analysis". Marken *et al.* (1997) was in agreement and remarked that "It has been noted that reproducible results using this method require a careful protocol for the treatment of the electrode surface. The rate of deposition has to be enhanced by stirring, rotating the electrode or the use of microelectrodes".

This would be inappropriate for field-based analysis. The use of microelectrodes (typically 0.1 – 10 μm), produced by modern microlithographic techniques using Pt, Au or carbon fibres, addresses some of these problems. Capacitive charging currents and increased mass transport rates, resulting in excellent signal-to-noise characteristics allow the determination of heavy metals, such as Cu, Pb, Cd and Zn, at ultralow concentrations ($<10^{-9}$ M) using ASV (Tercier and Buffle, 1993). However, the significant higher capita and fabrication costs make them less attractive for single use, on-site applications (Wang and Tian, 1999).

A more recent alternative as a substrate for the MFE are disposable screen-printed electrodes (SPE). These can combine ease of use and portability, with simple, inexpensive fabrication techniques. The coupling of SPEs with DPASV, offers greatly simplified designs and operation, in accordance with the requirements of field-based analysis.

1.7.4 Disposable screen-printed electrodes

Disposable SPEs are devices that rely on planar working, counter and reference electrodes on a plastic or ceramic substrate. These strips can be considered as self-contained electrochemical cells onto which the sample droplet is placed (Wang and Tian, 1999).

The fabrication of these electrodes is accomplished by using modern screen printing, thick-film processes as depicted in Figure 1.3. The technology relies on the sequential deposition of conductive and insulating inks with controlled thickness and shape onto the surface of a substrate (Palchetti *et al.*, 1999). The fabrication process requires application procedures and materials appropriate to the particular device being developed and it is therefore difficult to describe a general approach. However, several steps can be distinguished in the overall standard technology. A conductive film is applied to a substrate. The commonest inks used are carbon based for the working and counter electrodes and silver based inks for fabricating the reference electrode. The application of the film is done through a patterned screen contacting the substrate. The ink is placed onto the screen, and then forced through the screen with the aid of a squeegee. The film is baked to drive off solvents and cure the patterned paste.

Sequential depositioning of materials appropriate to the specific design and as often as necessary are required to realise the device (Galan-Vidal *et al.*, 1995). Such a process yields uniform and due to the low cost of fabrication, disposable electrodes of different shapes and sizes. A selection of reviews, covering the characteristics and applications of SPEs have been published by Fleet and Gunasingham (1992); Wang (1994b); Galan-Vidal *et al.* (1995); Brainina and Bond (1995); Hart and Wring (1994;1997); Wang and Tian (1999); Suzuki (2000) and Honeychurch and Hart (2003).

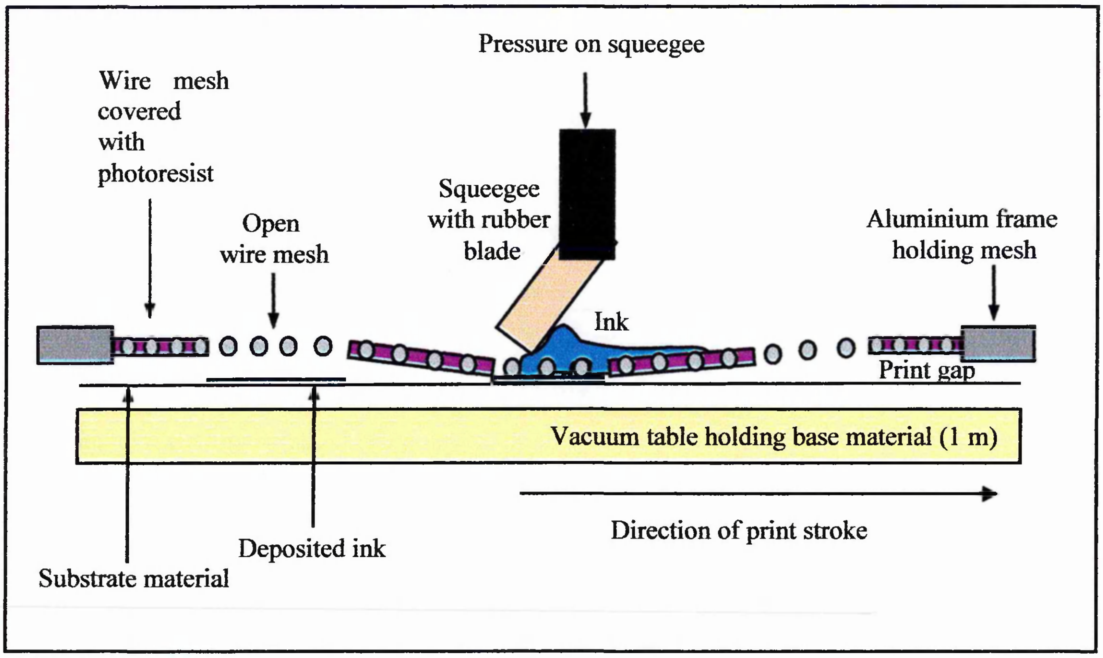


Figure 1.3 Schematic of the screen-printing process.

The attributes reported include flexibility of design and choice of materials, easy use, high selectivity and inexpensive fabrication techniques. Because no beaker based conventional electrochemical cell is required, SPEs are highly suited to field-based testing. SPEs are also for single use, which eliminates electrode preparation and cleaning procedures. Wang and Tian (1992) reported "convenient quantification of microgram per litre (ppb) concentrations is achievable using SPEs. Despite the very small non-dearated/unstirred samples and the low analyte concentration, the highly stable response coupled with extremely low cost make the SPE an attractive alternative to electrodes commonly used in routine (centralised) stripping operations".

To achieve such performance, proper attention is required towards selection of appropriate materials and printing conditions; including pressure, curing time, and temperature; due to their influence on the stripping and background signals. Desmond *et al.* (1998) concluded "Errors due to the screen printed sensor, such as differences in working electrode area and reference electrode potential can be responsible for variability in response". However, the use of very fine screen meshes allows reproducible quantities of ink to be applied very accurately. Screen-printing should not be considered an exact science, as many variables exist and precise, repeatable results will only be achieved if the process is applied correctly. This benefits from technical knowledge, good laboratory practice and experience.

Early development of SPE focused on the determination of glucose in blood samples. Hand-held glucose meters, widely used for home diabetes testing, represent the best example of a commercially successful electrochemical device utilising disposable enzyme electrode test strips. The first product was a pen-style device (Exac-Tech), launched by MediSense, Inc. in 1987; the working electrode was coated with a ferrocene-derivative mediator (Wang, 2002). Various commercial strips and pocket-sized test meters for self-monitoring of blood glucose have since been introduced. The success of the glucose sensors was the driving force behind the development of SPEs for other applications including other biomolecules, pesticides, herbicides and metals.

Another major incentive for the development of disposable SPEs was the initiative of the US Centres for Disease Control (CDC) aimed at large-scale screening of Pb in children's blood (Wang, 1994b). Such extensive screening requires a portable and yet highly reliable blood Pb measurement system. Leadcare (ESA Inc., Chelmsford, USA) is a self-contained analytical device able to perform on-site blood lead assays on finger stick samples smaller than 200µl within less than 5 minutes.

Surface modification schemes and the design of highly selective biological or chemical recognition layers (which can be incorporated into the carbon ink prior to electrode fabrication) has enabled applications of SPEs in biotechnology, clinical, food, and pharmaceutical analysis, agriculture, environmental studies and fermentation control, as well as the military and aerospace industries (Wang and Tian, 1999). Some recent studies are given in Table 1.6.

Table 1.6 Some applications of SPEs.

Analyte	Matrix	Reference
Trichlorfon, coumaphos	Drinking water	Gogol *et al.* (2000)
Ammonium, nitrate	Water, blood	Koncki *et al.* (1999)
Al	Water, soils	Akhtar *et al.* (1999)
Glucose, starch	Flour	Tao Hu *et al.* (1999)
Pesticides	Sheep wool extracts	Collier *et al.* (2002)
Ag	Photographic solutions	Dilleen *et al.* (1998)

Modification of the sensor surface involves a great variety of methods of surface activation and immobilisation of selective (bio)chemical systems on the sensor surface or in its bulk. Coupling highly specific biological recognition elements, such as enzymes, antibodies and micro organisms, with electrical transducers (biosensor), allows detection of the physico-chemical change; caused by the specific interactions between the target analyte and complementary bio-recognition layer, and hence provides quantitative and qualitative information.

Chemical sensors typically based on carbon SPEs as a substrate for the MFE (the MFE can be considered the recognition layer) have been widely exploited for the detection of the selected analytes (Cd, Pb, Cu, Zn) and are reviewed in Section 2.1. Carbon inks are well suited as a support for the MFE as they are relatively inexpensive, and exhibit the analytical requirements discussed in Section 1.7.3. Silver based inks are used for fabrication of the Ag/AgCl reference electrode. Other schemes, involving the use of CME are also reviewed. Schemes for the detection of As and Hg are reviewed in Section 5.1.

1.8 CONCLUSION

Concerns regarding heavy metal contamination and the effects to human health and the environment have resulted in extensive legislation (assessment, monitoring and control) which, in turn, has placed increasing demands on the field of analytical chemistry. Modern laboratory analytical instrumentation provides high sensitivities and low detection limits. However, field-based screening methods, although less accurate and precise, are now recognised as important tools in fulfilling legislative requirements. *In situ* testing greatly reduces costs, negates problems associated with sample contamination and storage, improves logistics of sending large numbers of samples to centralised laboratories, provides immediate decision-making potential, and allows more samples to be tested without increase in budget, all of which lead to more efficient and comprehensive environmental protection.

To this end, the hypothesis of this study can be defined as: (i) elevated levels of heavy metals in the environment pose a significant risk to human health and ecosystems, (ii) these metals can be detected using laboratory based electrochemical and spectrometric techniques (iii) specific development of electrochemical techniques may lead to a rapid and simplified multi-analyte *in situ* screening tool.

The following chapters detail the development and evaluation of an *in situ* electrochemical screening sensor for the analysis of the selected metals in soil and water samples. Chapter two concerns the fabrication of suitable carbon SPEs and development of an electrochemical assay for the simultaneous detection of Cd, Pb, Cu and Zn. In Chapter Three existing metal extraction methods for soils are reviewed and an alternative simple *in situ* extraction procedure is developed. This is an improvement in the state-of-the-art due to the lack of existing field protocol and provides a basis for further studies of this nature. Evaluation of the combined extraction + measurement system is performed by comparison with ICP-MS/AES data for a range of soil samples. The data obtained using the combined extraction + measurement system was linked to a newly developed data analysis tool, Fuzzy Areal Site Assessment (FASA) developed by partners within the PURE programme. This is based on a novel algorithm which aims at identifying all contaminant locations on a site using a limited number of samples. By linking the *in situ* screening sensor to the FASA site characterisation process, greater reductions in laboratory and drilling costs and rapid *in situ* risk assessments were achievable. This demonstrated a practical application of the developed extraction + measurement system.

Field demonstration, using portable instrumentation, was undertaken at a technical workshop organised by the European Commission funded EU Thematic Network on 'Sensors for Monitoring Water Pollution from Contaminated Land, Landfills and Sediment' (SENSPOL), the outcome of which is reported in Chapter four. Chapter Five concerns the development of Au SPEs, using a 'new' curable Au ink, for the detection of As and Hg. This is an improvement in the current state-of the-art as the Au ink can be cured at a low temperature (130°C), permitting electrode fabrication using disposable, cheap, plastic substrates. Furthermore, no surface modification or subsequent electrode adulteration to facilitate metal analyte depositioning is required. A patent; 'method and apparatus for trace level determination of metals', PCT/GB03/005101; has been filed for this work (Appendix A1). To de-convolute complicated stripping voltammograms, appropriate chemometric data analysis was employed.

By combining several techniques; materials selection, extraction, analysis, data treatment (chemometrics), output (FASA); the development of a complete field-based measurement solution has been possible.

CHAPTER 2

FABRICATION AND CHARACTERISATION OF CARBON *IN SITU* METAL SCREENING SENSOR

2.1 INTRODUCTION

To develop a sensor capable of providing end-users with an on-site analyte screening tool, allowing rapid *in-situ* measurements, issues regarding simplicity, speed, sensitivity, selectivity, low cost, and portability, must be addressed.

ASV can rapidly determine metal ions in the µg l^{-1} range using portable instrumentation (Section 1.7.1). Additionally, low-cost, disposal SPEs are an attractive alternative to classical bulky 'beaker-based' conventional cells, thus negating the need for solution stirring and deaeration, essential for simplified field-testing (Wang, 1994b). The use of microlitre sample volumes also greatly reduces solution waste in comparison to large volume conventional cells.

In addition, major problems associated with maintaining electrode stability and long term performance due to surface contamination by solution impurities and electrolysis products are eliminated by using SPEs. Fleet and Gunsingham (1992) suggested various possible solutions to these problems, but concluded, "Perhaps the approach recommended by the present authors of using low-cost, single analysis, disposable sensors may be the most effective approach".

Various studies have been undertaken to investigate the feasibility of exploiting carbon-based SPEs for trace metal determination. Wang and Tian (1992) first reported on the characteristics of SPEs for use in stripping analysis of trace metals. ExacTech blood glucose strips (Medisense Inc.) were employed for the determination of Pb by means of ASV and PSA in blood and urine samples. Comparison to the traditional HMDE indicated that sensitivity and signal-to-background properties were not compromised using the screen-printed strips. The simultaneous analysis of Cd, Pb and Cu was also demonstrated using standard solutions.

Desmond *et al.* (1998) described an environmental monitoring system for the determination of Cd, Pb and Cu based on ASV and SPE. A voltammogram of the simultaneous detection of Cd, Pb and Cu showed good resolution between stripping peaks. Single element calibration plots were reported for Zn, Cd, Pb and Cu, with detection limits of 55, 71, 64, and 123 $\mu g\ l^{-1}$ respectively.

Yarnitzky *et al.* (2000) developed a hand-held analyser for environmental samples, utilising disposal SPEs. The detection of Pb in drinking water samples was demonstrated. The use of SPEs greatly simplified the measuring procedure, and resulted in well-defined sigmoidal signals for Pb down to 5 $\mu g\ l^{-1}$.

Similar studies have been carried out using a range of carbon SPE designs for the determination of Zn, Cd, Pb or Cu. Wang *et al.* (1993) used a screen-printed ultra microelectrode array; Brainina and Bond (1995) investigated a graphite paste SPE; Desmond *et al.* (1996) developed a SPE incorporated into a 'Dipstick' analyser; Palchetti *et al.* (1999) characterised a carbon SPE; and Jung-Yuen Choi *et al.* (2001) developed a HgO-modified SPE.

More recently, debate over the use of Hg as an analytical tool has increased in scientific and environmental sectors due to concerns over occupational health and environmental issues. Palchetti *et al.* (2000) reported "even though the amount of Hg used for thin-film electrodes is neither significant nor represents a danger when properly used, the environmental lobby exerts strong pressure to develop mercury-free electrodes, which should be as reliable as the mercury electrodes". Zuman (2000) also suggested "Mercury in small amounts at room temperature is practically innocuous. It is only when heated above 100°C that mercury vapours are toxic. Inorganic salts of mercury have been used in medicine for centuries without ill effects, it is only organomercury compounds (not formed under laboratory conditions) which are toxic".

The question as to whether the benefits of providing an inexpensive and rapid on-site method of analysis, accessible to all industrial and regulatory sectors, for the assessment, monitoring and control of contaminated land, outweigh the possible threat of the use of Hg as an analytical tool, to the environment and human health is still very much a debatable one.

Thus, several mercury free approaches have been studied for the detection of Zn, Cd, Pb or Cu including; Wang *et al.* (2000) using a bismuth-coated SPE; Palchetti *et al.* (2000) developed a dithizone-modified SPE; Honeychurch *et al.* (2001) modified a SPE with 1-(2-pyridylazo)-2-naphthol; and Honeychurch *et al.* (2000;2002), examined the use of bare carbon SPEs.

This study acknowledges the work of others. However, none fully demonstrated a system capable of the simultaneous detection of Zn, Cd, Pb and Cu in soil and water samples, whilst keeping sensor design, fabrication techniques and analytical procedures simple and inexpensive. Furthermore, issues regarding implementation of the sensors, such as suitable sample preparation methods and instrumentation were not wholly addressed.

Commercial ASV metal analysers are available from the USA. The SA-1000 Scanning Analyser (Palintest Ltd) can detect Pb and Cu in water samples only, and the SA-5000 Scanning Analyser (Palintest Ltd) is for the measurement of Pb in water, soil, dust, and paint samples (www.palintest.com.au). However, the development of European technology, offering multi-analyte determinations in soil and water samples would be advantageous in terms of a more widespread acceptance of these devices by European industry, and better implementation of use through incorporation into standard methods.

Consequently, the envisaged electrochemical sensor will combine ASV with a Cranfield University 'in-house' SPE design (Section 2.2.3). Maintaining simple and rapid stripping protocols, using minimal reagents, while providing simultaneous determinations, were key factors during optimisation of the sensor. Sample preparation methods, and instrumentation suitable for the implementation of the sensor to on-site analysis of soil samples, will be addressed in subsequent chapters.

Carbon inks are composed of graphite particles, a polymer binder and other additives, selected for their dispersion, printing and adhesion tasks (Wang *et al.,* 1998b). Ink formulation is a specialised field, with most inks being proprietary in nature. As a result, selection of the optimal ink for a specific application is best achieved experimentally.

Therefore, three commercially available carbon inks were examined in order to determine the optimum ink formulation with respect to analytical performance and peak resolution for multi-analyte analysis. As the carbon inks are generally supplied in 1kg batches, often being used, then reclaimed from the stencil and re-used, it was also considered necessary to investigate the effects of ink contamination and ageing on the analytical performance.

The carbon SPEs were first characterised using CV, enabling electrochemical properties such as reversibility and active working electrode surface area to be determined. Key DPASV parameters were optimised and analytical performance was evaluated according to peak area (sensitivity), background characteristics, and reproducibility. As real sample analysis can be problematic due to complex mixtures of metals and interfering species, an essential selection criterion was also based on the degree of resolution between individual metal peaks on the resultant voltammogram.

Following carbon ink selection, the addition of Cd and Zn to the assay was evaluated. Finally, characterisation parameters, including limits of detection and linear range were established for Cd, Pb and Cu on the screen-printed carbon electrodes.

2.2 RESEARCH METHODOLOGY

Prior to commencing any laboratory procedures, COSHH (Control of Substances Hazardous to Health) assessments were completed. These identified hazards, disposal requirements, and control and emergency procedures. The recommendations were adhered to at all times. In addition, the location of fire extinguishers, spill kits, eyewash stations, and an appointed first aider, were identified.

2.2.1 General laboratory procedures and reagents

Good laboratory practice was adopted throughout all procedures, thus reducing indeterminate, or random, error caused by the day to day variation of instrumentation and reagents. Minor inaccuracies of the individual manipulations in the procedure are also minimised by adopting good laboratory practice.

Unless otherwise stated, all reagents used were of Analar grade, with minimal impurities, purchased from BDH (Poole, England), Sigma Chemical Company (Poole, England), and Aldrich Chemical Company (Gillingham, England). All solutions were prepared using HPLC grade water, being free of ions, organic compounds and micro-organisms, which can be present in distilled water.

All glassware was soaked overnight in a 10% nitric acid solution, rinsed with HPLC grade water, then oven dried at 80°C. This reduced contamination by residual metals from previous users and air-borne contamination in the laboratory. The same micropipettes were used for all volumetric measurements, as small variations between individual pipettes can occur.

High purity standard/stock solutions of 1000 mg l^{-1} were used to prepare 50 mg l^{-1} working standards for each analyte of interest. Standard mixtures were prepared at the required concentration, in 0.1 mol l^{-1} potassium chloride (KCl) and 1% v/v nitric acid (HNO_3) for subsequent ASV analysis. Potassium chloride was for use as a supporting electrolyte, and to stabilise the reference potential of the Ag/AgCl electrode. Nitric acid was used to obtain a low relatively constant pH and ensure the solubilisation of the analytes being determined.

A mercuric nitrate $Hg(NO_3)_2$ standard solution was also prepared by weighing out 2 g of $Hg(NO_3)_2$ and diluting to 100ml using a 1% v/v HNO_3 solution. This was used to prepare the mercury film in subsequent analyses. All solutions were stored in the refrigerator at 4°C, and used within 1 week.

Electrochemical measurements were performed using a computer controlled potentiostat-10, Autolab Electrochemical Analyser with a general-purpose electrochemical software operating system (GPES3) (Ecochemie, Utrecht, Netherlands). Conventional electrodes were used for comparative analysis and included; Platinum (Pt) disk working electrode; Pt wire counter electrode; Ag/AgCl solid reference electrode; purchased from BAS Technicol (Congleton, England).

2.2.2 Printing materials

Carbon inks Electrodag 423 SS supplied by Acheson Colloids (Plymouth, UK), I45 carbon ink, MCA Services (Cambridge, UK), C10903D14, Gwent Electronic Materials (Pontypool, UK). All inks were selected as they are widely used by the scientific community (Honeychurch *et al.,* 2000), (Wang *et al.*, 1996, 1998b) and were easily obtainable.

Ag/AgCl biosensor grade (15% w/w silver chloride) C2DR15 ink, was supplied by MCA Services (Cambridge, UK). Blue protective epoxy-resin based polymer insulating ink 242-SB was supplied by Agmet (ESL, Europe). Surface treated polyester sheets, 228x305mm (0.25mm thickness); ST725, were purchased from Cadillac Plastics Ltd (Swindon, England). The different SPE will be referred to as Acheson new ink, Acheson old ink, Gwent and MCA.

2.2.3 Printing procedure

Three-electrode SPE's were fabricated in-house by a multi-stage screen-printing procedure using an automated DEK 248 machine (Figure 2.1) (DEK Ltd, Weymouth, England) and screens with appropriate designs (DEK precision screen division, Weymouth, England). For the basal track a stainless steel stencil with 125 wires/cm and for all other layers a 77 wires/cm were used. The mesh was mounted at 45° to the print stroke and covered with 13 μm emulsion. The optimised printing parameters are summarised in Table 2.1.

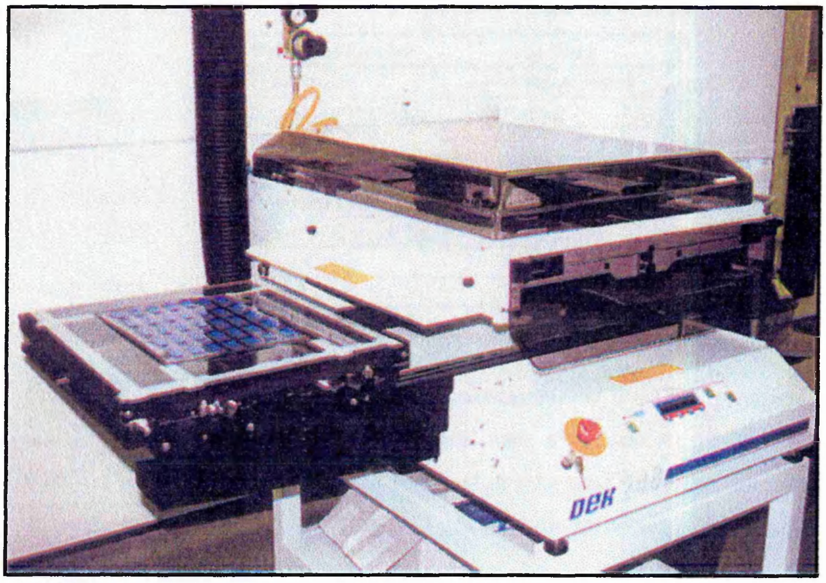


Figure 2.1 DEK 248 automated screen-printing machine

Table 2.1 Optimised parameters for printing SPEs, using DEK 248.

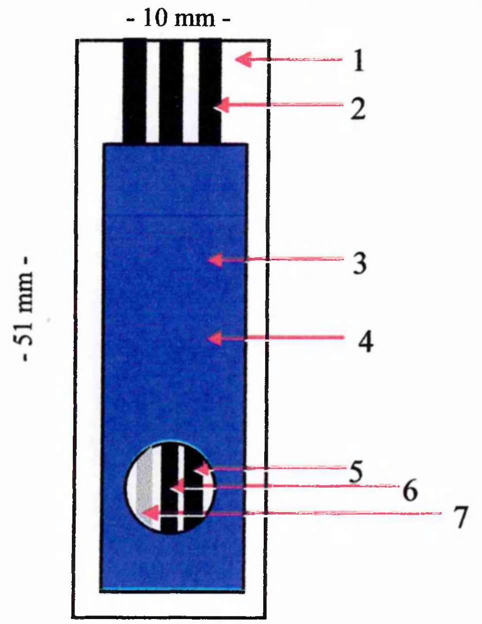
Print parameter	Setting
Print mode	Print/Flood
Squeegee pressure	4 kg/m^2
Print gap	2.5 mm
Deposits	1
Forward carrier speed	50 mm/s
Reverse carrier speed	50 mm/s
Front limit	50 mm
Rear limit	450 mm
Separation speed	70 %

Fabrication involved the sequential deposition of multiple ink layers onto the chosen substrate. The ink was placed onto the screen, and forced through the screen with the aid of a squeegee. The order in which the inks were deposited was determined by their function. The carbon ink was deposited first, forming the basal track, working and counters electrodes, followed by the Ag/AgCl ink to form the reference electrode, and finally the insulating ink.

Between the printing steps, the layers of ink were dried. For the carbon and Ag/AgCl ink layers, the drying step was performed at 60°C for a minimum of 2hrs. The insulation ink was dried at 120°C for a minimum of 2 hours to cure the epoxy resin.

2.2.4 Electrode design

An 'in-house' electrode design, which has previously proved successful at Cranfield, was adopted for this application, shown in Figure 2.2. This compact design allows the fabrication of one hundred electrodes per substrate sheet, as well as straightforward connection to the electrochemical analyser using standard connectors (RS Components, UK), as shown in Figure 2.3. It also allows easy and uniform addition of sample droplets onto the 3-electrode working area.



Legend
1:Base material "substrate"
2:Contact pad
3:Basal track (under insulating ink)
4:Insulating ink
5:Carbon auxillary electrode
6:Carbon working electrode
7:Ag/AgCl reference electrode

Figure 2.2 Screen-printed electrode design.

Figure 2.3 Standard electrode connector allowing electrode linkage to the electrochemical analyser

2.2.5 Characterisation of SPE by Cyclic voltammetry

The SPEs were initially characterised by cyclic voltammetry (CV). A simple potential waveform that is often used in electrochemical experiments is the linear waveform. The potential (E) is continuously changed as a linear function of time (t), depicted in Figure 2.4. The rate of change of potential with time is referred to as the scan rate (v). A commonly used variation of this technique is cyclic voltammetry (Figure 2.4).

In CV, the working electrode potential is initialised at a potential where minimal electrolysis occurs and hence there is minimal faradaic current. As the potential is scanned in a negative direction, across the formal potential of the analyte of interest, the analyte is reduced. At a particular set value, the scan direction is reversed and the analyte that was reduced in the outward excursion is re-oxidised, provided the analyte electrochemistry is reversible. CV is performed in unstirred solutions so the measured current (I) is limited by analyte diffusion to the electrode surface.

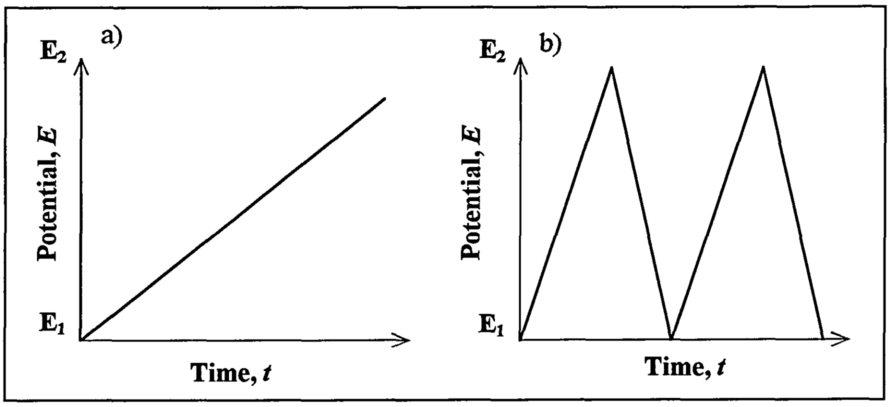


Figure 2.4 a) Linear scan excitation waveform b) cyclic voltammetry waveform.

The ferricyanide anion, $Fe(CN)_6^{3-}$, in which the iron atom is initially in the 3+ oxidation state was used to characterise the screen-printed carbon electrodes. A typical voltammetric response is depicted in Figure 2.5. At the surface of the working electrode, a single electron is accepted by each ferricyanide anion, causing it to be reduced to the ferrocyanide anion form $Fe(CN)_6^{4-}$, the iron atom is in the 2+ oxidation state.

On the reverse scan $Fe(CN)_6^{4-}$ is reoxidised to $Fe(CN)_6^{3-}$. Repeated reduction and oxidation of the analyte causes alternate cathodic and anodic currents to pass through the working electrode. By utilising a well-characterised redox couple such as the ferri/ferrocyanide couple, a basic electrochemical investigation of the physical properties of the electrode surface is possible.

A signal of primary interest is the height of the peak. The peak current, I_p, is described by the Randles-Sevcik equation:

$$I_p = (2.69 \times 10^5)\, n^{3/2}\, A\, D^{1/2}\, C\, \nu^{1/2} \qquad (1)$$

Where n is the number of electrons transferred, A is the electrode area (cm^2), D is the

dWhere n is the number of electrons transferred, A is the electrode area (cm^2), D is the

cdiffusion coefficient of the species being oxidised/reduced ($cm^2\ s^{-1}$), C is the (concentration of this same species in the bulk solution ($mol\ l^{-1}$), and ν is the scan rate ($V\ s^{-1}$).

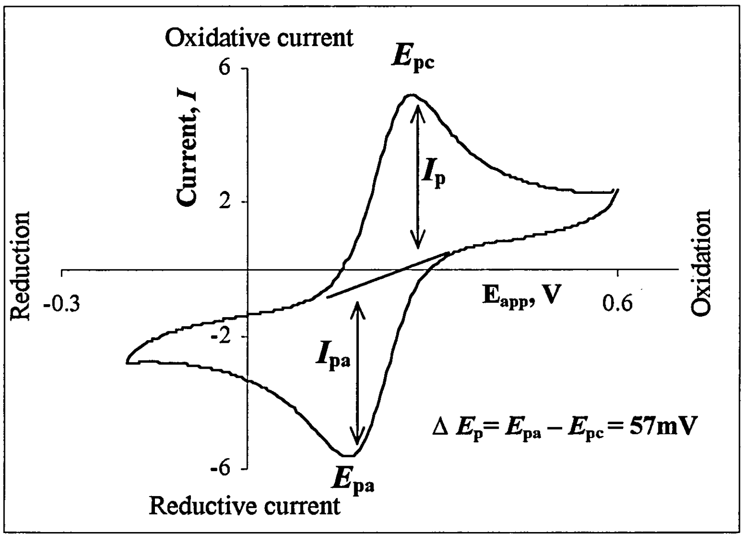


Figure 2.5 Typical CV (current-potential) curve for the $Fe^{2+}/Fe^{3+}(Cn)_6$ couple at a Pt disk working electrode (vs. Ag/AgCl reference electrode). E_{pc} = cathodic peak potential; E_{pa} = anodic peak potential; I_{pc} = cathodic peak current; I_{pa} = anodic peak current; ΔE_p = peak separation.

The potential difference between the reduction and oxidation peaks is theoretically 57 mV for a one electron reversible reaction. In practice, the difference is typically 70-100 mV. Larger differences, or non-symmetric reduction and oxidation peaks are an indication of poor reversibility. These parameters make cyclic voltammetry a powerful tool for characterisation and mechanistic studies of redox reactions at electrodes.

2.2.6 Cyclic voltammetry procedure

A 0.1 mmol l^{-1} potassium ferricyanide ($K_3Fe(CN)_6^{3-}$) was prepared in a 0.1 mol l^{-1} KCl solution. The SPE was connected to the electrochemical analyser (Section 2.2.3) and a 100μl sample droplet placed onto the SPE, such that it completely covered the 3-electrode assembly as described by the circular zone in the insulation layer. The potential range was set at +0.7V to -0.6V. The electrochemical behaviour of $K_3Fe(CN)_6^{3-}$ on the SPE was studied by varying the scan rate (v) between 10-300 mV s^{-1}. For each scan rate 10 complete cycles were performed to establish system equilibrium. The peak height of the anodic currents of the tenth scan was taken as the equilibrated peak currents.

A plot of peak current (amps) versus the square root of the scan rate ($v^{1/2}$) should transform the data into a linear relationship as described by the *Randles-Sevcik* equation. The equation can be modified to give an expression for the slope of the line;

$$\frac{Ip}{v^{1/2}} \text{ (Slope)} = (2.69 \times 10^5)\, n^{3/2}\, A\, D^{1/2}\, C \tag{2}$$

Since the diffusion coefficient of ferricyanide is well documented, the effective working electrode area (A) can be readily calculated.

For comparison purposes, CV's were also performed on a Platinum disk working electrode, where the surface area is very smooth and well characterised. This will give an indication of the accuracy of the procedure. For this, a 50 ml glass beaker, a large area Pt auxiliary electrode and a standard Ag/AgCl reference electrode were utilised. The Pt working electrode was polished every three experiments, using a HPLC water slurry of 0.3 μm alumina on a disc pad and rinsed thoroughly with water, to obtain a clean renewed electrode surface.

2.2.7 Differential Pulse Anodic Stripping

The mercury film electrode (MFE) was prepared by the *in-situ* method discussed in Section 1.7.3, and involved addition of 100μl of $Hg(NO_3)_2$ solution previously prepared to 10 ml of the test sample, giving an optimal of 20 mg l^{-1} $Hg(NO_3)_2$. The SPE was connected to the electrochemical analyser and a 100μl droplet of the solution pipetted onto the working area of the SPE.

Varying the deposition time (t_{dep}), deposition potential (E_{dep}), and $Hg(NO_3)_2$ concentration identified the optimal stripping parameters for each carbon ink type.

Once these had been established, (Section 2.3.4) the stripping performance of the carbon inks could be correctly determined.

2.2.8 Data analysis

All experimental/exploratory stripping analysis was repeated three to five times using individual SPEs for all tests. All data reported represent the mean of three replicate measurements, unless otherwise stated.

The relative standard deviation (RSD), expressed as a percentage, was used as a measure of assay precision and is defined as 100 s/x, where s=standard deviation and x=arithmetic mean (Miller and Miller, 2000). The correlation coefficients (r), slopes (b) and intercepts (a) of the calibration curves were calculated by regression and correlation analysis using Microsoft Excel.

2.3 DATA COLLECTION AND ANALYSIS

2.3.1 Carbon working electrode characterisation

The electrochemical properties of the carbon working electrode were examined using the well characterized redox couple ferro/ferricyanide ($[Fe(CN)_6^{4-}/[Fe(CN)_6^{3-}]$). Cyclic voltammograms of a 0.1 mmol l^{-1} $K_4Fe(CN)_6$ obtained using the various carbon working electrodes are depicted in Figure 2.6a-d. The peak at +0.05 V (vs. Ag/AgCl) is discussed at the end of this section.

All sensors exhibited the expected dual-peak shape characteristic of a reversible redox reaction, with an increase in peak currents (I_p) evident with increasing scan rate (v). At low v (20 mV s^{-1}), the anodic to cathodic peak separation (ΔE_p) obtained for both Acheson old and MCA SPEs was 220 mV, whilst Acheson new ink electrodes gave a value of 210 mV. Electrodes fabricated using Gwent ink showed the most reversible behaviour with the smallest ΔE_p of 190 mV.

This is considerably greater than the theoretical value of 58mV expected for Nernstian one-electron transfer reactions (Bond *et al.*,1997). A ΔE_p of 90 mV was obtained for ferrocyanide using a solid Pt disc working electrode (Figure 2.7).

The decrease in the electron transfer rate of the SPE, which manifests itself as an increase in ΔE_p, may be attributed to low carbon content of the ink and the presence of the polymer binder and other additives used for the printing process. As a result, uncompensated bulk resistances in the film (related to the dimensions and geometry of the film) and charge transfer resistances (related to the physical nature of the carbon ink) have been identified as possible causes of shifts in peak separation (Erlenkotter *et al.*, 2000).

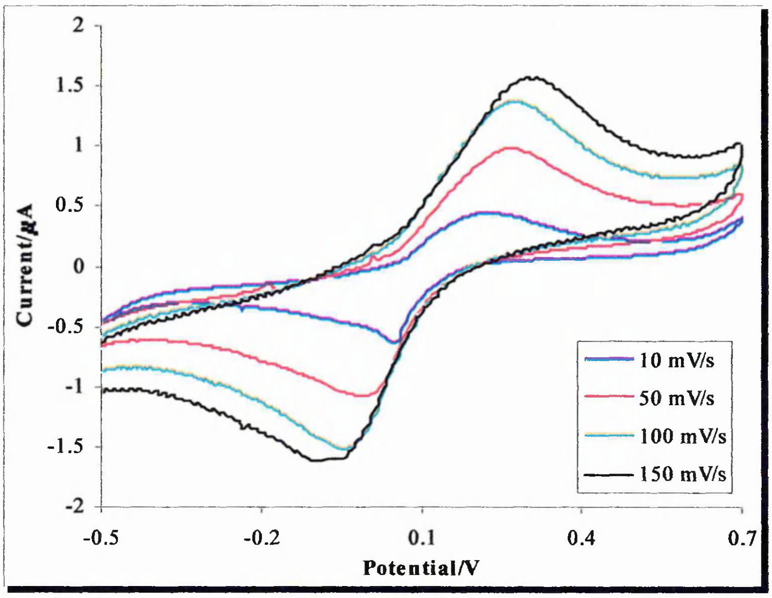


Figure 2.6a Cyclic voltammograms of a 0.1mmol l^{-1} $K_3Fe(CN)_6^{3-}$ at SPE fabricated using Acheson new ink. Initial potential +0.7V; switching potential -0.6V, scan rate (v) 10-150 mV s^{-1}.

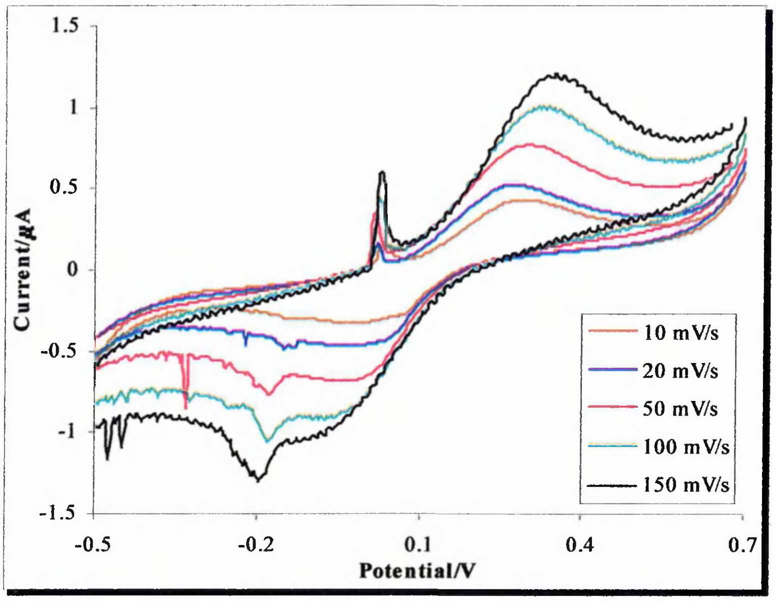


Figure 2.6b Cyclic voltammograms of a 0.1mmol l^{-1} $K_3Fe(CN)_6^{3-}$ at SPE fabricated using Acheson old ink. Initial potential +0.7V; switching potential -0.6V, scan rate (*v*) 10-150 mV s^{-1}.

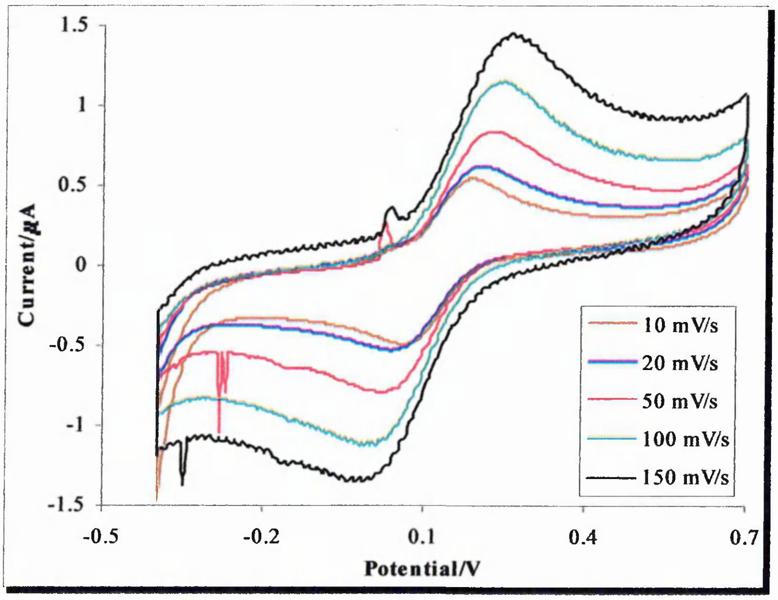


Figure 2.6c Cyclic voltammograms of a 0.1mmol l^{-1} $K_3Fe(CN)_6^{3-}$ at SPEs fabricated using Gwent ink. Initial potential +0.7V; switching potential -0.6V, scan rate (*v*) 10-150 mV s^{-1}

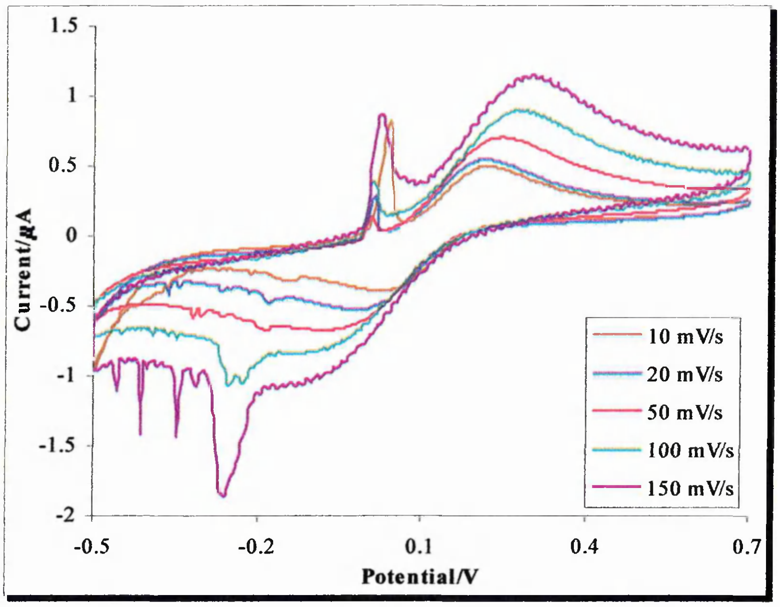


Figure 2.6d Cyclic voltammograms of a 0.1mmol l^{-1} $K_3Fe(CN)_6^{3-}$ at SPEs fabricated using MCA ink. Initial potential +0.7V; switching potential -0.6V, scan rate (*v*) 10-150mV s^{-1}.

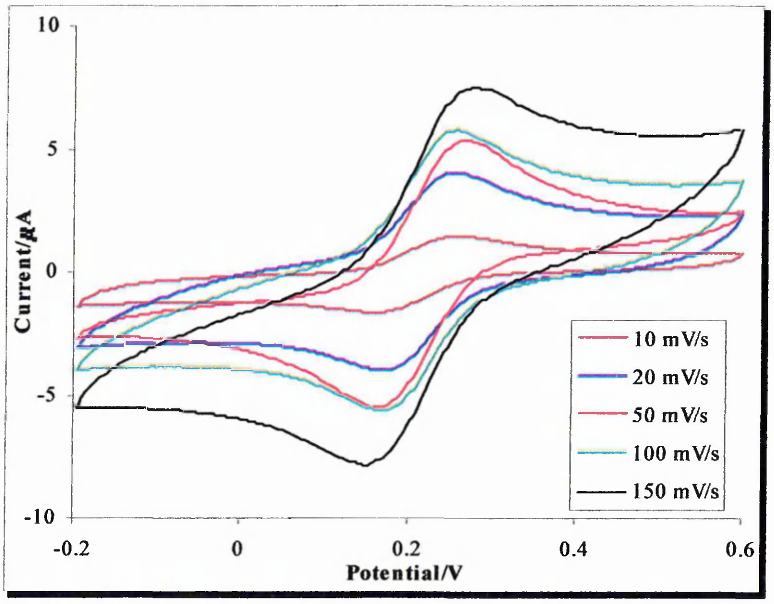


Figure 2.7 Cyclic voltammograms of a 0.1mmol l^{-1} $K_3Fe(CN)_6^{3-}$ Pt disc working electrode (vs. Ag/AgCl, Pt counter). Initial potential +0.7V; switching potential -0.6V, scan rate (*v*) 10-150mV s^{-1}.

Seddon *et al.* (1997) found the carbon to polymer ratio strongly affected the magnitude of the ΔE_p. As carbon content increased, an observed drop in the magnitude of ΔE_p from an estimated 320 mV for low carbon content to 100 mV for higher carbon content was observed. It was concluded that since any uncompensated bulk resistances in the film would contribute to shifts in ΔE_p of only a few millivolts, the trend was a consequence of the charge transfer resistances of the dry film.

Grennan *et al.* (2001) compared a GCE and a SPE fabricated from Gwent Ink. The heterogeneous electron transfer rates were calculated according to the method of Nicholson (1965), with values of 1.2×10^{-2} cm s^{-1} and 2.2×10^{-3} cm s^{-1} for the GCE and SPE respectively. The poorly reversible redox reaction of ferri/ferrocyanide at SPE was attributed to the presence of the polymeric binder.

Similar results were reported by Wang *et al.* (1996) who concluded "such decrease in the transfer activity is consistent with the presence of the binder, which leads to a decrease in the redox activity of a particular reaction and also the overall analytical performance of the SPE".

The increase in ΔE_p, which also increased with scan rate, shown in Figure 2.6 and the reported decreases in the electron transfer rates, suggests the voltammetric responses at screen printed carbon electrodes can be classed as quasi-reversible according to the definition of Desmond *et al.*, (1998).

SPE fabricated using Acheson old and MCA carbon inks also exhibited additional redox reactions taking place, with an anodic peak present at a potential of approximately 0.05 mV. SPE fabricated using Acheson new and Gwent inks also display similar behaviour but to a much lesser degree. These irreversible redox reactions may be due to contaminants or other components present in the ink.

Kroger and Turner (1997) also found this effect when using SPEs in 0.1 mol l^{-1} KCl. CVs ran in the absence of KCl did not display such peaks. It was suggested the chloride ions in solution could be reacting with the chloride ions present in the printed

Ag/AgCl reference electrode. The chloride ions are required as a supporting electrolyte and to stabilise the reference potential of the Ag/AgCl electrode and should not be omitted. However, this effect has not been reported using alternative working electrode inks with this Ag/AgCl reference ink, indicating some property of the carbon ink or contamination of the ink is responsible for the additional redox peak.

2.3.2 Electrochemical active area

In order to investigate the differences in ΔE_p displayed by the SPEs, and to determine if carbon content could be the possible source of these differences, the electrochemical active area for the ferrocyanide reaction on the carbon electrode surface was calculated using the *Randles-Sevcik* equation, modified for a quasi-reversible reaction, as detailed in Section 2.2.5.

Using the slopes obtained from plots of anodic I_p versus square root of scan rate ($v^{1/2}$) for 0.1 mol l^{-1} ferrocyanide, the *Randles-Sevcik* equation was applied to all the carbon ink types and the active surface areas were calculated. The results are as follows:

Ink type	**Active surface area**	**% of measured geometric area**
Acheson old	0.0277cm^2	19.78%
Acheson new	0.0207cm^2	14.78%
Gwent	0.0272cm^2	19.92%
MCA	0.0191cm^2	13.64%

The percentage value relates to the measured geometric (planar) area of the working electrode of 0.14cm^2 (0.7 x 0.2 cm). As expected the area of active carbon is much less than the geometric area, due to additional components in the ink. SPE fabricated using Acheson new and Gwent inks show a greater active surface area. This correlates with the exhibited smaller ΔE_p, for these sensors, suggesting these inks have higher carbon content and improved electron transfer kinetics.

Erlenkotter *et al.* (2000) examined a Pt SPE using Scanning Electrochemical Microscopy (SEM) and also found that compared to the geometrical area, the measured active electrode areas were significantly reduced. It was concluded that "this observation leads to the conclusion that only a small percentage, one third or one fourth of the surface area, comprised Pt readily available for the electrochemical reaction. The residual of the Pt surface seems to be blocked by the polymer".

From the investigations carried out using CV, it can be assumed that the Gwent ink has the highest carbon content, followed by Acheson new ink, due to the calculated active surface area and observed ΔE_p.

2.3.3 Optimisation of DPASV parameters for Pb and Cu

Initially, optimisation of DPASV parameters for Pb and Cu measurements was performed using Acheson new SPEs. This involved varying the E_{dep}, t_{dep} and Hg concentration. Stripping performances were then compared.

Deposition potential – (E_{dep})

The effect of E_{dep} on the analysis of a 200 µg l^{-1} Pb and Cu solution is summarised in Figure 2.8. The I_p for Pb increased with more negative E_{dep} up to –0.9 V. Further reduction in the E_{dep} resulted in diminished I_p and increased background currents, due to the evolution of hydrogen gas at negative potentials. At potentials more positive than –0.75 V no response was observed, as Pb is not reduced at these potentials on a carbon SPE. The maximum peak current for Cu was obtained at –0.7V. The Cu peak became broader and less well defined at potentials more positive than -0.7 V and no peak currents were recorded at -0.55 V. Taking into account sensitivity, low background current, and the requirements of multi-analyte analysis, an E_{dep} of -0.8 V was considered a suitable compromise for subsequent analysis of Pb and Cu.

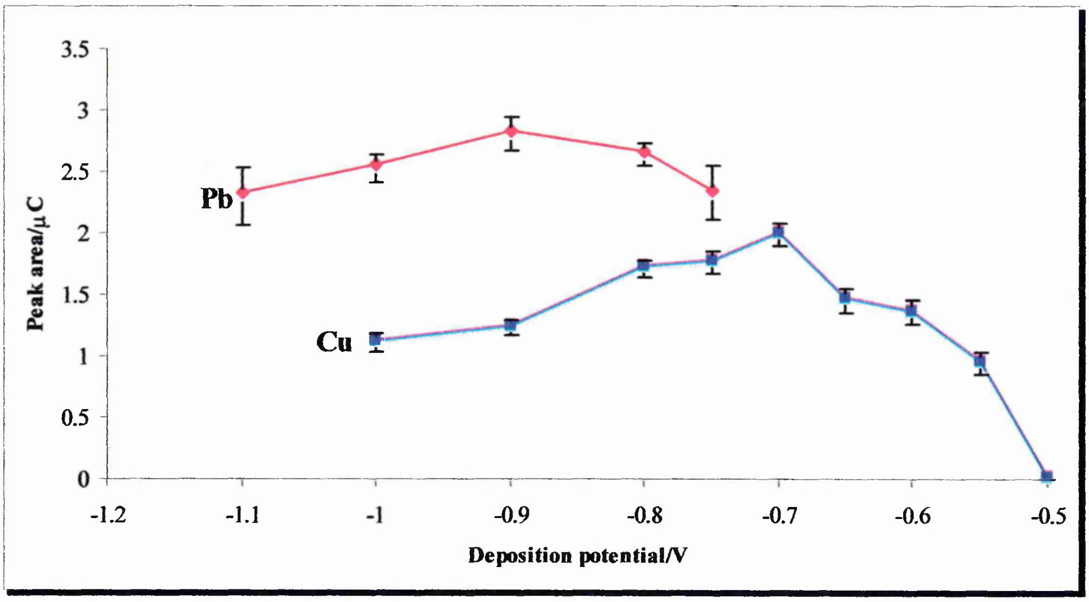


Figure 2.8 Effect of E_{dep} on the magnitude of stripping peak currents for 200 μg l^{-1} Pb and Cu, in 0.1 mol l^{-1} KCl, 20 mg l^{-1} $Hg(NO_3)_2$, (n=3). t_{dep} 165 sec; E_{scan}:-1.1v ⇒ -0.2v @ 20 mV s^{-1}; interval time 0.5 s.

Deposition time – (t_{dep})

Figure 2.9 shows the effect of t_{dep} on the I_p for a solution containing 200 μg l^{-1} Pb and Cu. As would be expected, the longer the t_{dep}, the larger the response, with the plot of I_p versus t_{dep} exhibiting linear behaviour up to 240 s. This results from an increase in the amount of Pb, Cu and Hg ions being reduced at the electrode surface. After 240 s, no significant change in the response was seen, which may be attributed to surface saturation of the working electrode.

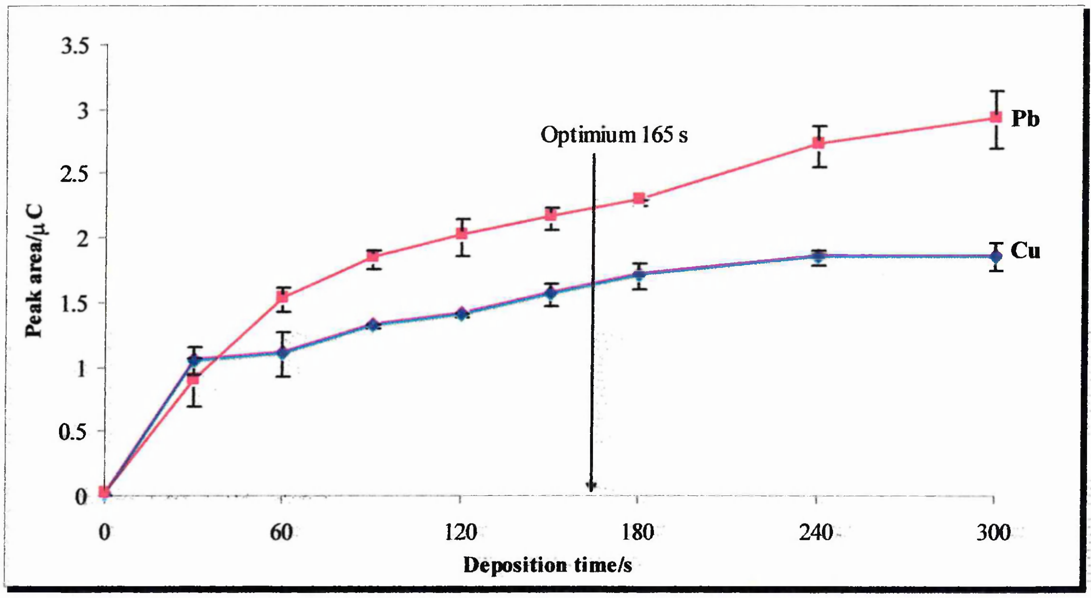


Figure 2.9 Effect of t_{dep} on the magnitude of stripping peak currents for 200 μg/l Pb and Cu, E_{dep} -0.8 V; all other experimental conditions are as defined in Figure 2.8.

The preferred t_{dep} would be no longer than that required to obtain a well-defined stripping peak. Unduly long plating times may cause interferences arising from the formation of intermetallic compounds or saturation of the Hg film (Reeder and Heineman, 1998). Therefore a t_{dep} of 165 s was selected for DPASV analysis of Pb and Cu ions at microgram per litre concentrations. This value was considered a compromise between sensitivity and analysis time.

Mercuric nitrate concentration

Figure 2.10 shows the effect of varying the $Hg(NO_3)_2$ concentration between 10 and 60 mg l^{-1}. No significant difference in the peak current was observed over 20 mg l^{-1} although the slope for Pb between 20-40 mg l^{-1} was higher than for Cu. Considering the conflicting issues of high sensitivity and use of low Hg concentrations, a $Hg(NO_3)_2$ concentration of 20 mg l^{-1} was selected for subsequent analysis.

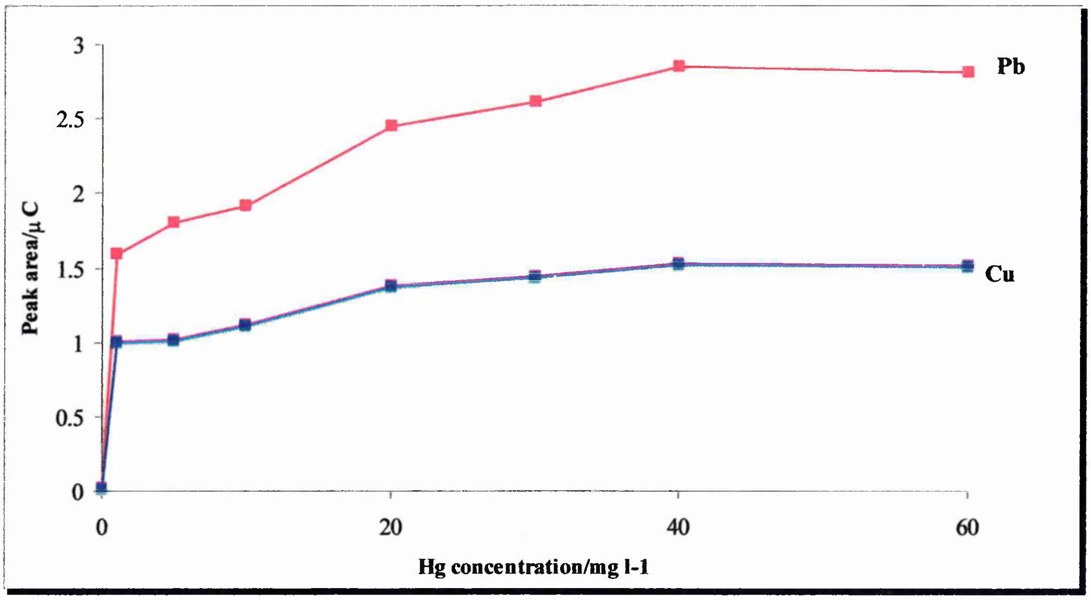


Figure 2.10 Effect of $Hg(NO_3)_2$ concentration on the magnitude of stripping peak currents for 200 µg/l Pb and Cu (n=3), t_{dep} 165 s; E_{dep} -0.8 V; all other experimental conditions are as defined in Figure 2.8.

2.3.4 Comparison of sensor stripping performance

Using the optimised parameters, sensors were evaluated using a 200 µg l^{-1} Pb and Cu solution. Figure 2.11 displays stripping voltammograms for each sensor and Table 2.2 contains the mean I_p and RSD following 10 repeat measurements.

All sensors provided voltammograms that allowed the simultaneous analysis of Pb and Cu, although, the stripping peak for Cu is broader and less well defined. This is presumably due to the low solubility of Cu in Hg and the irreversibility of Cu reduction in a chloride media. Bradshaw and Scollary (1997) reported "the determination of Cu using a MFE is affected by more problems than are encountered for most other metals usually determined by this technique. These problems are a consequence of the proximity of the Cu oxidation peak to that for the oxidation of the mercury in the film electrode, as well as the irreversibility of the Cu^{2+} reduction".

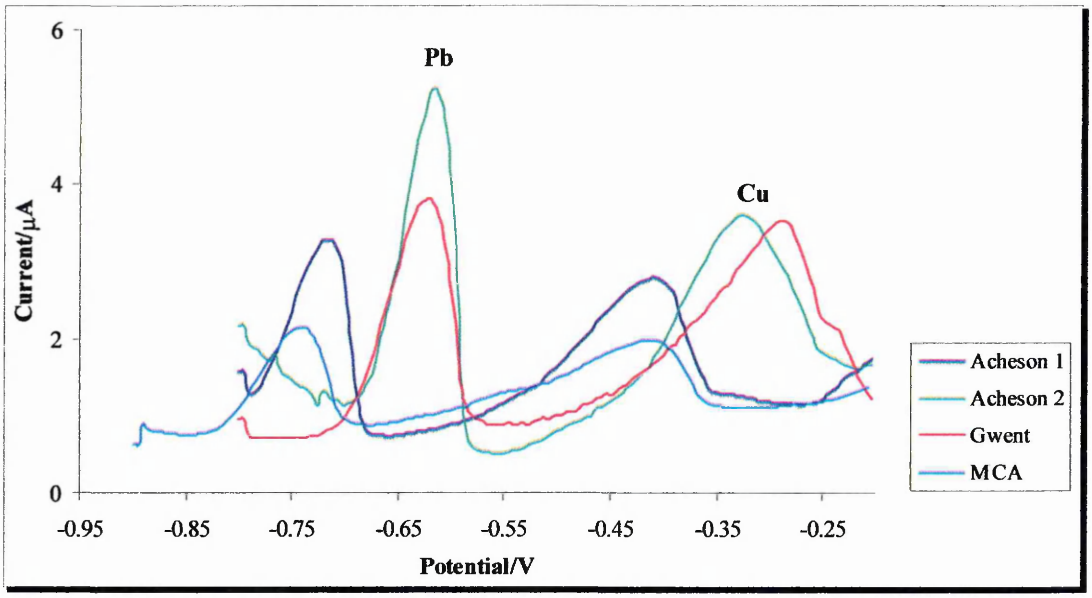


Figure 2.11 Stripping voltammograms of 200 μg l^{-1} Pb and Cu at SPE's fabricated from different carbon inks. In 0.1 mol l^{-1} KCl, 1%v/v HNO_3; 20 mg l^{-1} $Hg(NO_3)_2$; t_{dep} 165 sec; E_{dep} -0.8 V; E_{scan}:-0.8v ⇒ -0.2v @ 20 mV s^{-1}; interval time 0.5 s.

Wang and Luo (1984) also reported chloride ions have a suppressive effect on Cu stripping peaks. However, the chloride ion concentration needs to be kept constant to stabilise the Ag/AgCl reference electrode and be sufficiently high to negate the effects of residual levels of chloride ions in the sample matrix. A chloride concentration of 0.1 mol l^{-1} was used for the simultaneous analysis of Cd, Pb and Cu.

SPE fabricated using Acheson old ink gave the largest analytical response, shown in Table 2.2. This was unexpected due to the data obtained from CV analysis (i.e. smaller active surface area and highest ΔE_p). It is possible that the increase in response was caused by metal contaminants present in the ink due to repeated ink reclamation from the screen and re-usage. The same screen is used to fabricate a number of 'in-house' electrodes, which could lead to ink contamination. The evaporation of the polymeric binder over time, similar to effects of high curing temperatures, could also have an effect.

Table 2.2 Mean peak currents for 200 μg/l Pb and Cu at sensors fabricated from different carbon inks.

	Acheson new		**Acheson old**		**Gwent**		**MCA**	
Analyte	**Pb**	**Cu**	**Pb**	**Cu**	**Pb**	**Cu**	**Pb**	**Cu**
Peak potential – V	-0.68	-0.38	-0.68	-0.36	-0.62	-0.34	-0.74	-0.42
Peak area - μC	2.42	1.38	3.57	2.37	3.39	2.14	1.32	1.02
RSD - % (n=10)	4.9	5.7	3.8	4.5	14.3	15.6	13.8	9.1

Wang *et al.* (1996) reported "high curing temperatures lead to decomposition and evaporation of organic components of the carrier, and hence to an increase in the relative carbon content". These observations have indicated that inks used in the fabrication process should be properly stored and used for a limited time, in order to fabricate reproducible electrodes.

While the CV data demonstrated the SPE fabricated using Gwent ink had the most reversible nature and was less affected by additional redox reactions, ASV analysis resulted in irreproducible responses, with RSD ranging from 1.5% to 21% (n=10). A visual inspection of the Gwent SPE sheets showed the layer of carbon ink formed looked pitted and uneven, with rough edges, compared to the alternative carbon inks. Further reproducibility investigations were carried out, where electrodes were selected from random points across the SPE sheet. However, variability remained unchanged and it was concluded that some property of the carbon ink was causing the poor quality printed surface.

Although the Gwent ink SPEs exhibited a variable response, it achieved the highest peak area for the analytes, with the exception of Acheson old SPEs due to additional metal contaminants, whilst MCA SPEs had the lowest peak area. This would be expected since Gwent SPEs had the highest calculated active surface area; suggesting more support for the Hg film formation and hence more analyte taking part in the analysis, with MCA SPEs having the lowest active surface area.

Figure 2.12 shows background voltammograms exhibited by the sensors. All show rising background currents, with peaks at approximately -0.3 V, especially for

Acheson old and MCA SPEs. As previously discussed, this value is in close proximity to the oxidation potential of mercury and this may be as a result of the Hg film.

Zakharchuk *et al.* (1999) also suggest "insoluble mercury compounds are formed on the electrode surface modified with mercury *in situ* both during anodic oxidations and electrolysis at potential scans from -0.3 to -1.2 V in solutions containing chloride ions. This effect therefore could also be contributing to the observed background current.

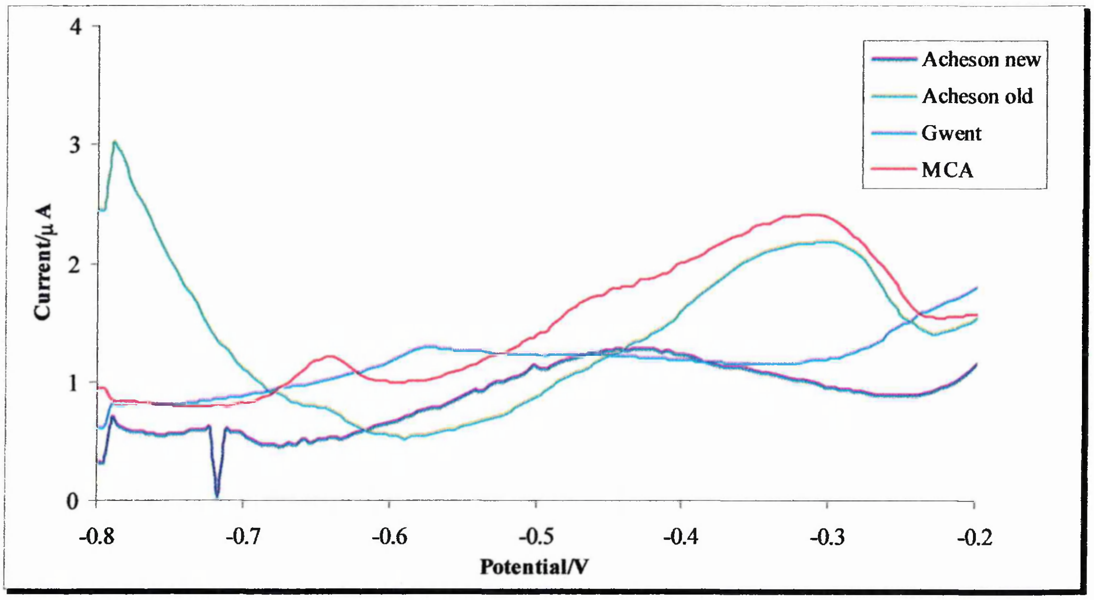


Figure 2.12 Background voltammograms at SPEs fabricated from different carbon inks. All experimental conditions are as defined in Figure 2.11.

As well as the observed differences in reversibility and active surface area, related to differences in carbon and polymeric binder content, exact ink formulations are regarded as proprietary information. This makes the variation in response exhibited by these carbon inks difficult to fully elucidate. Acheson ink is produced using natural graphite and may contain metal contaminants whilst Gwent and MCA inks are produced using synthetic grades of graphite. As such, unknown characteristics including the quality of the carbon particles, uniformity, particle size and porosity, ingredients for the printing and adhesion tasks and the mutual ratio of these components will vary from ink to ink.

Wang *et al.* (1998b) investigated a variety of commercially available carbon inks, including the Gwent ink, for performance. It was found that differences in the ink composition and in the printing and curing conditions have a profound effect upon the analytical performance of the resulting carbon sensors.

Since ink selection was based on an acceptable compromise between peak area (sensitivity), background characteristics, reproducibility, and resolution of stripping peaks, SPEs fabricated using Acheson new ink was selected as the most appropriate ink for this application. Well-defined, reproducible stripping currents for Pb and Cu, and lower background currents were exhibited using this SPE. As a result, all subsequent analyses were performed using SPEs fabricated using Acheson Electrodag 423 SS carbon ink.

2.3.5 Detection of Cd and Zn

To broaden the applicability of the sensor, investigations were conducted to include Cd and Zn in the assay. As suggested in Section 2.1, the ability to perform multi-analyte determinations was an important criterion in sensor development. The more analytes that can be measured in a single analysis process, without the need for additional reagents or adjustments to the assay, the more attractive the sensor becomes, in terms of cost, analysis time and end-user perception.

To produce an acceptable stripping peak for Cd, the optimal E_{dep} was investigated using a reagent blank; which includes HNO_3, $Hg(NO_3)_2$ and KCl; and a 200 $\mu g\ l^{-1}$ Cd solution. All other experimental conditions were kept the same as for Pb and Cu. The E_{dep} was varied from -1 V to -1.3 V, as illustrated in Figure 2.13.

The E_{dep} has to be sufficiently negative for the reduction of Cd; however, this should be kept to a minimal to reduce increased background noise due to the evolution of hydrogen at these more negative potentials. A well-defined stripping peak for Cd was achieved using a E_{dep} of -1.1 V.

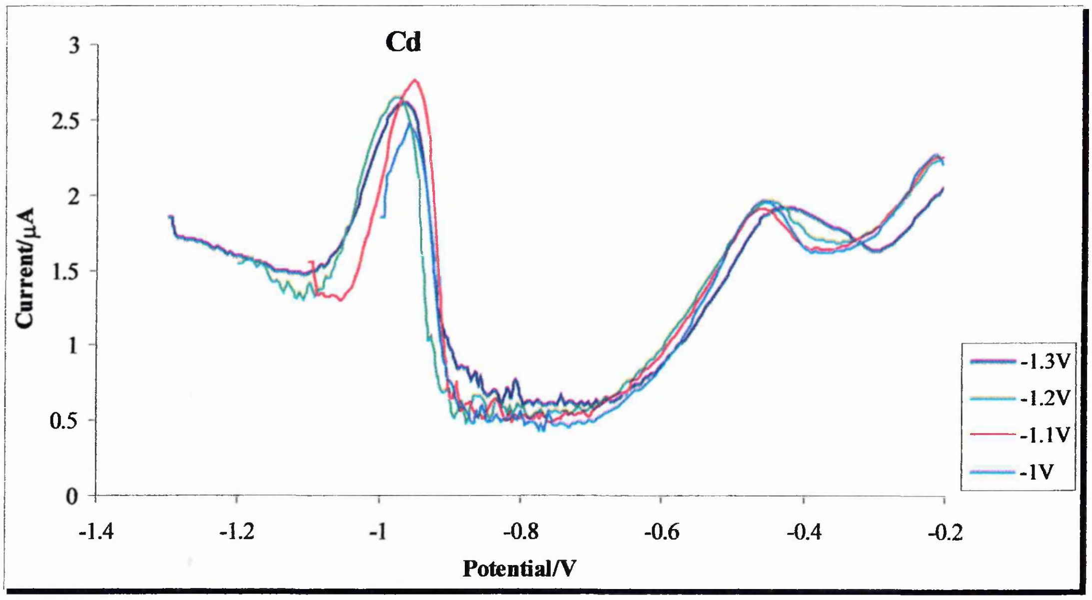


Figure 2.13: Effect of E_{dep} on 200 µg l^{-1} Cd, in 0.1 mol l^{-1} KCl; 20 mg l^{-1} $Hg(NO_3)_2$; 1% v/v HNO_3; t_{dep} 165s; end potential -0.2 V; E_{scan}:-1.1v ⇒ -0.2 V @ 20 mV s^{-1}.

A mixed solution containing 200 µg l^{-1} Cd Pb and Cu was analysed to evaluate the effect of this E_{dep} on the response for Pb and Cu. Figure 2.14 shows well resolved stripping peaks were obtained for the analytes. Although a slight decrease in the stripping response for Pb and Cu was observed using a more negative potential, adequate discrimination between the background current and analyte peak current was obtained.

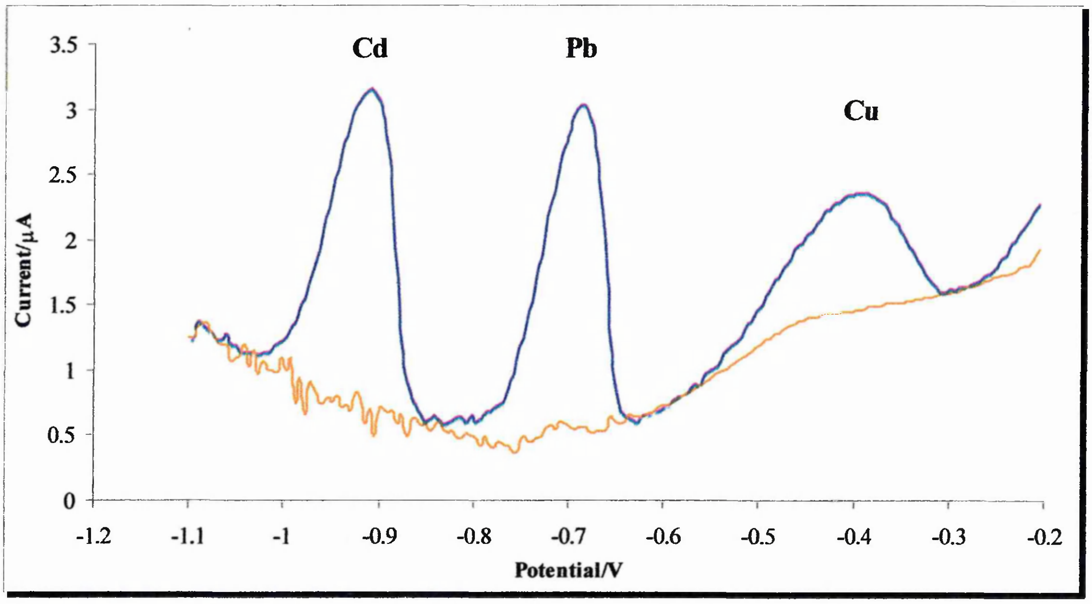


Figure 2.14: Stripping voltammogram of 200 μg l^{-1} Cd Pb Cu, and a blank solution using a E_{dep} of −1.1V. All other experimental conditions are as defined in Figure 2.13.

Since Zn oxidises at negative potentials (Figure 2.15), the measurement of Zn was next evaluated by adjusting the pH to increase the potential window of the sensor. The potential range in which the sensor can operate is limited by hydrogen evolution at sufficiently negative values. The potential window shifts towards less negative potentials and narrows progressively upon decreasing the pH of the solution (Van Venrooij *et al.*, 1996). The oxidation (stripping) of the Hg film limits the potential window at positive values.

The E_{dep} was taken to −1.5 V and the pH varied between 1 and 4. Figure 2.15 shows the effect of pH on a solution containing 200 μg l^{-1} of Zn, Cd, Pb and Cu.

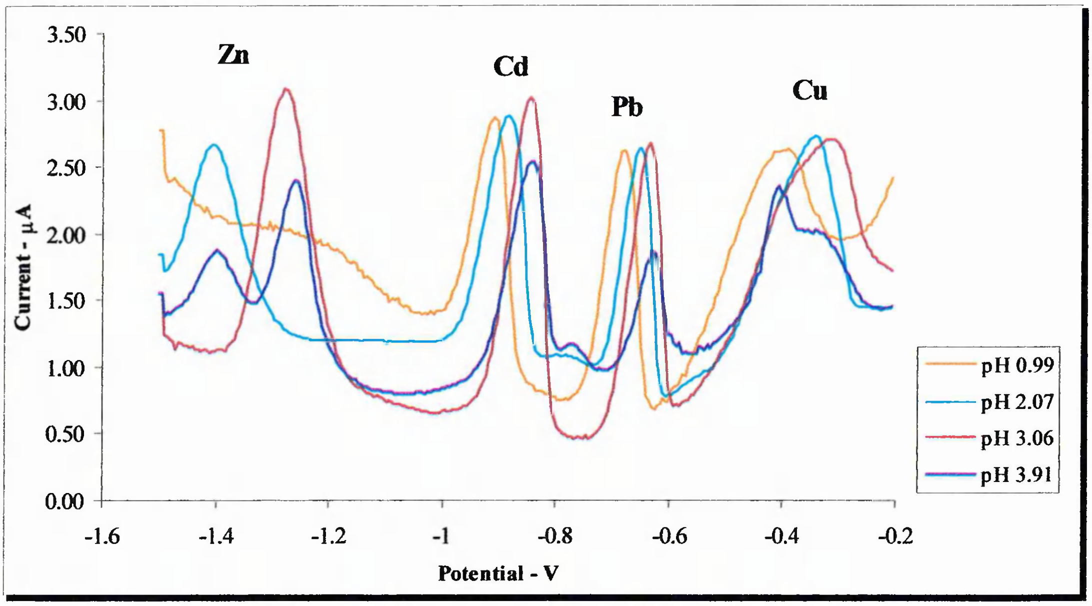


Figure 2.15: Stripping voltammogram of 200 µg l^{-1} Zn Cd Pb Cu using a E_{dep} of –1.5 V. All other experimental conditions are as defined in Figure 2.13.

At low pH, no stripping response for Zn was obtainable due to interference from H_3O^+ reduction. By changing the pH the hydrogen evolution wall is shifted to more negative potentials and a stripping response for Zn was obtained at an optimal pH value of 3. Stripping responses for the other analytes were not compromised at pH values of up to 3. However operating at low pH is otherwise advantageous for a number of reasons. Firstly, working at low pH increases the extractability of most metals from the soil matrix and further pH adjustments would complicate *in situ* protocols, that must, by definition, be simple. Secondly, Cu analysis can be complicated due to its tendency to bind strongly to many organic and inorganic ligands and the occurrence of the Cu stripping peak at a potential where many organic species are adsorbed onto the mercury electrode (Bradshaw and Scollary, 1997). Low pH values may be used to prevent complexation with organic compounds. Finally, problems associated with the formation of intermetallic compounds also require consideration.

Intermetallic compounds may form between metals deposited into the mercury electrode, or between the electrode material and a deposited metal. When an intermetallic compound is formed, the stripping peaks for the constituent metals may be severely depressed or shifted (Wang, 1985).

Numerous intermetallic compounds have been reported, although much focus has been placed on the Cu-Zn compound because of the presence of these metals in a diversity of analytical samples. Under certain conditions, the simultaneous deposition of Zn and Cu results in the formation of CuZn, $CuZn_2$ and $CuZn_3$ (Wang, 1985). These compounds are stripped near the potential for Cu, resulting in an enhanced copper peak and a diminished Zn peak. The magnitude of the Zn peak is determined by the ratio of the Cu and Zn concentrations deposited in the MFE, and will decrease as the Cu concentration in solution increases.

Svancara *et al.* (1994) investigated the use of a MFE for the determination of Zn, Cu, and Pb in drinking water samples. It was reported "Zn forms a number of intermetallic compounds such as Cu-Zn, and these compounds are responsible for a diminishing of the Zn peak, which may even disappear. This response was dependent on the mutual concentration ratio between Cu and Zn and on the type of electrode; however the Cu metal ions do not interfere on the response of Zn if they are present in concentrations lower than Zn^{2+}". It was also reported "with MFEs the smaller the volume of mercury deposited onto the electrode support the more pronounced is the effect of the intermetallic compound".

Desmond *et al.* (1996) reported "intermetallic compound formation, inherent with MFEs was found to be a real problem for the determination of Zn and Cu in one analysis. A complex formed between Zn and Cu and the oxidation of this complex occurs at the same potential as that of copper, resulting in a decrease in the Zn peak".

Various approaches have been suggested for minimising or eliminating errors caused by intermetallic compound formation. The addition of a 'third element' which preferentially binds the copper, for example Gallium, results in a copper-gallium intermetallic instead of a copper-zinc intermetallic, thus the zinc concentration can be directly determined (Oi-Wah Lau and Oi-Ming Cheng, 1998). However, this would add to the complexity of the analysis, which is obviously undesirable for *in situ* analysis.

Desmond *et al.* (1996) report on a multiple scan method to counteract this problem. This involves two voltammetric sweeps, with the initial scan having a preconcentration potential more positive than that of zinc, allowing the copper concentration to be accurately determined. A subsequent sweep is then carried out in which all the metals are accumulated and the increase in the copper peak height is used to calculate the correct zinc concentration.

Due to these considerations, together with the added procedure of pH adjustment, a second analysis would have to be performed to obtain quantitative data for Zn. As the principal aim was to provide a rapid, simple and cost-effective sensor device, using simple stripping protocols, further studies focused on development of the assay for the simultaneous analysis Cd, Pb and Cu at pH 1.5.

2.3.6 Calibration curves for Cd Pb Cu.

The role of an analyte screening sensor is to provide an expeditious response. As discussed in Section 1.6.5, this often entails compromises in assay performance. However, Williams and D'Silva (1994) suggested 'it is not always necessary to have an accurate calibration graph as a single-point determination can be used as a threshold level to trigger an alarm. For instance, an instrument calibrated at 50 $\mu g\ l^{-1}$, the EC limit for lead in drinking water, could be used to alert the analyst when the value is exceeded'. However, to evaluate the constraints of the electrochemical sensor, calibration curves were constructed using mixed standards of Cd, Pb and Cu in the range of 0-200 $\mu g\ l^{-1}$. Precision was evaluated by repetitive measurements (n=6) at each concentration. Figure 2.16 shows well-defined and resolved stripping peaks for each analyte. The peak potentials generally shifted to more positive potentials with increasing concentration.

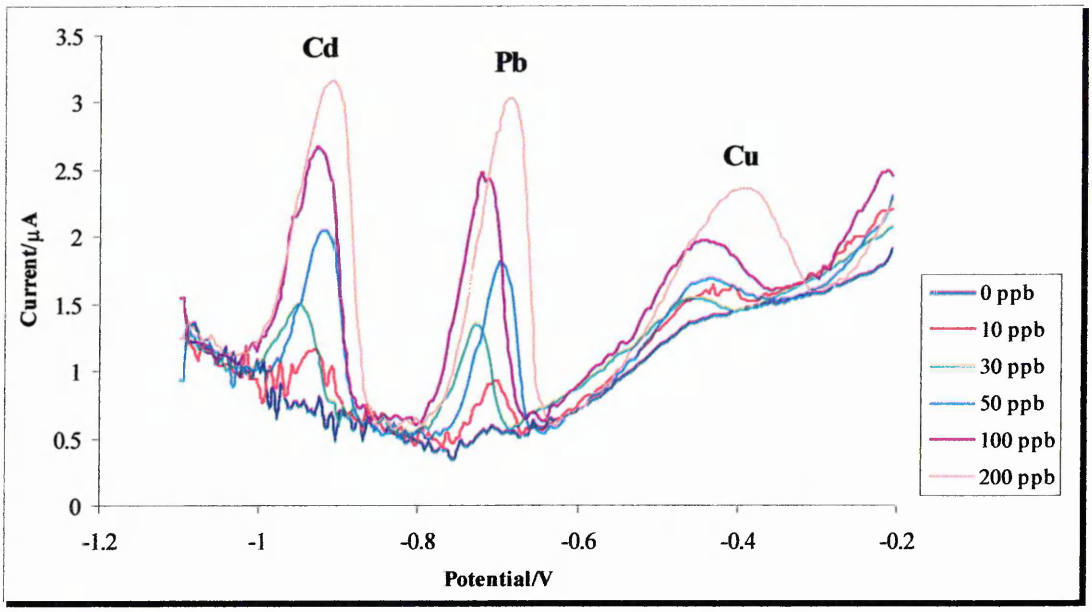


Figure 2.16 Stripping voltammograms for increasing concentrations of Cd, Pb, Cu in 0.1 mol l^{-1} KCl; 1%v/v HNO_3; 20 mg l^{-1} $Hg(NO_3)_2$; t_{dep} 165 sec; E_{dep} -1.1 V; E_{scan}:-1.1v ⇒ -0.2v @ 20 mV s^{-1}; interval time 0.5 s.

Calibration plots of the analyte stripping responses versus its concentration in the range of 0-200 µg l^{-1} exhibit a polynomial relationship, yielding r^2 values of 0.9964 for Cd, 0.9987 for Pb and 0.9970 for Cu (Figure 2.17). Relative standard deviations (RSD) values of less than 6% for Pb and Cd and less than 8% for Cu were obtained (n=6) at each concentration increment. The limits of detection, calculated according to the IUPAC convention ($S/N = 3$) of the blank measurement, were 9.1 µg l^{-1} for Cd, 1.4 µg l^{-1} for Pb and 32.6 µg l^{-1} for Cu.

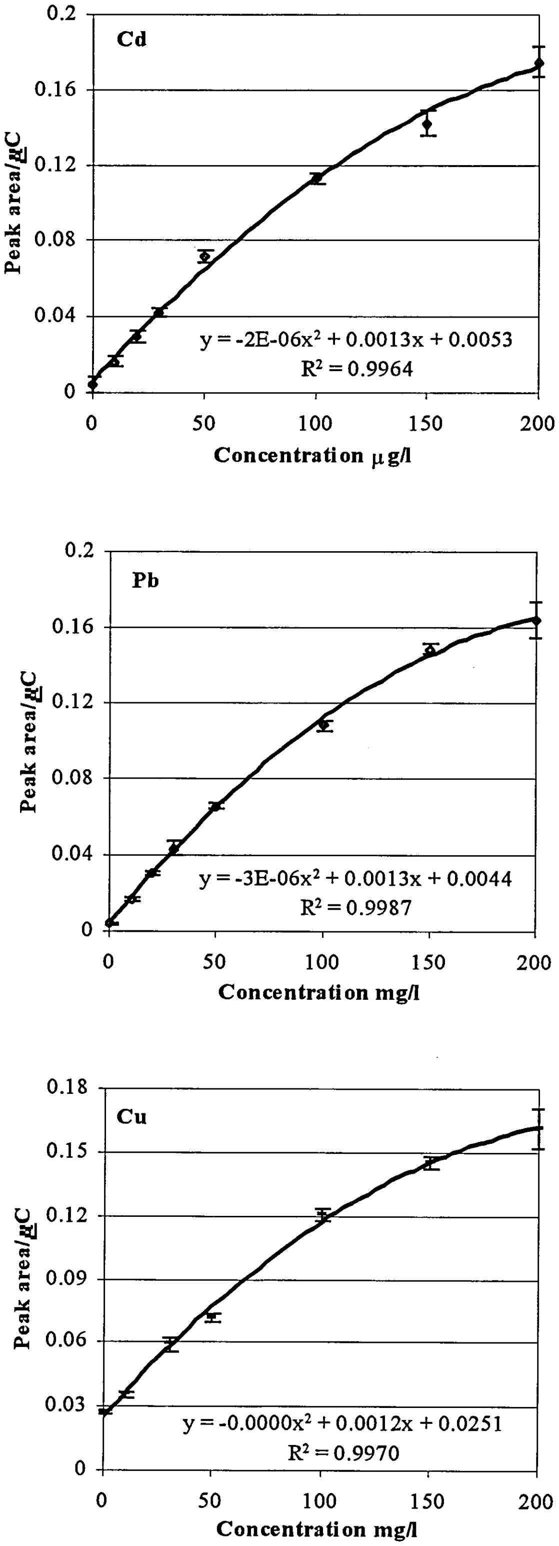


Figure 2.17 Calibration for Cd, Pb, Cu in the range of 0-200 µg l^{-1} using mixed standards in 0.1 mol l^{-1} KCl; 1% v/v (HNO_3); 20 mg l^{-1} $Hg(NO_3)_2$; t_{dep}:165 s; E_{dep}:-1.1 V; E_{scan}:-1.1V ⇒ -0.2 V @ 10 mV s^{-1}; interval time 0.5s.

2.4 CONCLUSION

The purpose of an *in situ* analyte screening sensor device is to provide end-users with rapid measurements on the chemical composition of the selected samples. Rapid screening offers immediate decision-making potential usually not possible with centralised laboratory analysis, whilst reducing the cost/amount of samples ultimately being sent to a central laboratory for a final detailed analysis.

Consequently a sensor device capable of providing such measurements was developed for the simultaneous detection of Cd, Pb, and Cu. Suitable SPEs were fabricated using an automated DEK 248 screen-printing machine. Characterisation of a selection of commercially available carbon inks was performed and the most appropriate selected. A rapid and simple DPASV procedure was developed for the simultaneous analysis Cd, Pb and Cu. Zn may also be included in the assay using a simple pH adjustment procedure. The following conclusions may be drawn from this work:

- Screen-printing technology provided a reproducible method for the fabrication of low-cost disposable electrodes. The use of disposal electrodes negates problems associated with electrode fouling and cleaning. The electrode design enabled easy and uniform depositioning of the sample droplet onto the 3-electrode working area, as well as simple connection to the electrochemical analyser. The use of SPEs has provided an alternative to conventional 'bulky' electrochemical cells for on-site analysis.

- The carbon ink used in the fabrication of the SPEs strongly affects the stripping performance of the fully optimised sensor, including sensitivity, reproducibility and background noise. Acheson Electrodag 423 SS was selected as the most appropriate carbon ink for the development of the electrochemical sensor, based on these criteria.

- Generally, screen printed carbon working electrodes show quasi-reversible behaviour, with the active carbon area being around 20% of the geometric area.

- The use of SPE and DPASV, together with simple experimental protocols, has provided an *in situ* electrochemical screening sensor capable of simultaneous detection of Cd, Pb and Cu, obtaining detection limits of 9.1 μg l^{-1}, 1.4 μg l^{-1} and 32 μg l^{-1} respectively, with a RSD of less than 8%.

CHAPTER 3

IN SITU SOIL SAMPLE PREPARATION AND ANALYSIS

3.1 INTRODUCTION

Since ASV analysis is a solution based technique, dissolution of solid samples, prior to analysis is necessary. This is often a vital step, which may significantly affect the outcome of the assay. Several laboratory based sample dissolution techniques are available, however as discussed in Section 1.5 wet digestion techniques have seen particular widespread use in soil pollutant studies.

Traditionally, total sample digestion procedures involved using hydrofluoric acid (HF) in combination with other acids, including nitric (HNO_3), sulphuric (H_2SO_4), perchloric ($HClO_4$) and hydrochloric (HCl) acid. Since most pollutant inputs are not silicate-bound, total analysis involving the use of HF generally has little significance in pollution studies. Elements contained in these minerals are unlikely to have any effect on sediment/water interactions or be biologically available. In addition, the use of HF leads to long, dangerous and cumbersome schemes and its use is not recommended for routine analyses (Sastre *et al.*, 2002).

Thus, in soil pollutant studies, analyte extraction procedures; also referred to as leaching, has become a common alternative to total digestion. This enables specific metal fractions or phases i.e. biovailable fraction, to be measured and which are, in most cases, sufficient for the purpose of environmental policy (Rauret *et al.*, 1999).

These extraction schemes are based on the assumption that ions associated with soil components will be displaced from adsorption sites by the presence of an excess of competing ions in the extract solution. For example, the exchangeable phase is assumed to be easily mobilised by soil-soil solution ion exchange reactions and are therefore available for root uptake (bioavailable). Oxidisible phases, as measured by chemically more aggressive extractants, are assumed to remain in the soil for longer periods but may be mobilised by decomposition processes (e.g. weathering and microbial activity) (Kennedy *et al.*, 1997).

An extensive inventory of extraction schemes have been published, though two main groups have emerged; the single reagent extraction procedures, where one extractant is used on one soil sample; and the sequential extraction procedures, where several extractants are used sequentially on the same sample (Lopez-Sanchez *et al.*, 2002).

3.1.1 Single reagent extraction schemes

Single extraction schemes provide a relative empirical method for evaluating the potential availability of pollutants for plant uptake and mobility in the soil profile. The data is used in the assessment or prediction of the environmental effects of the pollutant concentration in the environment, and the promulgation of guidelines or objectives for soil quality (Lopez-Sanchez *et al.*, 2002).

Various reagents are utilised in single extraction schemes, ranging from very strong acids, such as aqua regia (3:1 HCl/HNO_3), to neutral unbuffered salt solutions, mainly calcium chloride ($CaCl_2$) or sodium nitrate ($NaNO_3$). Buffered salt solutions or chelating agents ethylenediaminetetraacetic (EDTA) are also used as they form very stable water-soluble complexes with a wide range of cations (Lopez-Sanchez *et al.*, 2002). Some common extractants and the metal fractions associated with each are listed in Table 3.1.

The choice of reagent will strongly depend on the data requirements of the end-user. 'Pseudo' total analysis results in the dissolution of all except the most resistant silicate-bound fractions (dissolved using HF) and would be used to assess the extent and long-term potential toxicity effects of pollution. In most countries, the quality standards are based on the pseudo total fraction as it provides an estimate of the extent of metal build up in soils (Gupta *et al.*, 1996). Moderate extractants (EDTA) are useful for studies of trace metal mobility, and weak extractants ($CaCl_2$) for plant uptake studies.

Table 3.1 commonly used reagents in single extraction schemes.

Type	Extractant	Metal Fraction	Reference
'Pseudo' total	HNO_3/HCL, HNO_3/H_2SO_4, $HClO_4$/ HNO_3, H_2SO_4/H_2O_2	Residual	Wray (1998) Bettinelli *et al.* (2000) Marin *et al.* (2001) Devkota (2001)
Moderate	EDTA, DTPA, CH_3COOH	Exchangeable carbonate/organically bound	McGrath (1996) Kim *et al.* (2003) Fangueiro *et al.* (2002) Sun *et al.* (2001)
Weak	H_2O, $CaCl_2$, $NaNO_3$	Easily exchangeable/ water soluble	Dold (2003) Maiz *et al.* (2000)

(DTPA); diethylenetriaminepentaacetic acid, (CH_3COOH) acetic acid; hydrogen peroxide H_2O_2.

Many single extraction schemes have been published which vary in experimental protocols including heating and/or shaking the sample; length of extraction, extractant types; and extractant volume:sample ratios. However, standard accredited and validated schemes do exist including; The International Organisation for Standardization (ISO 11466:1995) (corresponding to BS 7755-3.9:1995); USEPA SW-846 Series; Official & standardized methods of analysis 3rd edition (Royal Society of Chemistry); and Soil Science Society of America (SSSA) methods of soil analysis book series.

3.1.2 Sequential extraction schemes

Metal fractions can be isolated more specifically using a sequential extraction scheme carried out in a prescribed order. This allows the assessment of metal fractions between various phases in one soil or sediment sample.

Numerous sequential extraction schemes have been developed, many of which are variants on that of Tessier *et al.* (1979). In this scheme the sediment/soil total metal content was divided into five fractions: exchangeable, carbonate bound, iron oxide or manganese oxide bound, organic bound, and residual fraction. Other schemes mainly differ in the extraction conditions used. For example, the method of Salomons and Forstner (1980) is based on conditions that allow the iron/manganese oxide fraction to be subdivided into three fractions; easily reducible; moderately reducible; and non-silicate iron (Abollino *et al.*, 2002). The metal fractions are termed according to the geochemical phase attacked by a particular extractant (e.g. CH_3COOH for the carbonate bound fraction).

However, it is now generally accepted that extractants (used in both single and sequential schemes) are not selective and that minor variations in extractants and experimental protocols have significant effects on the measured concentrations (Lopez-Sanchez *et al.*, 2002). As such, metal fractions should be related to the extractant used. For example, metals extracted by EDTA should be referred to as the extractable element and not 'bio-available' or mobile forms, which are interpretations of data rather than results of actual measurements (Quevauviller, 1998).

Quevauviller (1998) termed extraction schemes as 'operationally defined', meaning the 'forms' of metals are defined by the determination of extractable elements, using a given procedure, and therefore the significance of the analytical results is highly dependant on the extraction procedures used.

This situation has resulted in a lack of uniformity in environmental studies, preventing comparison of data and validation of methods used. In an effort to harmonise measurements for extractable metal contents in soil, the European Commission; through the BCR Programme (Community Bureau of Reference) and its successor (Standards, Measurements and Testing Programme), launched a collaborative project (BCR 4, 1987) which aimed at; (1) design a three-step sequential extraction scheme; (2) test the selected scheme in interlaboratory studies involving expert European laboratories; and (3) certify the extractable trace element contents of a sediment reference material (Rauret *et al.*, 1999).

Table 3.2 contains an outline of the BCR sequential extraction scheme. Full accounts of BCR methodologies, and related aspects including the origin and history of activities of the BCR programme, are detailed in; Quevauviller (1996;1998;1999), Rauet *et al.* (1999); Quevauviller *et al.* (1997); Whalley and Grant (1994); Sahuquillo *et al.* (2003) and Lopez-Sanchez *et al.*, (2002).

Table 3.2 Outline of BCR sequential extraction scheme.

Step	Extractant	Metal fraction	Interpretation
1	CH_3COOH 0.11 mol l^{-1} pH 5.0, 16 h shake	Exchangeable fraction + carbonates	Weakly absorbed metals retained on sediment surface, metals released by ion-exchange processes, metals that can be co-precipitated with carbonates
2	$NH_2OH.HCl$ 0.5 mol l^{-1} pH 1.5,16 h shake	Easily Reducible fraction	Metals bound to iron/manganese oxides
3	CH_3COONH_4 1 mol l^{-1} H_2O_2 30%, pH 2	Oxidising fraction	Oxidisable fractions bound to organic matter/sulphides
Residual	Aqua regia	All except alumosilicate fraction	All but tightly silicate bound fractions

($NH_2OH.HCl$) hydroxylammonium chloride; (CH_3COONH_4) ammonium acetate

When implementing single or sequential extraction schemes, long mechanical shaking or heating times are necessary; the BCR scheme requires a 16 h end-to-end shake at each step; the ISO 11466:1995 method requires slow oxidation over a period of 16 h, followed by heating for 2 h. Heating systems generally used include hotplates, sand-baths and aluminium blocks.

To improve these time-consuming conventional techniques, closed vessel microwave heating has seen widespread use in wet digestion procedures. EPA SW-846 #3051 is an alternative microwave assisted method to the open-vessel #3050B method. 3051A also includes an option of adding HCl to the analysis, to improve the efficiency of the microwave method, making it comparable to #3050B (Link *et al.,* 1998).

The application of closed pressurised vessels in combination with microwave heating allows high temperatures to be attained at relatively low pressures, reducing digestion times from several hours to minutes (Link *et al.,* 1998). Other benefits include; an improved recovery of volatile elements and compounds; reduced interferent levels; minimal volumes of reagents; more reproducible procedures allowing better transfer of standard methods between laboratories; and precise temperature control (Agazzi and Pirola, 2000).

Detailed accounts on the fundamentals, validation and applications of microwave assisted digestion procedures may be found in Link *et al.* (1998;1999); Florian *et al.* (1998); Bettinelli *et al.* (2000); and Sastre *et al.* (2002).

Ultrasonic extraction is also merging as an alternative to conventional hotplate techniques. The ultrasonic technique applies intense, high-frequency sounds/energy to liquids, producing intimate mixing and powerful chemical and physical reactions. The process of 'cavitation' is in effect cold boiling and results from the creation and collapse of countless micro-bubbles in the liquid, producing shock waves. Cavitation accelerates both chemical and physical reactions (Marin *et al.,* 2001).

Perez-Cid *et al.* (1998) investigated speeding up the BCR sequential extraction method using ultrasound. The complete extraction scheme was reduced from 51 h to 22 min, with no statistically significant differences in the recoveries of Cu, Ni, Pb and Zn. Studies have also been carried out by Al-Merey *et al.* (2002) for Cu, Pb and Zn in soil samples; and Ashley *et al.* (2001) for Pb in dust wipe samples.

Although an extensive catalogue of laboratory based extraction schemes are available, there appears to be a gap in current technology concerning simple, inexpensive and rapid *in situ* extraction schemes, which can be easily modified according to the requirements of the end-user, and are suitable to use with this type of sensor device. Brainina *et al.* (2001) reported 'UV radiation, ozonation, γ-irradiation, chemical digestion, and electrochemical oxidation are utilised as laboratory based sample preparation techniques, unfortunately none have been realised as a portable version'.

Consequently, a simple, rapid sample preparation method, which can operate in conjunction with the *in situ* sensor device, was investigated. The purpose of the method was to 'screen' samples; to provide a semi-quantitative determination, identifying samples containing analyte levels above or below legal threshold values and evaluation of the potential likelihood of analyte release. As such, the proposed method will be a rapid single reagent extraction procedure, with short extraction times. Such a method would also provide a basis for further studies in this area.

Following exploratory research, a soil certified reference material (CRM) was used to evaluate the precision and accuracy of the proposed *in situ* extraction method. 82 soil samples supplied by partners within the European Union PURE programme were analysed and data compared against known analyte concentrations previously obtained using ICP-MS. This enabled further evaluation of the usefulness of the *in situ* method using a larger data set.

3.2 RESEARCH METHODOLOGY

All general reagents and procedures detailed in Section 2.2 were adopted for the sample analysis studies. An adjustment in the preparation of the calibration standards; from a 0.1 mol l^{-1} KCl electrolyte to 1 mol l^{-1} aqua regia electrolyte; was performed in order to matrix match the standards and samples. Unless otherwise stated, all other assay parameters including the *in situ* MFE formation and DPASV parameters were performed as detailed in Section 2.2.

3.2.1 PURE Soil sample standard preparation and analysis

82 soil samples were supplied in 2 sets (set 1 = 25 samples; set 2 = 57 samples) by partners within the PURE programme for analysis of Cd Pb and Cu. The samples were alluvial sediments, varying in colour (from light tan to dark brown). The samples were supplied air dried and sieved to obtain the <2mm fraction for analysis. All samples were stored in airtight containers at 4°C. Known analyte values were obtained by an independent laboratory using EPA method #3051A (microwave

extraction for 'pseudo' total using concentrated aqua regia and H_2O_2) and EPA #6020 A/98 (ICP-MS) (Appendix A2).

3.2.2 Proposed *in situ* analyte extraction method

The *in situ* analyte extraction method was developed according to the requirements discussed above. The use of aqua regia was selected as the reagent of choice for the following reasons:

a) EDTA was considered as Pb and Cu have high complexation constants with EDTA and being highly associated with Fe oxides and hydroxides, can be remobilised because of the complexation of Fe with EDTA (Sahuquillo *et al.*, 2003). However, an increase in acidity causes a considerable increase in extractability for those elements which are more fixed or of intermediate mobility. Therefore, the effect caused by a progressive acidification of the soil, or a rapid change, for instance, due to an 'acid rain' episode, would be significantly better predicted using a more aggressive extractant.

b) By operating at low pH (ASV is compatible with strongly acidic solutions), individual sample pH changes are minimized. In addition, pH adjustments can complicate experimental protocol and lead to irreproducible results. The BCR original protocol was modified due to the strong influence of sample pH in step two of the extraction. This lead to insufficiently reproducible conditions when the procedure was validated through inter-comparison exercises (Sahuquillo *et al.*, 2003). The increase in the acidic concentration with pH values down from 2 to 1.5, along with an increase of the reagent concentration resulted in an increase in the amounts of extracted metal and hence allowing more reproducible and comparable results to be obtained.

c) A 1:3 HNO_3:HCl mixture results in higher recoveries than when using just HNO_3. HCl may be used to dissolve carbonates and some phosphates and sulphates, with the notable exception of pyrite. Nitric acid is a strong oxidising agent and is therefore preferred where sulphides or significant amounts of organic matter are present. The resultant chlorides and nitrates are typically water soluble. The oxidation of most metals in nitrates and chlorides is as follows:

$$M^0 + HNO_{3(aq)} \rightarrow M(NO_3)_{x\,(aq)} + NO_{(g)} + H_2O$$
$$M^0 + 2HCl_{(aq)} \rightarrow M(Cl)_{2(aq)} + H_{2(g)}$$

Where M^0 refers to the elemental metal. Link *et al.* (1999) suggested 'by restricting to just the use of HNO_3 limits are placed on the chemistry of the method. It has been shown that some analytes are unstable either in the oxidising environment of HNO_3 only leach, or in environments containing only trace amounts of chlorides. Solution stabilities and recoveries of these analytes can be increased by raising the concentration of chlorides in the leaching solution through Cl^- ion complexation'.

The proposed *in situ* analyte extraction was performed as follows:

1g of soil was weighed into a small acid resistant vial and 5ml of aqua regia (1mol l^{-1}) added (HCl 32% v/v, HNO_3 65% v/v) supplied by Fisher Scientific, Loughborough, England. The amount of sample to acid ratio was taken as recommended by the Official and Standardised Methods of Analysis (ed. Watson, 1994). The sample was manually shaken for 3 min (replaced by sonication in Section 3.3.1). The resulting extracts were filtered using Whatman cellulose nitrate membranes (0.2μm) and diluted to 50 mL using high purity grade water. A Millipore Sterifil Aseptic filtering system (Millipore, Watford, England), which is supplied with a syringe pump facilitating field use, was used for all sample filtering. Samples were diluted as necessary and DPASV performed.

3.2.3 Certified reference material.

A natural matrix soil CRM (Resource Technology Corporation (RTC), Laramie, WY USA) was used for quality control purposes and to evaluate precision and accuracy of the proposed *in situ* analyte extraction procedure. The certified values were obtained using USEPA SW846 Methods #3050 and #6010.

3.2.4 Data analysis

All experimental/exploratory stripping analysis was repeated three to five times using individual SPEs. All data reported represent the mean of three replicate measurements, unless otherwise stated.

Final soil analyte concentrations (mg kg^{-1}) were calculated using the method of direct calibration. Calibration was carried out at the beginning of the analysis. Standards, CRMs and blanks were inter-dispersed with the samples and run throughout the analysis for validation purposes. Regression and correlation analysis was performed using Microsoft Excel.

The relative standard deviation (RSD), expressed as a percentage, was used to define assay precision. CRM certified values and PURE soil sample ICP-MS values were used as a reference point for *in situ* ASV accuracy appraisal, utilising regression analysis, the *two-sided paired t*-test and recovery values for data interpretation. Recovery values; defined as "the proportion of the amount of analyte, present in or added to the analytical portion of the test material, which is extracted and presented for measurement" (Cuadros-Rodriguez *et al.*, 2001); were calculated as:

Recovery = (*in situ* ASV/ICP-MS (certified value)) x 100%

3.3 DATA COLLECTION AND ANALYSIS

3.3.1 *In situ* soil extraction method

The *in situ* extraction method was performed using a range of aqua regia concentrations (Figure 3.1). Extracting into 1 mol l^{-1} aqua regia resulted in well-defined stripping responses. Higher aqua regia concentrations led to depressed and distorted stripping responses for all analytes. Since no significant changes in pH (1.5) were observed, it was assumed saturation by chloride ions was causing this effect.

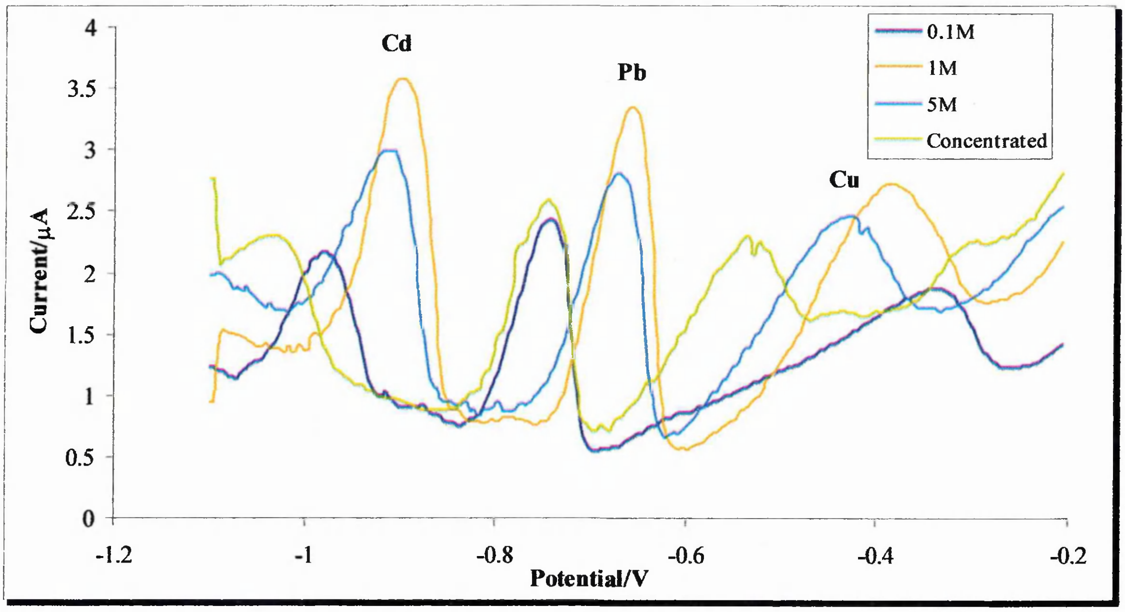
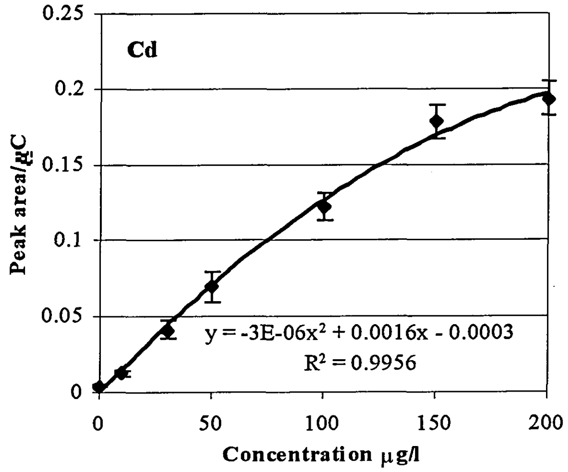


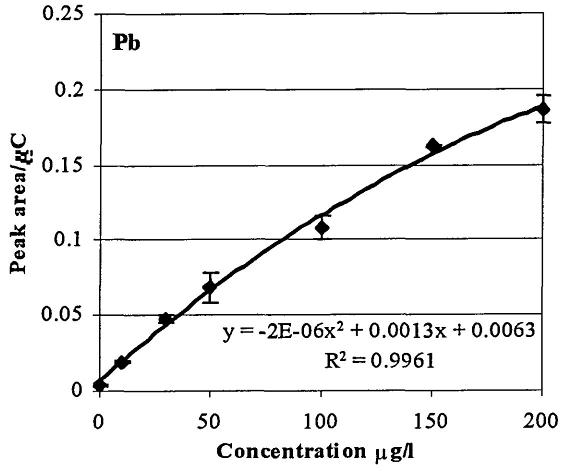
Figure 3.1 Soil CRM extracted into various concentrations of aqua regia. t_{dep} 165 s; E_{scan}:-1.1v ⇒ -0.2 V @ 10 mV s^{-1}; interval time 0.5 s.

Using 1 mol l^{-1} aqua regia, the soil CRM was extracted in 10 replicates and DPASV analysis performed in triplicate on each resulting soil extract. Final analyte concentrations and recovery values were calculated as detailed in Section 3.2.4. Figure 3.2 shows calibration curves for Cd, Pb and Cu using 1 mol l^{-1} aqua regia as the supporting electrolyte.

The extraction resulted in approximately 50% recovery of the analytes (Table 3.3). Variation in extraction efficiency between replicate samples was reflected by the fairly high RSD values of 9-14%. This was thought to be a consequence of the very short extraction time (3 min) and irreproducibility of the sample agitation (i.e. manual shake, but is necessitated by the desire for a field-based assay).

In an attempt to improve the extraction; while maintaining simplicity and speed, the use of an ultrasonic bath, as an alternative to manual shaking was investigated. As discussed in Section 3.1 ultrasonic extraction has been employed to speed up laboratory based extraction methods and is easily adopted for on-site analysis by utilising commercially available portable battery-operated ultrasonic probes.





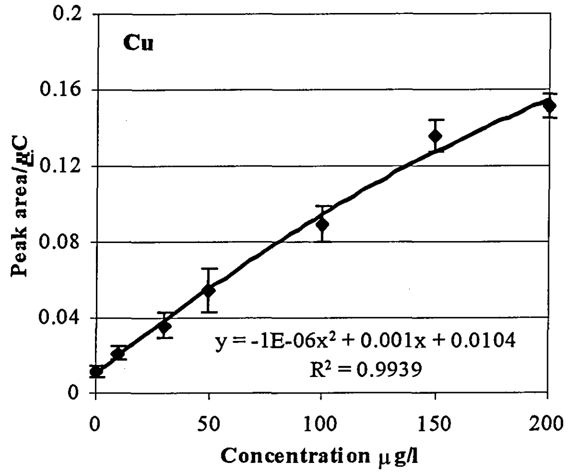


Figure 3.2 Calibration for Cd, Pb, Cu 0-200 µg l^{-1} mixed standards in 1 mol l^{-1} aqua regia, 20 mg l^{-1} $Hg(NO_3)_2$: t_{dep}:165 s; E_{dep}:-1.1 V; E_{scan}:-1.1V ⇒ -0.2 V @ 10 mV s^{-1}; interval time 0.5s.

Table 3.3 Recoveries and RSD of *in situ* extraction and analysis using soil CRM following 3 min manual shake.

Analyte	Mean (mg kg^{-1})	RSD (%)	Certified (mg kg^{-1})	Recovery (%)
Cd	6.05 ± 0.98	11.69	11.7	51.7
Pb	12.89 ± 0.92	9.82	25.6	50.4
Cu	9.65 ± 1.2	13.95	18.8	51.3

Recovery = 100%(certified value/mean value)

Table 3.4 shows data obtained for the soil CRM after 3 min sonication, all other conditions were kept constant. Recovery values were slightly improved with increases of 12.4%, 1.3% and 5% for Cd, Pb and Cu respectively. A decrease in the RSD from 9-14% to between 4-8% suggests employing ultrasonic extraction results in a more effective and uniform sample agitation procedure compared to end-to-end manual shaking. The use of an ultrasonic probe in place of a bath; which would be the method of choice for field use; may result in increased extraction efficiencies as unlike baths that dissipate the vibrational energy over a large area, probes focus the energy on a localised sample zone hence providing a more effective cavitation in the liquid (Perez-Cid *et al.*, 1998).

Table 3.4 Recoveries and RSD of *in situ* extraction and analysis using soil CRM following 3 min sonication.

Analyte	Mean (mg kg^{-1})	RSD (%)	Certified (mg kg^{-1})	Recovery (%)
Cd	7.77 ± 0.46	6.69	11.7	64.1
Pb	13.23 ± 0.39	4.82	25.6	51.7
Cu	10.77 ± 0.75	7.95	18.8	57.3

The recovery values of 51-64% achieved by the *in situ* method reflect both the very short extraction time (3 min) and the use of a 1 mol l^{-1} aqua regia extractant (matrix effects are considered in Section 3.3.2). The certified values refer to dissolution of all except the silicate-bound metal fractions (USEPA Method #3050, using concentrated aqua regia). As such, the recovery values for the *in situ* extraction method would be expected to be less than the certified values. However, from the precision observed,

(<8%) it can be assumed that the recovery values indicate the efficiency of the extraction method and the values are not random.

From these data, the proposed *in situ* extraction method has provided a means of 'screening' samples; obtaining a semi-quantitative determination, whilst using simple and rapid *in situ* experimental protocols. The method could be further developed and modified, according to the requirements of the end-user, similar to laboratory based single extraction methods. For example, for a more aggressive extraction, extraction times could be increased, or to evaluate water soluble fractions (i.e. bioavailability), $CaCl_2$ could be used. The final choice of extraction method is ultimately at the behest of the site owner or regulator and will depend upon the particular analytical information required.

These data were presented and discussed at the SENSPOL, Response to New Pollution Challenges Workshop, Kings College, London, 4-7 June 2002. The general consensus was the level of precision and accuracy attained was acceptable for a rapid screening field-based tool.

3.3.2 Matrix effects

The recovery values reported above assume that there are no sample matrix effects on the electrochemical measurement process; that is the recovery values are solely a consequence of the *in situ* extraction efficiency. For this to be true, the relationship between the analyte response value and analyte concentration using test solutions (calibration curve Figure 3.2), must be the same for all samples. Cuadros-Rodriguez *et al.* (2001) reported "Recovery is an estimation of the systematic error of the global process, but does not consider whether this error is a consequence of changes in the amount of analyte or is due to matrix effects".

The relationship between analyte response and analyte concentrations can change unpredictably due to complexation and adsorption, which take place during the stripping analysis, when samples containing organic components are determined

(Brainina *et al.*, 2001). In fact, matrix effects occur even with methods such as plasma spectrometry, which have a reputation for being relatively free from interferences (Miller and Miller, 2000).

Frenzel (1993) reported "The adsorption of organic substances onto the electrode can be a serious limitation in the applicability of SV. Polymeric organic materials such as surfactants, humic acids, and proteins, tend to adsorb at the electrode surface, strongly affecting the deposition of trace metals leading to reduced sensitivity or even causes complete failure of the electrode".

In most cases, a decrease in the peak current is observed due to the hindered transport to the surface, by the sorption of an organic film during the deposition step, and the changed reversibility of the metal oxidation reaction (Wang, 1985). The peak potential may also shift to more positive values as a result of the increased irreversibility of the stripping process.

However, not all surface-active substances exhibit a depression effect and others may in fact result in significant enhancement of the stripping peaks, with no general trend being established (Wang and Tian 1992). The effect ultimately depends on their chemical structure, concentration, their environment, the metal determined, the deposition potential, and the type of electrode used.

To investigate sample matrix effects, a selection of soil samples provided under the PURE project, and a soil CRM, were extracted using the *in situ* extraction method. The soil extracts were analysed before and after a 50 $\mu g\ l^{-1}$ single-point standard addition.

As can be seen from the data presented in table 3.5 only a portion of the analyte 'spike' was recovered. The stripping response decreased between 5-37% for Cd, 5-26% for Pb and 10-25% for Cu, with one outlier of 68% recorded for Cd. The observed peak depression is probably a consequence of adsorption processes rather than complexation, due to the strongly acidic conditions, under which no complexation occurs (Wang & Luo, 1984).

This phenomenon was also apparent from examination of the resulting voltammograms; an example of which is shown in Figure 3.3 in which no significant peak distortion or overlap was observed. The relatively minor changes in the peak shape and potential (small changes in potential are concentration dependent) indicate the main effect of surface-active compounds at the *in situ* MFE is to hinder the transport of the metals to the surface during the deposition step and thus to change the magnitude of the peak current (Wang & Luo, 1984).

Table 3.5 Percentage of signal decrease in soil extracts following standard addition.

Cd	**CRM**	**6**	**8**	**11**	**15**	**17**
Original extract value (μg l^{-1})	80.6±5.1	0	0	0	0	24.8±4.9
Original + spike (50μg l^{-1}) (μg l^{-1})	122.7±2.7	31.6±7.8	15.7±2.4	42.0±8.1	38.6±2.8	65.2±3.2
Spike signal decrease %	5.98	36.9	68.7	15.9	22.9	12.8
Pb						
Original extract value (μg l^{-1})	137.1±9.5	75.8±9.5	138.1±5.6	187.8±16.5	83.0±5.2	156.5±15.1
Original + spike (50μg l^{-1}) (μg l^{-1})	190.1±9.1	101.8±7.9	178.2±3.2	182.3±10.2	98.7±7.6	178.7±16.5
Spike signal decrease %	13.7	19.1	5.2	23.4	25.8	13.5
Cu						
Original extract value (μg l^{-1})	111.6±7.3	51.5±2.1	69.4±19.1	125.6±5.5	68.2±5.6	87.7±24.1
Original + spike (50μg l^{-1}) (μg l^{-1})	122.1±7.9	89.5±3.8	75.2±12.9	148.7±4.1	105.4±6.7	112.7±17.5
Spike signal decrease %	24.5	11.8	37.1	15.3	10.9	18.1

Sagberg and Lind (1982) investigated the effects of synthetic surfactants and naturally occurring humic substances on the DPASV response of synthetic sea water samples spiked with Cu Pb and Cd. Using a HMDE it was found that all peaks decreased in height. However, heights did not vary to any great extent in an acidic solution and were not much affected by pH changes. For Pb and Cu, the peak heights decreased in alkaline solutions, probably due to formation of hydroxo-complexes of these metals. The decrease in peak heights was accompanied by a cathodic shift in the peak potentials, which indicates complex formation. It was concluded "if a marked increase in the peak heights is observed upon acidification of a neutral water sample, this may indicate the metals are released from particles or complexes in solution, even if surfactants are present in the samples".

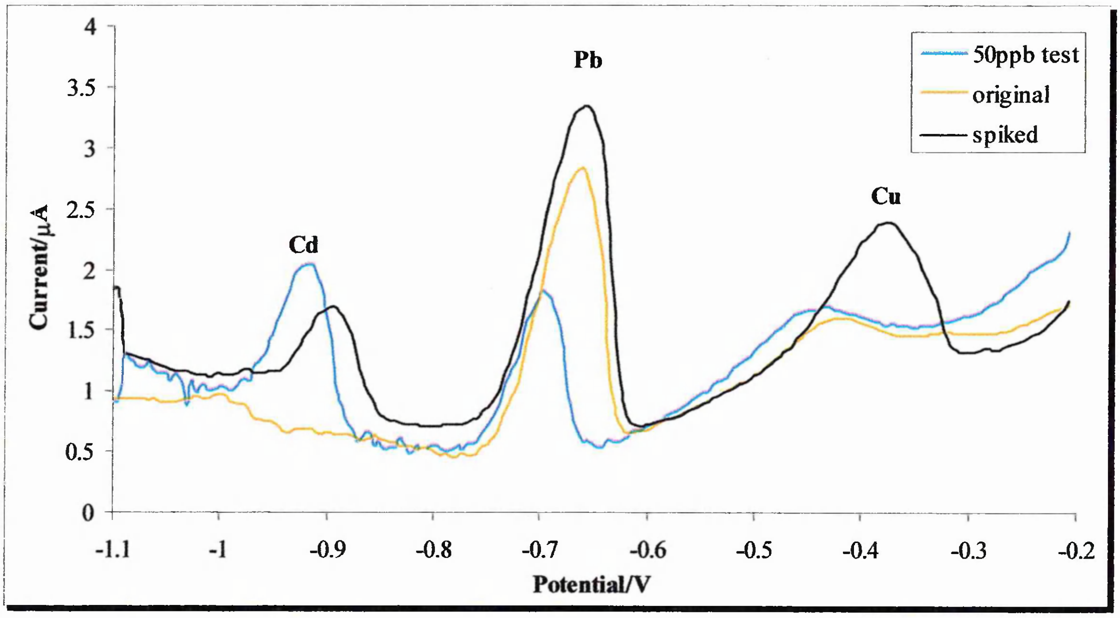


Figure 3.3 Stripping voltammograms of test solution and sample six spiked (50 μg l^{-1}) and un-spiked. All experimental conditions are as defined in Figure 3.2.

The variation in the extent of signal decrease seen can be related to differences in soil type; their physical and chemical properties; the determined analyte; and the amount/type of interfering components present in the sample. This could include naturally occurring organic carbon, and/or industrial pollutants, such as long-chain amines and alcohols (Wang, 1994a).

Wang and Luo (1984) studied the effects of gelatine, humic acid, camphor and starch on the stripping responses of Cd, Pb and Cu using a MFE on glassy carbon disk. It was found effects varied from metal to metal and from one organic compound to another. In all cases, the Cu peak was most severely affected. This was attributed to complexation processes rather than adsorption. Peak depressions of between 15% (slight) and 85% (severe) were recorded for different metal/organic compound mixtures. Except for Cu no effect on the reversibility of the stripping reaction was observed. It was concluded the relatively stable response observed may be a feature of the *in situ* plated MFE at which a continuously renewed surface is maintained.

These data indicate that the stripping responses of the analytes are affected by unknown components present in the soil matrix. To account for this, a 'spiked' sample can be used to estimate the extent of signal depression and the data adjusted accordingly. For example, the *in situ* extraction recoveries obtained using the soil CRM (section 3.3.1) may be adjusted to take into account the decrease in response due to matrix effects; 64% recovery for Cd, 5.98% signal decrease of spike, leading to an adjusted analyte recovery value of 69.98% (64% + 5.98%)

Alternatively, the standard addition method typically used in ASV; involving the addition of several small volumes of a known standard to a known volume of sample, can be employed. After each addition, a voltammogarm is obtained. From the linear increases in the response associated with each addition, a linear calibration model, incorporating matrix effects, can be used too determine the analyte concentration of unknown samples (Donachie *et al.*, 1999).

The main disadvantages of this technique are that it is very time-consuming and labour-intensive and can lead to sample contamination and some interference may still hinder the measurements. Brainina *et al.* (2001) suggested "when actual materials are analysed in the field, the calibration curve method is obviously preferable to the method of standard addition for the following considerations; The analysis procedure and the program of statistical processing of analysis data are largely simplified; the number of operations and total measurement time are reduced; and most importantly, the field laboratory need not be outfitted with additional reagents". In addition, interactions between the soil matrix and the spike can often result in incorrect determinations (Kennedy *et al.,* 1997).

Polymer modification is also a widely used approach for reducing adsorption phenomena. These polymers can either represent a new electrode surface, i.e. conducting polymers, or allow only the electroactive species of interest to reach the electrode underneath. Thus, selectivity can be enhanced by membrane porosity, excluding large molecules such as proteins, or by ion-exchange characteristics, if it is desired to make the membrane selective to charged species (Brett,1999). Cation-exchange polymers, including Nafion and size exclusion polymer coatings, such as cellulose acetate, are commonly used for metal ion determination by ASV.

A number of studies have been carried out for the detection of metal ions using this approach and include; a Nafion coated glassy carbon electrode (Lio and Wu, 1992) and (Agra-Gutierrez *et al.,*1999); carbon paste electrode modified with *N-p-*Chlorophenylcinnamohydroxamic acide (CPCHA) (Degefa *et al.*, 1999); and a poly(ester sulphonic acid) coated MFE with batch injection analysis (Brett and Fungaro, 2000).

Although the use of polymeric coatings can reduce interferences from metal ion determinations, electrode fabrication and experimental protocols are complicated. In addition, modified electrodes often lack robustness and reproducibility (Stulik, 1999). Therefore, this approach was considered superfluous for the purposes of obtaining semi-quantitative data at this stage.

Sample matrix effects have resulted in reduced stripping responses for all analytes. However, low sample pH values (1.5) and the inherent features of the MFE have served to minimise this, with a large percentage of the 50 $\mu g\ l^{-1}$ spike being recovered.

These analyses were performed on samples with analyte levels below legal threshold values, and although organic content was unknown, sampling was carried out across a portion (50 hectares) of an ex-industrial site, located in a river valley. As such, organic material would be expected to be present in the samples and be highly variable. Since the sensor was able to distinguish between spiked and un-spiked samples at low analyte concentration, it can be assumed matrix effects would have minimal effect when screening samples with analyte levels above legal threshold values, allowing provision of a yes/no response without difficulty. When site histories and initial site assessment indicate possible high organic levels, the single-point standard addition could be used to prevent false negatives being recorded.

3.3.3 Analysis of PURE soil samples

The *in situ* extraction method was tested using soil samples supplied by partners within the PURE programme. The objective was to further evaluate the usefulness of the combined *in situ* analyte screening device (extraction+analysis) using a larger sample size, of varying soil types and analyte concentrations.

The soil samples were supplied in 2 sets; set 1 = 25 samples; set 2 = 57 samples. Known analyte concentrations; determined by an independent laboratory using the standard methods detailed in Section 3.2.1 and a site map showing sample locations across the industrially contaminated site, are included as Appendix A2. Data obtained by the *in situ* method were validated by comparison with ICP-MS data (EPA #3051A extraction and #6020A ICP-MS). Regression and correlation analysis was performed on each data set separately, and combined to evaluate repeatability (systematic errors) over time.

Lead

Figures 3.4-3.6 show regression lines of each data set for Pb, and Table 3.6 summarizes the validation parameters.

The results show good correlation between methods, reflected by the R^2 values, with little difference between sample batches. The statistical significance of this correlation was tested using a *two-sided* *t*-test. From this it was assumed that the relationship was highly significant as the calculated values far exceeded the tabulated *t*-value.

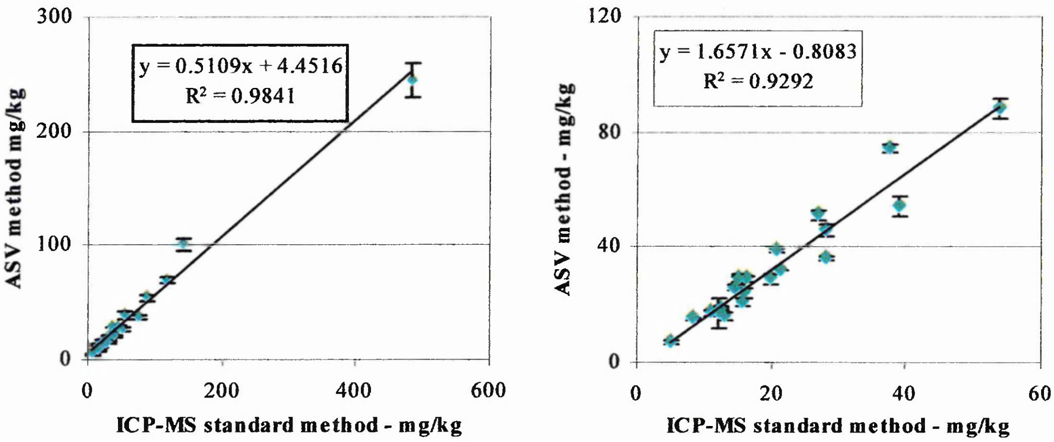


Figure 3.4 Comparison of *in situ* extraction + ASV vs. EPA #3051A extraction + ICP-MS (EPA #6020A) for Pb using data set 1 over the whole data range and <100 mg kg^{-1} (1 sd = error bars; n=3).

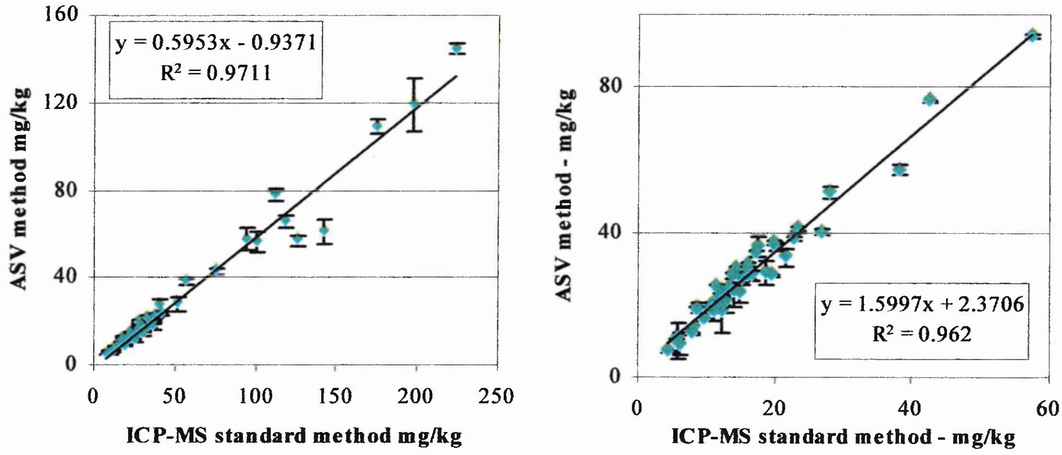


Figure 3.5: Comparison of *in situ* extraction + ASV vs. EPA #3051A extraction + ICP-MS (EPA #6020A) for Pb using data set 2 over the whole data range and <100 mg kg^{-1} (1 sd = error bars; n=3).

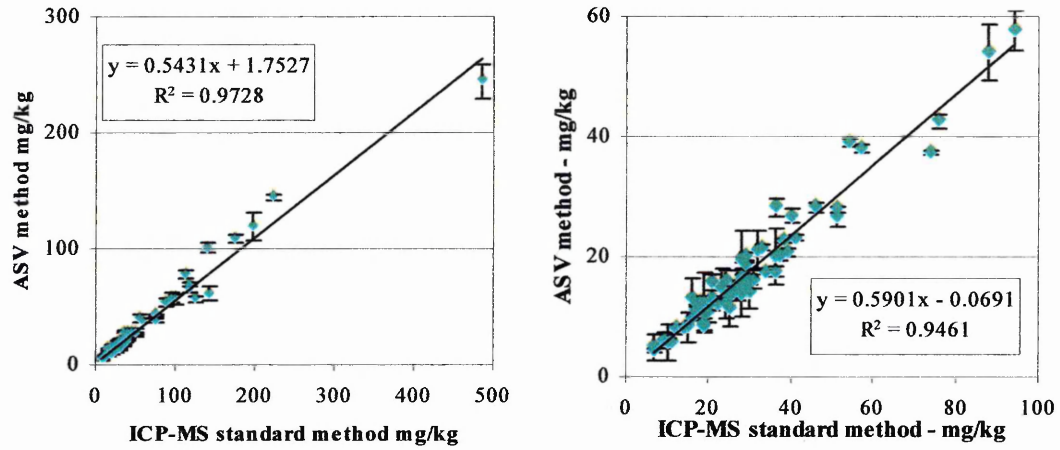


Figure 3.6: Comparison of *in situ* extraction + ASV vs. EPA #3051A extraction + ICP-MS (EPA #6020A) for Pb using data set 1+2 over the whole data range and <100 mg kg^{-1} (1 sd = error bars; n=3).

Table 3.6 Pb analysis of PURE soil samples (mg kg^{-1}).

	Data set 1 - 25	Data set 2 - 57	Data set 1+2 - 82
Mean recovery %	63.32	56.94	58.89
SD	9.32	7.19	8.38
RSD	14.7	12.6	14.2
Trendline	y = 0.5109x + 4.4516	y = 0.5953x – 0.9371	y = 0.54310x + 1.7527
R^2	0.9841	0.9711	0.9728
RSD* <5%	8	33	41
5-10%	13	17	30
10-20%	4	7	11
>20%	0	0	0
CRM recovery %	55.27	53.1	54.21
SD	2.50	1.49	2.25
RSD (n=5)	4.53	2.8	4.15

* Soil extracts measured in triplicate using separate SPE – measure of ASV precision

The mean recoveries of the combined *in situ* acid extraction and ASV analysis were similar to those achieved using the soil CRM. The higher RSD of 14.2% reflects the variation in extraction efficiency and/or matrix effects of different soil samples. Of the 82 samples, 61 (75%) had Pb concentrations of below 40 mg kg^{-1}. The remaining 22 samples had concentrations up to 232 mg kg^{-1}, with one high sample of 484 mg kg^{-1}. However no apparent trend between recovery values and analyte concentration was observed. A small bias was present as the confidence limits of the intercept did not include the zero value. Acceptable DPASV precision was obtained, with 50% of samples achieving RSD of <5% and 86% of samples <10%.

Cadmium

Of the 82 samples supplied, only 5 samples from set 1 and 18 from set 2 (total 23) were analysed as either no Cd was present or levels were below the ASV limit of quantification following sample preparation. Figure 3.7 shows the regression line for data set 1 and 2, with validation parameters summarised in Table 3.7.

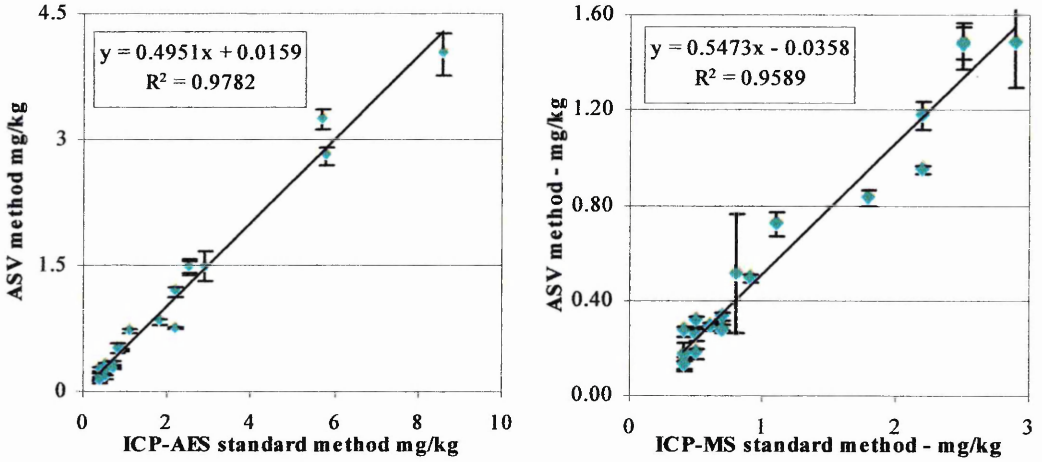


Figure 3.7 Comparison of *in situ* extraction + ASV vs. EPA #3051A extraction + ICP-MS (EPA #6020A) for Cd using data set 1+2 over the whole data range and <4mg kg^{-1} (1 sd = error bars; n=3).

The first data set was only based on five samples; ICP-MS values of 0.4 mg kg $^{-1}$ (3 samples); 0.5 mg kg $^{-1}$; and 2.2 mg kg $^{-1}$. Since 4 of these were very close to the *in situ* limit of quantification following sample preparation, which in turn resulted in low recoveries and high RSD values; the higher value of 2.2 mg kg^{-1} was responsible for the observed correlation between methods using this data set. The level of uncertainty will increase when working close to the limits of quantification; due to an increase in the relative contribution of the background current to the stripping response; also reflected by the high RSD of the DPASV analysis.

Table 3.7: Cd analysis of PURE soil samples (mg kg^{-1}).

	Data set 1 - 5	Data set 2 - 18	Data set 1+2 - 23
Mean recovery %^{1}	34.90	53.20	49.24
SD	4.08	8.42	10.81
RSD^2	11.7	15.8	21.96
Trendline	y = 0.3373x + 0.0063	y = 0.4894x + 0.0652	y = 0.4951x + 0.0159
r^2	0.9964	0.9833	0.9782
RSD* <5%	1	9	10
5-10%	0	6	6
10-20%	2	3	5
>20%	2	0	2
CRM recovery %	70.60	68.4	69.49
SD	3.11	4.14	3.65
RSD	4.40	6.06	5.25

* Soil extracts measured in triplicate using separate SPE – measure of ASV precision

The combined data set, having a wider spread of Cd values, achieved a lower mean recovery value and higher RSD values. Although concentration dependent, the correlation between methods was assumed to be significant as the calculated *t* value exceeded the tabulated *t* value using a *two-sided t*-test.

Copper

Figures 3.8-3.10 show regression lines of each data set for Cu and Table 3.8 summarizes the validation parameters.

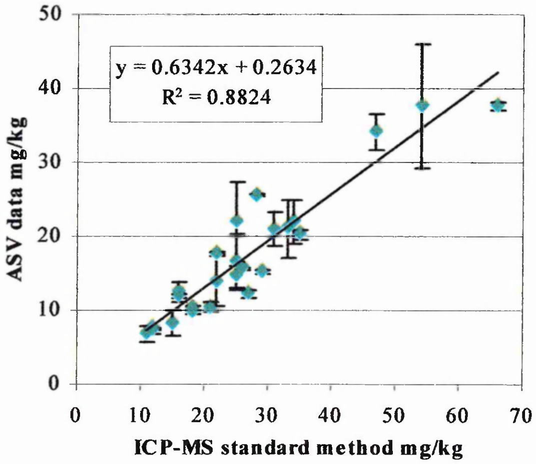


Figure 3.8 Comparison of *in situ* extraction + ASV vs. EPA #3051A extraction + ICP-MS (EPA #6020A) for Cu using data set 1 over the whole data range (1 sd = error bars; n=3).

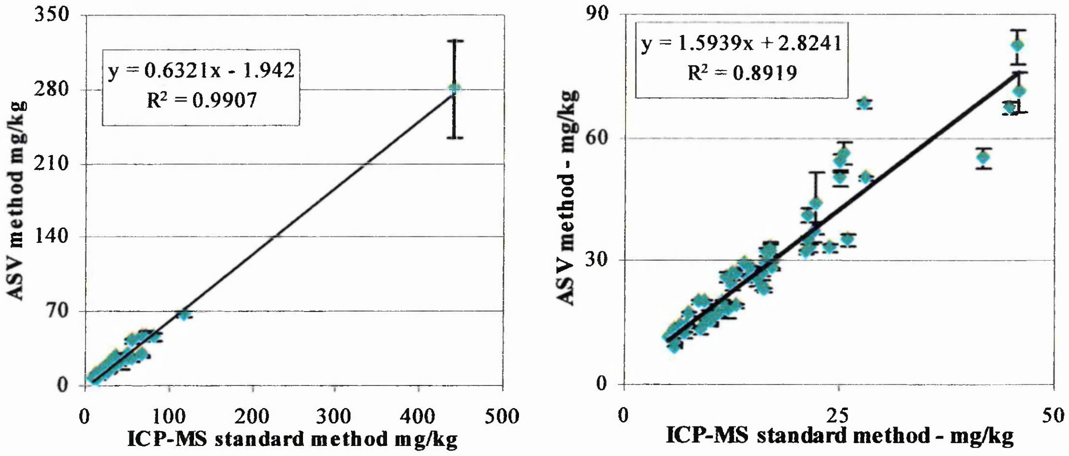


Figure 3.9 Comparison of *in situ* extraction + ASV vs. EPA #3051A extraction + ICP-MS (EPA #6020A) for Cu using data set 2 over the whole data range and <100 mg kg^{-1} (1 sd = error bars; n=3).

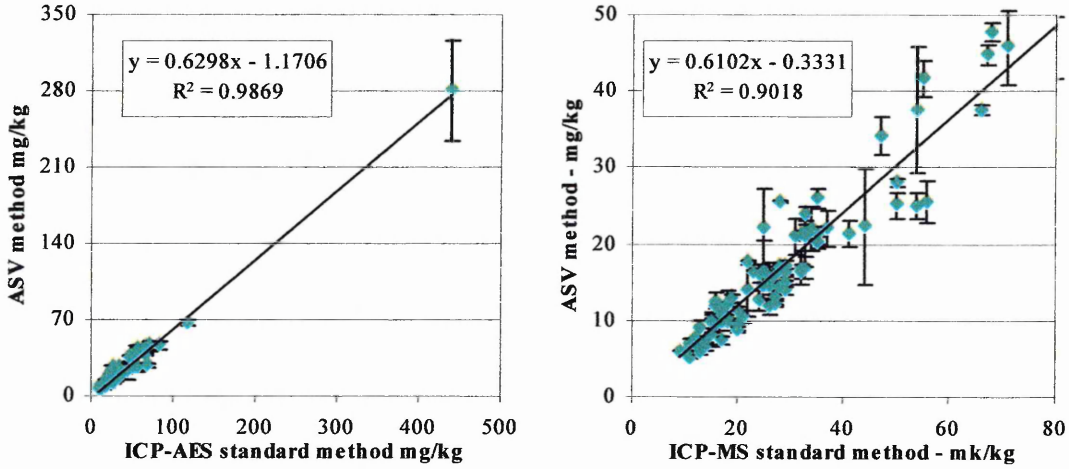


Figure 3.10 Comparison of *in situ* extraction + ASV vs. EPA #3051A extraction + ICP-MS (EPA #6020A) for Cu using data set 1+2 over the whole data range and <100 mg kg^{-1} (1 sd = error bars; n=3).

Table 3.8 Cu analysis of PURE soil samples (mg kg^{-1}).

	Data set 1 - 25	Data set 2 – 57	Data set 1+2 – 82
Mean recovery %^{1}	63.98	57.10	59.20
SD	11.55	8.85	10.19
RSD^2	18.1	15.5	17.2
Trendline	y = 0.6342x + 0.2634	y = 0.6321x – 1.942	y = 0.6298x – 1.1706
r^2	0.8824	0.9907	0.9869
RSD* <5%	11	23	34
5-10%	3	27	30
10-20%	6	7	13
>20%	5	0	5
CRM recovery %	47.56	46.82	47.18
SD	2.6	2.52	2.45
RSD	5.46	5.39	5.18

* Soil extracts measured in triplicate using separate SPE – measure of ASV precision

The results for the combined data set suggest good correlation between methods, reflected by the statistically significant (*two-sided t*-test) R^2 values. However, of the 82 soil samples, 78 (95%) had Cu concentrations of below 70 mg kg^{-1}. The remaining 4 samples had concentrations of 71, 82, 118 and 440 mg kg $^{-1}$. Removing the 4 higher values from the data set, resulted in a linear equation of 0.5626x + 0.7391 and an r^2 value of 0.8442. This was similar to the results obtained using set 1 which did not contain any high values. This indicates the ASV method had little correlation with ICP-MS data when analysing samples with small changes in concentration near to the limit of quantification, following sample preparation. However, the method has classified samples into low and higher values. The high RSD in some cases also reflect the relatively imprecise nature of ASV when measuring this analyte. The observed background current and the complicated nature of copper analysis (discussed in Section 2.3.4) contribute to the disparities between results observed.

Overall, the combined *in situ* electrochemical screening method yields data of sufficient analytical quality to classify soils according to their metal content, and is comparable to ICP-MS analysis. Although systematic error is evident; quantifiable stripping responses for all analytes, allowing the identification of samples containing analytes above or below legal threshold values, has been demonstrated.

3.3.4 Integration of site characterisation tools

When operated independently, the *in situ* electrochemical screening sensor is a powerful site characterisation tool. It permits more samples to be tested without increase in budget, leading to more efficient environmental protection. However, the opportunity of having an extensive sampling strategy, which comprehensively maps contamination across large sites, is rare. Instead a sample of smaller size is taken and it is assumed to be representative of a larger area from which it is taken (Kennedy *et al.*, 1997).

To improve this situation partners within the PURE programme developed a data analysis tool for site characterisation, aimed at identifying all the contaminant locations on a site using a limited number of samples. Fuzzy Areal Site Assessment (FASA) is based on a novel algorithm which identifies contaminated zones in potentially contaminated sites (Ozdamar and Demirham, 2002). The approach partitions a given site into smaller sections in an iterative manner. This leads to a dynamic grouping of sample data which are utilised in calculating an aggregate areal measure for each sub-region.

Areas of interest are selected for re-partitioning according to their areal values and they are further partitioned with the aim of refining the boundaries of the contaminated zones that they possibly contain (Ozdamar *et al.*, 2000). Using these measures, FASA provides non-statistical estimates for the locations of non-contaminated blocks in the site. Partitions classified as non-contaminated are stored and reported, thereby significantly reducing the area of the site to be re-investigated.

Fuzzy (overlapping) partitioning leads to improved information retrieval from available data, because samples are shared simultaneously by multiple spatially related clusters. Thus, data contribute to the fuzzy assessment of more than one block (cluster) depending on the rate of overlapping among neighbouring blocks.

Primary goals of FASA were to reduce drilling and laboratory costs, to carry out *in situ* risk assessments, and to identify hot-spots accurately. The performance of FASA has been tested using chemical data obtained from standard laboratory methods (Ozdamar and Demirham, 2002). However, greater reductions in laboratory and drilling costs and rapid *in situ* risk assessments can be achieved by linking the *in situ* electrochemical screening sensor to the FASA site characterisation process.

To evaluate the usefulness of the *in situ* electrochemical screening sensor in FASA, experiments were conducted using the data obtained for the PURE soil samples detailed in this chapter. The legal threshold values for the heavy metals considered according to the Italian National DL.471 (1999) valid for an industrial use of the area are 15, 1000 and 600 mg kg^{-1} of dry soil for Cd, Pb and Cu, respectively. Since the site was not contaminated from a legal point of view, in order to test the FASA methodology it was decided to use 'virtual' threshold values, lower than the legal values and such that some of the soil samples had measured concentrations higher than the virtual limits. The 'virtual' threshold values were set at 0.31, 31.7 and 22.5 mg kg^{-1} for Cd, Pb and Cu, respectively.

FASA experiments were carried out in 3 phases. The locations of the samples across the contaminated site are shown in Figure 3.11. The first phase included 25 samples (less than one third of the whole data set). Hence data density was approximately 15,000 m^2 per sample. The second phase included 30 more data from the regions in the site that were declared by FASA to have potential of contamination. The third phase was performed on the whole set of 82 samples with a data intensity of 4750 m^2 per sample.

Using 30% of ASV data for Pb (Phase 1), FASA was able to cover 95% of the contaminated data, missing only 1 contaminated sample (Figure 3.12a). This reduced the area of the site by one third. In phase two, using two thirds of the available data for Pb, FASA included all contaminated data in its coverage and reduced the size by half (Figure 3.12b). For Cd and Cu, FASA was able to identify more than 70% of contaminated data from the whole data set (82 samples).

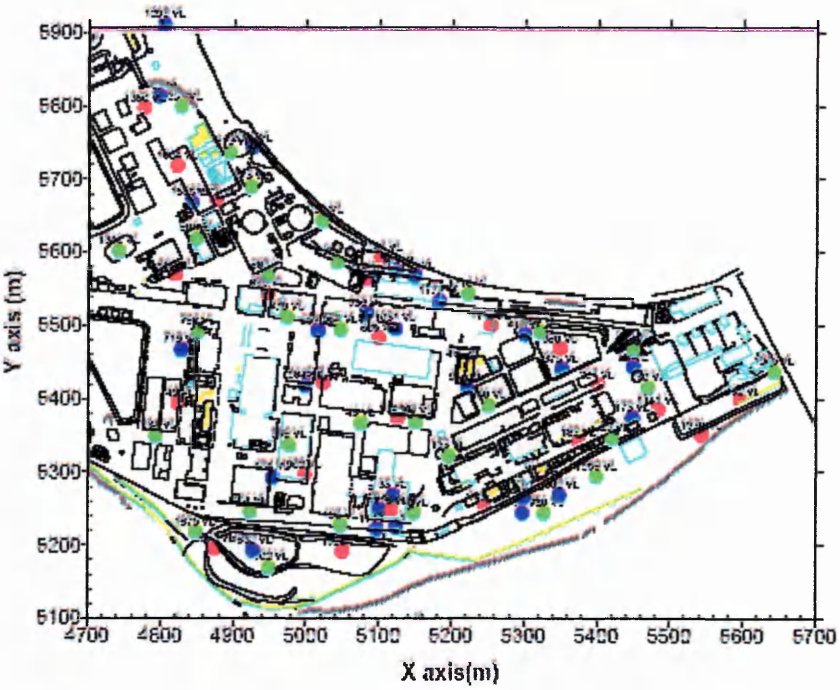


Figure 3.11 Site map showing location of boreholes where soil samples of the first set (25, red circles), of the second set (30, green circles) and of the third set (27, blue circles) were collected.

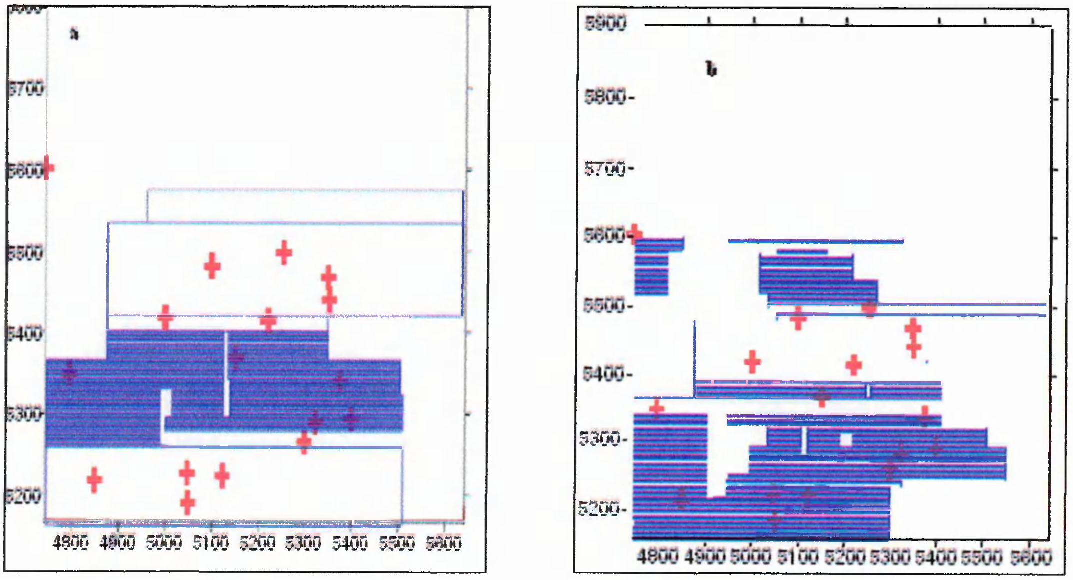


Figure 3.12 FASA results for Pb using (a) 25 data; (b) 55 data. Contaminated data are illustrated as a red cross.

This demonstrates FASA is able to map the spatial distribution of contamination using less precise and accurate chemical data obtained by the *in situ* electrochemical screening sensor. A cost comparison (Section 4.3.5) indicated a value of £9.77 *vs.* £43.77 for the *in situ* electrochemical screening sensor and standard laboratory analysis per test, respectively. The greater saving in laboratory costs and the rapid and flexible nature of the *in situ* electrochemical sensor, provide FASA with a more effective tool for chemical data collection. This has illustrated an additional utility of the *in situ* electrochemical screening sensor in the rapid characterisation of large potentially contaminated sites.

3.4 CONCLUSION

A simple non-exhaustive *in situ* extraction method has been developed for use in conjunction with the *in situ* analyte screening sensor. The extraction method has addressed the lack of existing protocol, regarding sample preparation methods of field based soil analysis, for *in situ* sensors.

The *in situ* extraction method was developed with the aim of providing a soil extract suitable for the *in situ* sensor to obtain a semi-quantitative determination. This permits classification of samples containing analyte levels either above or below legal threshold values, along with the potential likelihood of analyte release.

The approach employed was designed for field-based application where speed, simplicity and extraction stability were primary considerations. To this end, a 1 mol l^{-1} aqua regia extractant and a rapid sample agitation method were selected to meet this requirement. Using a 3 min sonication resulted in improved accuracy and precision compared to a 3 min manual shake. Recoveries of 64%, 52% and 57% and RSD of 7%, 5% and 8% (n=10) for Cd, Pb and Cu, respectively were obtained using a soil CRM.

The achieved recoveries were a consequence of extraction efficiency and/or sample matrix effects. The observed peak depression was attributed to adsorption rather than complexation processes due to the highly acidic conditions of the test solutions. However, use of the direct calibration method has demonstrated that a simple means of quantification can be accomplished; without the need for more complicated methods, such as electrode polymer modification or standard addition.

Comparative analysis of 82 soil samples showed good correlation with ICP-MS analysis. Soil extracts containing Cu and Cd levels near to the limit of quantification resulted in increased error. Although appearing less effective than laboratory methods; which provide increased accuracy/precision and are able to distinguish between different metal fractions; the combined sample extraction-DPASV *in situ* method has successfully classified soils according to their metal content.

The method is rapid and simple to perform in field conditions, without the need for an electricity supply. Rapid screening offers immediate decision-making potential usually hindered by central laboratory sample analysis, whilst reducing the cost/amount of samples ultimately being sent to a central laboratory for a final detailed analysis. The site characterisation specialist must make the choice between the less accurate and precise but reduced cost and improved logistics offered by the *in situ* screening extraction + sensor compared with the higher cost, improved accuracy and higher precision offered by the centralised inflexible laboratory method. In most cases, the analyst is most concerned with rapid site screening, in which situation; the data suggests the *in situ* extraction + ASV method to be a valid option. Operation of the *in* situ method either independently or combined with FASA is a powerful tool for site characterisation.

CHAPTER 4

SENSOR SITE CHARACTERISATION PERFORMANCE

4.1 INTRODUCTION

Demonstration under field conditions permits evaluation of the operation of the *in situ* electrochemical sensor and identification of necessary improvements to enhance practical performance of the sensor.

Consequently, the device was successfully tested within the closely controlled framework of a technical workshop held in Seville, Spain 6-9 November 2002, organised by the European Commission funded EU Thematic Network on 'Sensors for Monitoring Water Pollution from Contaminated Land, Landfills and Sediment' (SENSPOL).

The aim of the meeting was to achieve progress in the use and implementation of sensors and analytical techniques to be used in field conditions, by evaluating their performance in characterising a contaminated metal mining site, located in the mining area of Aznalcollar (Andalucia, Spain). Sensors were also evaluated for their use in assessing the effectiveness of a water treatment plant for mine wastes at the contaminated site.

Extracted soil and water samples were provided at the site, the results of which are presented here. The soil/sediments samples were extracted using water, acetic acid and aqua regia, according to SENSPOL organising committee chosen protocols. The extracts were also bought back to the laboratory in order to complete the analysis due to limited time on site. This provided a means of evaluating the *in situ* electrochemical sensor when samples are prepared in different extractants. The corresponding solid soil samples were extracted in the laboratory for comparison purposes, using the proposed *in situ* extraction method. Suitable hand-held instrumentation was investigated prior to the site visit.

4.1.1 Study area

The mining area of Aznalcollar is located on the southeast of the Iberic pyrite zone (IPZ), between Spain and Portugal. The IPZ is a volcanic area with massive sulphide resources, the most extensive in Europe. Massive sulphides are mainly constituted by pyrite (FeS), chalcopyrite (CuS), espheralite (ZnS) and galena (PbS) and trace metal components such as As, Cd, Ag, Sb and Co. The remaining mineral components are mostly silicates.

Since the Roman period (2200 B.C.), mining activity has been mainly directed at extracting Fe, Ag, Cu, Zn and Au. This has provided a basis for considerable investment in metal related industries but has also resulted in a strong impact on the environment, namely acidic metal containing solutions and the solid wastes from the mining activity. Aznalcollar has been one of the most representative examples of such impact and was also seriously contaminated by the well-known spill of mine tailings in 1998.

The spill occurred in the surroundings of Donana Park, the largest bird reserve in Europe. In this type of mine, ore is milled, washed and after treatment with several reagents, the valuable metal sulfides are separated by flotation from uneconomic sulfides such as pyrite (Grimalt *et al.*, 1999). Huge volumes of acidic wastes and tailings are generated in the process. These tailings are composed of water-rich muds that are generally stocked behind a dam. The main aspects to be controlled in these installations concern wall resistance and water draining. The tailings reservoir is situated near the Agrio River, a small tributary of the Guadiamar River. The waters used in the mining operations are currently dumped in this small tributary.

In 1998 a serious breach of the dam took place, resulting in approximately 4 million cubic metres of acidic water and 2 million cubic metres of toxic mud containing high amounts of heavy metals being released into the Guadiamar River (Grimalt *et al.*, 1999). The Agrio and Guadiamar Rivers overflowed and the toxic mud-waters flooded a zone of approximately 400 m both sides of these rivers, affecting the vegetation and crops of both margins. Many fish, shellfish, vertebrates and amphibians died. Remediation activities included removal of polluted mud, construction of walls to prevent the polluted waters entering into Donana National Park, and removal of dead fauna. Soil was treated and sowing, irrigation, harvesting, pruning and grazing programmes were implemented.

Irrespective of these remediation actions, regular site assessments and monitoring is necessary to manage the long-term effects of the accident and evaluate the effectiveness of remediation actions. The *in situ* analyte screening sensor would permit rapid site characterisation and reduce centralised laboratory analysis. Significant reductions in costs and immediate decision-making potential are clearly beneficial in environmental protection programmes.

4.2 RESEARCH METHODLOGY

4.2.1 SENSPOL sampling and pre-treatment

Sampling, treatment and analysis protocols were developed by *Grup de Tecniques de Separacio-Universitat Autonoma de Barcelona* (GTS-UAB), the company *Techicas de Proteccion Ambiental, S.A.* (TPA), both partners of DIMDESMOTOM EU project, and the *Institute of Environmental Chemistry* (IIQAB-CSIC, Barcelona).

Soil sampling and sample homogenisation was carried out by TPA, sample treatment and analysis by GTS-UAB (heavy metals) and IIQAB-CSIC (hydrocarbons). The treated samples (homogenised and dried soils/sediments and corresponding extracts) were released as samples for analysis during the technical meeting.

Polluted soils, sediments and mine waters were sampled from areas of industrial acitivity across the Aznalcollar site, illustrated in Figure 4.1. The main contaminants are toxic heavy metals. Soil samples were taken by a percussion drilling method. The samples were dried, ground and sieved to obtain particle sizes of <100 μm.

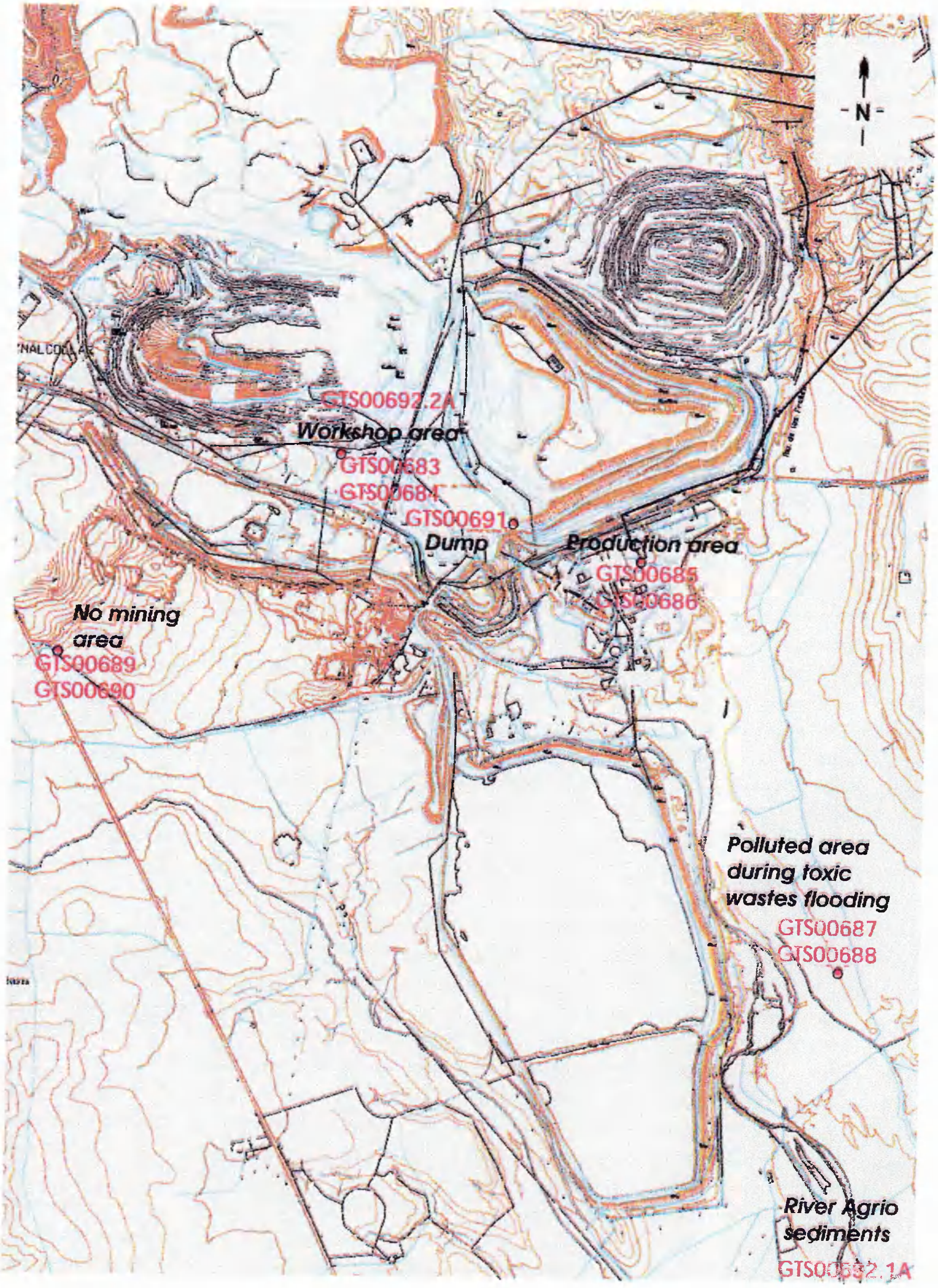


Figure 4.1 Sampling locations across the Aznalcollar mining site, Spain.

Aliquots of mine water samples were filtered with a 0.2 μm pore size filters. The metal content in the filtered solution was evaluated by ICP-OES or ICP-MS depending on the level of the metal concentration.

Soil and sediment samples were treated using three different extractants:

a) Water extraction. This is a compulsory test demanded by regulation in several EU countries to classify the type of contaminated soil. The method may be applied to evaluate the metal release from contaminated soils under rain conditions.

b) Acetic acid extraction. This was carried out to determine the first fraction of the BCR protocol (Report EUR 19502 EN), SES-BCR and constitutes the mobile fraction of heavy metals in soil (ion exchangeable and bound to carbonates).

c) Aqua regia. This provides pseudo-total content of heavy metals in soils.

Heavy metal analysis was performed using ICP-OES model ARL 3410 and ICP-MS model PQ Excell VG Elemental (Appendix A3), and was used for comparative analysis. Samples were diluted 1/10 or 1/100 using supporting electrolyte to meet instrument compatibility.

4.2.2 Samples

Samples supplied by the SENSPOL organising committee included nine soil samples (GTS00683-691), a sediment (GTS00692) and two reference materials (GTS00721/2), and four mine water samples (GTS00693-696). GTS00721 (CRM 601) was from Flumendosa Lake (Italy), certified acetic acid extract for BCR-SES by BCR, and GTS00722 (MR6) was from the Aznalcollar site certified for pseudo-total metal content by the Laboratory of Reference Materials, Department of Analytical Chemistry (University of Barcelona, Spain). The samples were stored at 4-8°C and analysed within 1 month.

4.2.3 *In situ* analysis

Standard solutions were prepared prior to the site analysis using Analar grade reagents purchased from BDH (Poole, England), Sigma Chemical Company (Poole, England) and Aldrich (Gillingham, England). Distilled water was used for all additional preparations while on site. DPASV analysis was performed using identical protocols to that performed in the laboratory detailed in Section 2.2, using a hand-held PalmSens electrochemical analyser (Palm Instruments BV, Utrecht, Netherlands) (Figure 4.2). This provides 6-8 hours of operation using rechargeable batteries and fits into a briefcase. Full instrumental specifications can be found at *www.palmsens.com.* Comparative analysis prior to the site visit confirmed the applicability of the instrument to this application (Section 4.3.1).

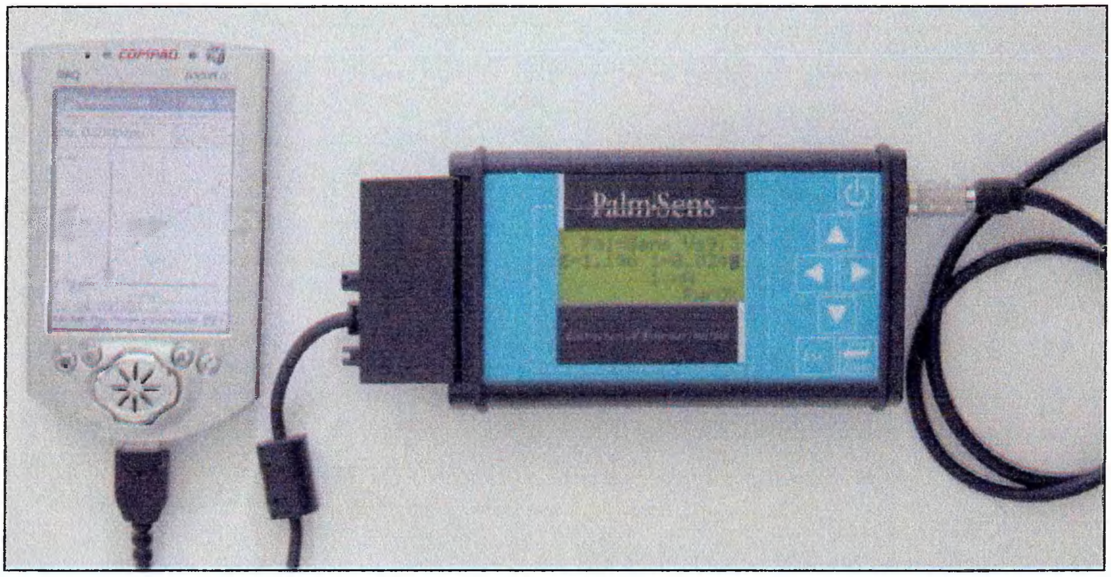


Figure 4.2 PalmSens electrochemical analyser (dimensions: 155 mm x 85 mm x 35 mm; weight: 0.45 kg; power: 2 AA cells NiMH for 6-8 hrs operation).

4.2.4 DPASV laboratory analysis

The sample extracts were bought back to the laboratory in order to complete the analysis due to limited time on site. Solid samples were extracted using the developed *in situ* extraction method (Section 3.2.2) for comparison purposes.

4.3 DATA ANALYSIS AND COLLECTION

The recovery values for the soil and water samples supplied as extracts reflect the accuracy of the *in situ* ASV method. The recoveries for the samples extracted using the *in situ* extraction method are a measure of both the extraction efficiency and ASV analysis combined.

4.3.1 Comparison of Desktop and Palmsens electrochemical analysers

PalmSens performance was evaluated by comparative analysis with a laboratory based computer controlled potentiostat-10, Autolab Electrochemical Analyser (Ecochemie, Utrecht, Netherlands). Calibration standards (0-200 $\mu g\ l^{-1}$) were analysed using both instrumentation. Regression lines were plotted to check for systematic instrumental error.

It can be seen from Figure 4.3a/c that the PalmSens did not give results that were significantly higher or lower than the bench top analyser, with positive correlation coefficient and slope values of close to unity being obtained. The hand-held PalmSens instrument was therefore used for subsequent analysis.

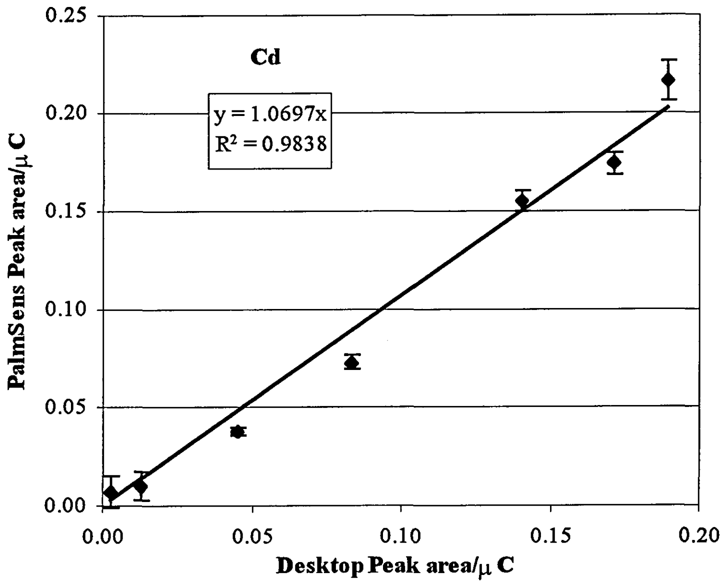


Figure 4.3a Comparison of desktop and PalmSens instrumentation using calibration standards (0, 10, 30, 50, 100, 150, 200μg l^{-1}) for Cd. t_{dep} 165 sec; E_{scan}:-1.1v ⇒ -0.2v @ 20mV s^{-1}; interval time 0.5secs.

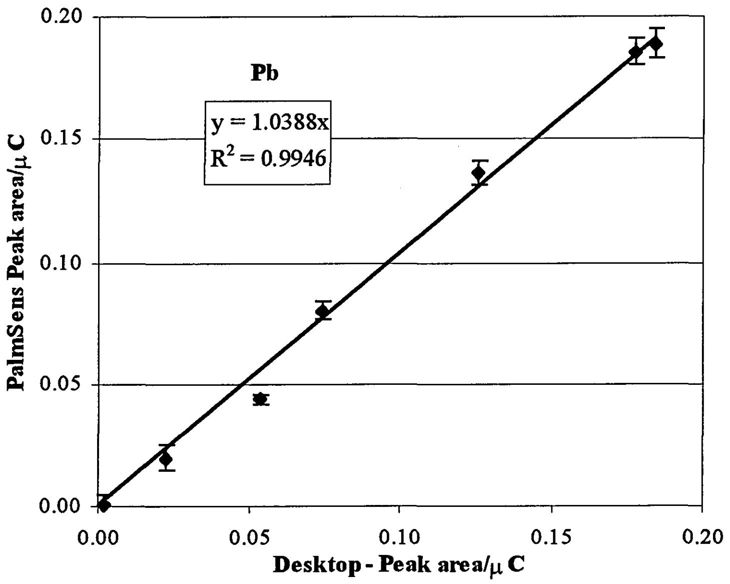


Figure 4.3b Comparison of desktop and PalmSens instrumentation using calibration standards (0, 10, 30, 50, 100, 150, 200μg l^{-1}) for Pb. All experimental conditions are as defined in Figure 4.3a.

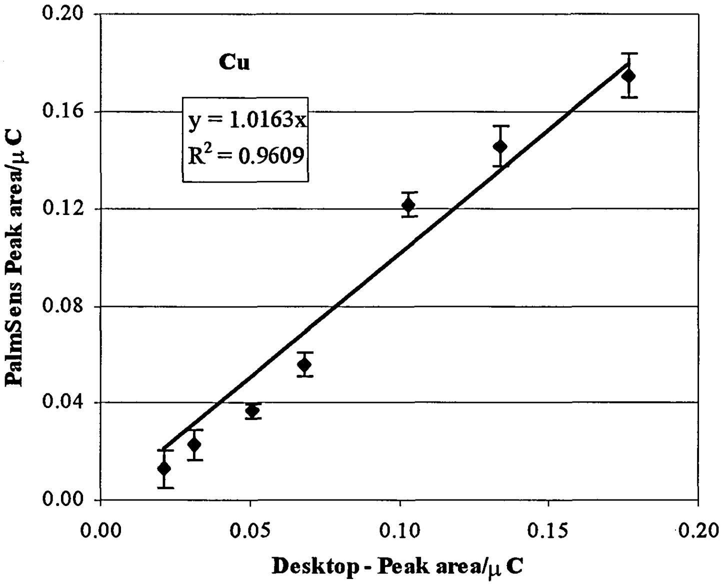


Figure 4.3c Comparison of desktop and PalmSens instrumentation using calibration standards (0, 10, 30, 50, 100, 150, 200μg l^{-1}) for Cu. All experimental conditions are as defined in Figure 4.3a.

4.3.2 *In situ* soil/sediment analysis – SENSPOL Technical Meeting, Seville

Table 4.1 shows data obtained on-site against DPASV laboratory data and ICP-OES data, for samples extracted using the aqua regia method. ICP, ASV field and ASV lab refers to data obtained by the ICP-OES, field-based ASV and laboratory-based ASV procedures respectively. The relative standard deviation (RSD) term relates to the precision of the laboratory-based ASV procedure (n=3). All data obtained in the field were provided to the SENSPOL organisers at the time of analysis.

The field ASV data for Pb and Cu are comparable to those achieved by ICP-OES, clearly identifying and discriminating between those samples containing high and low analyte concentrations. Recovery values for samples containing higher Pb concentrations were close to 100%, although more variation in recoveries was observed at lower Pb concentrations. Considering the ASV field data represent a single analysis (akin to a rapid field-based screening method), compared to the more accurate but lengthy ICP-OES laboratory analysis, the data obtained would be more advantageous to the site assessor than higher accuracy levels, in terms of immediate decision-making possibilities.

•

Table 4.1 Pb, Cd and Cu in soil/sediments extracted by aqua regia (mg kg^{-1}).

Sample	**683**	**684**	**685**	**686**	**687**	**688**	**689**	**690**	**691**	**692**	**721**	**722**
Pb												
ICP	872	977	292	47	140	179	13	40	1116	1111	245	1853
Asv field	**1072**	**1033**	**310**	**46**	**60**	**41**	-	**25**	**1412**	-	-	-
Asv lab	972	1072	286	46	120	157	6.9	33	646	775	255	1998
$\%^1$	123	106	106	98	43	23	-	63	127	-	-	-
%	112	110	98	97	85	88	53	82	58	70	104	107
RSD	6.2	3.3	8.9	0.6	4.7	1.9	3.2	8.8	6.8	1.9	4.2	2.3
Cu												
ICP	666	349	254	34	66	72	12	20	518	508	199	419
Asv field	**460**	**196**	**278**	**93**	**167**	**162**	**143**	**147**	**662**	-	-	-
Asv lab	440	328	158	18	51	56	12	17	518	387	206	327
$\%^1$	69	56	109	273	253	225	1^{+3}	735	128	-	-	-
%	66	94	62	54	77	77	100	84	100	76	103	97
RSD	9.6	6.1	13.9	16.1	10.2	7	12.1	4.7	8.4	4	3.7	8.7
Cd												
ICP	4	<2	<2	<2	<2	<2	<2	<2	20	19	16	12
Asv field	**0.72**	**0.81**	**0.07**	**0.08**	**<**	**0.09**	**0.03**	**0.07**	**0.11**	-	-	-
Asv lab	<2	<2	<2	<2	<2	<2	<2	<2	10	12.1	10	12.3
%									52	64	63	103
RSD									7.7	6.3	11.5	6.1

%: recovery of ICP value (lab/icp*100); $\%^1$ recovery of ICP value (field/icp*100) RSD (n=3); - samples not analysed. 683-691 soil; 692 sediment; 721/2 soil CRM

The field ASV data for Cu were considerably higher than ICP-OES for most samples. This disparity was thought to be due to a miscalculation during dilution of samples at the time of the analysis, as laboratory analysis of the same extract did not result in similarly high recovery values. However, the samples were still classified as either above (samples 683-865, 691) or below (686-690) the legal threshold action limit of 190 mg kg^{-1} (Dutch List, Section 1.3), thus fulfilling the objective of a rapid field based screening tool.

Analysis carried out in the laboratory, using the same field methodologies, resulted in slightly improved accuracy, for all analytes, however this data represent mean values. RSD values for the laboratory based ASV data indicate adequate precision with most values <10%. A quantifiable stripping current was obtained in samples containing higher levels of Cd, as shown in Figure 4.4.

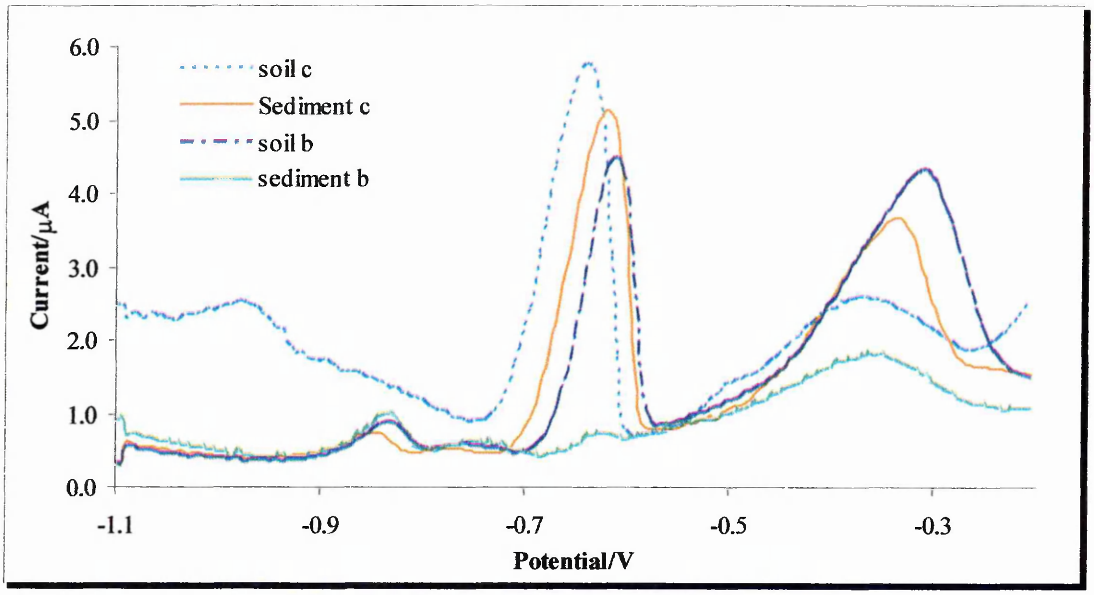


Figure 4.4 DPASV assay of Cd (-0.85 V), Pb (-0.65 V) and Cu (-0.35 V) in a soil and sediment sample, extracted using either acetic acid (b) or aqua regia (c). All experimental conditions are as defined in Figure 4.3a.

The samples extracted by acetic acid (Table 4.2) represent the 'mobile' analyte species; that is species recovered by this relatively non-aggressive extraction method. The laboratory analysis demonstrates the method operates well in different extractants (Figure 4.4) and at lower analyte concentrations, with values comparable to ICP-OES results. Recovery values for Cu and Cd are overall lower than for Pb. This may be reflecting complexation processes of these analytes in this type of extractant, however dilution with high purity grade water served to minimise this.

Water extracts were not analysed as these were below the current limit of detection for this assay using a deposition time of 165s (extract ICP-MS values Cd <5, Pb/Cu <10 μg l^{-1}). It may be possible to improve assay limits of detection by increasing the DPASV depositioning time.

Table 4.2 Pb, Cd and Cu in soil/sediment extracted by acetic acid (mg kg^{-1}).

Sample	683	684	685	686	87b	688	689	690	691	692	721
Pb											
ICP	119	59	6	2	1.2	7	<0.4	<0.4	109	14	2.4
Asv lab	112	66	5	3	0.8	7	<0.4	<0.4	120	14	1.2
%	94	112	82	150	66	100			110	100	49
RSD	3.5	4	13.7	5	3.2	3.4			1.6	6	22.2
Cu											
ICP	47	10	39	1.5	2	5	<0.4	<0.4	72	276	9
Asv lab	46	9	38	0.97	0.88	3.4	<0.4	<0.4	68	234	8.4
%	97	90	96	65	44	68			95	85	95
RSD	12.9	12.4	16.7	7.9	9.3	4			10.2	0.62	7.5
Cd											
ICP	2.37	0.64	1.04	<0.2	<0.2	<0.2	<0.2	<0.2	7.49	4.1	3.8
Asv lab	1.69	0.42	0.99	<0.2	<0.2	<0.2	<0.2	<0.2	4.87	3.24	2.56
%	71	66	95						65	79	67
RSD	3.1	2	8.3						4.7	7.5	13

%: recovery of ICP value (lab/icp*100); RSD: relative standard deviation (n=3); 683-691 soil; 692 sediment; 721 soil CRM

4.3.3 *In situ* water treatment plant sample analysis

Water samples were taken before and after entering the treatment plant at the contaminated site. Table 4.3 shows the data obtained *in situ* and at the laboratory DPASV are comparable to the ICP-OES values, although a slight increase was detected for Pb and Cd. Figure 4.5 illustrates the effectiveness of the assay in monitoring remediation processes, with well-defined stripping currents obtained from samples prior to treatment, but not post-treatment. This is clear evidence that the remediation has been successful in radically decreasing contaminant concentrations.

Table 4.3 Cd, Pb and Cu analysis of water samples before (693, 694) and after (695, 696) treatment (mg l^{-1}).

Sample	693	694	695	696
Pb				
ICP	0.89	0.74	<0.05	<0.05
Asv field	1.11	1.02	0.02	0.02
Asv lab	1.09	0.84	<0.05	<0.05
Lab %	123	114		
RSD	9.3	8.3		
Cu				
ICP	0.18	0.18	<0.05	<0.05
Asv field	-	1.78	0.05	0.05
Asv lab	0.18	0.12	<0.05	<0.05
Lab %	100	68		
RSD	18	15		
Cd				
ICP	0.62	0.63	<0.05	<0.05
Asv field	0.65	0.99	0.02	0.02
Asv lab	0.74	0.89	<0.05	<0.05
Lab %	119	141		
RSD	8	3.9		

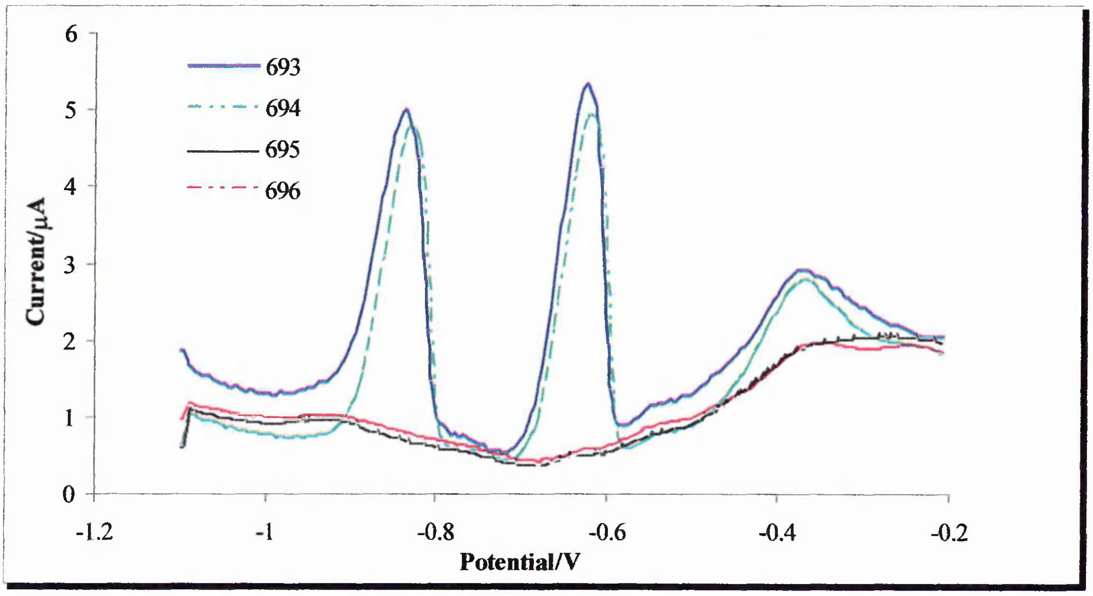


Figure 4.5: DPASV voltammograms for Cd, Pb and Cu in water samples pre and post treatment plant. All experimental conditions are as defined in Figure 4.3a.

4.3.4 *In situ* extraction method

The solid samples were extracted in the laboratory using the proposed *in situ* extraction method. As discussed in the previous chapter, the applicability of *in situ* sensors is questionable with respect to soil analysis unless suitable field based preparation methods are available.

Table 4.4 contains data obtained for the soil/sediment samples after preparation with the proposed *in situ* extraction method. ASV analysis recovered approximately 55% Cd, 58% Pb, and 48% Cu, when compared to the ICP-OES reference values. These are similar to the recoveries of the PURE soil samples and CRM (Chapter 3). As expected, the mean values are lower than those achieved using the samples prepared by the standard pseudo-total method (Table 4.1) reflecting constraints posed by the field-based nature of the extraction method. However, semi-quantitative data have been obtained, identifying those samples containing either high or low analyte levels.

Table 4.4 Cd, Pb and Cu analysis of soil/sediment samples extracted using the *in situ* extraction method.

Sample	**683**	**684**	**685**	**686**	**687**	**688**	**689**	**690**	**691**	**692**	**721**	**722**
Pb												
ICP	872	977	292	47	140	179	13	40	1116	1111	245	1853
Lab	557	582	129	29	85	133	7	22	663	659	173	980
%	64	49	44	61	60	75	55	56	47	42	71	53
RSD	6	5.5	6.3	8	4.1	2.4	8.9	3.2	2.1	3.4	1	3.7
Cu												
ICP	666	348	254	34	66	72	12	20	518	508	199	419
Lab	303	138	99	14	36	33	5.8	10	286	275	117	220
%	45	40	39	40	54	46	48	49	54	54	61	68
RSD	4.4	6.2	9.2	11	28	9.4	13	19	4.3	4.7	5.8	9.4
Cd												
ICP	4	<2	<2	<2	<2	<2	<2	<2	20	19	16	12
Lab	2.3	-	-	-	-	-	-	-	11.8	9.67	9.3	6.6
%	59								59	51	58	55
RSD	6.5								4.6	2.6	2.6	8

4.3.5 *In situ* electrochemical screening sensor costing

An approximate cost for soil analysis using the *in situ* electrochemical screening sensor is contained in Table 4.5. This assumes a maximum sample throughput of 18 per operator per day (4680 per annum). The number of samples which can be analysed in a given time period is controlled by the rate-limiting extraction step. The cost for the SPEs was calculated based on the production of 100,000 per year at Cranfield University and the reagents and consumables were based on the amount of each used per analysis (Appendix A4). The profit was set at 30% assuming a high volume, low cost product.

Table 4.5 Estimated cost of soil analysis using the *in situ* electrochemical sensor.

	Cost – per annum £
Personnel	30,000.00
Capital equipment – PalmSens (based on 3 yr depreciation)	666.67
Pipette	120.00
Reagents and consumables	
Potassium chloride	5.40
Nitric acid	11.79
Hydrochloric acid	31.17
Ultrapure water	117.94
Metal standards	100.00
Filtration equipment	2808.00
Pipette tips (100 μl)	46.80
Pipette tips (5 ml)	280.80
Universals	877.50
Glassware	100.00
SPEs	18.72
Sub total	35159.79
30% profit	10547.94
Total for 4680 tests	**45707.73**
Per test	**9.77**

Conventional laboratory analysis costs will vary between testing facilities. However, the Environment Agency National Laboratory Service provided a quotation of £43.77 per sample for ICP-OES analysis excluding appropriate sample containers and delivery (Appendix A5). A cost reduction of 77% is therefore indicated for the *in situ* electrochemical screening sensor procedure.

In addition, samples submitted for laboratory analysis require appropriate storage and transportation to the laboratory. The samples are analysed using high precision instrumentation and highly trained personnel. Data then require distribution to the relevant on-site personnel. As such, a minimum turnaround time of 7 days is expected, although times of up to 21 days are not uncommon.

The cost and logistical benefits offered by the *in situ* electrochemical screening sensor allow a more dynamic approach to site assessments and routine monitoring of remediative processes and are attractive to both the stakeholder and site characterisation specialist. However, assay simplification generally results in precision and accuracy losses and when absolute values are required, laboratory testing is obligatory. In this case, samples failing the *in situ* electrochemical screening assay can be sent to a centralised laboratory for detailed analysis. In this way, the amount of samples requiring more costly analysis is dramatically reduced.

For example, the data contained in Table 4.1 show analysis using laboratory methods identified 5 out of 12 samples having measured concentrations of Pb above the legal threshold value of 530 mg kg^{-1} (the new Dutch list, Section 1.3). The cost of laboratory analysis of 12 samples would be £525.24 based on the above quotation. The same 5 samples were also identified using the *in situ* extraction + analysis methods, at a cost £117.24. In addition, a charge of £218.85 would be incurred for detailed laboratory analysis of the 5 identified samples, making a total of £336.09 + transportation costs. In this case, a potential cost saving of 36% could be achieved by pre-screening prior to detailed laboratory analysis.

4.4 CONCLUSION

Overall, the results show that the field-based DPASV-SPE method is capable of simultaneous analysis of Cd, Pb and Cu, in a range of soil types, extractant solutions and across a range of concentrations. Use of the direct calibration method provided a rapid means of quantification, without significant adverse matrix effects. The method has been successfully proven as an *in situ* screening tool for the detection of these common heavy metal pollutants.

The method is able to classify soils according to their heavy metals content, thereby reducing the number of samples requiring transfer to a centralised laboratory for further analysis. This reduction in more costly analytical treatment has clear economic benefits and hence is attractive to site owner, regulator and legislator combined. It can also allow more samples to be tested without increase in budget, leading to more efficient and comprehensive environmental protection.

The samples containing high metal concentrations were from dump and workshop areas, whilst samples with low contaminant concentrations were from surrounding areas where either no history of mining was evident or where toxic floods had not previously occurred. This indicates the sensor device has successfully mapped pollution at the contaminated site and is capable of locating toxic 'hotspots'.

The *in situ* extraction method resulted in lower analyte values than for samples extracted using the laboratory aqua regia extraction and does not determine specific metal fractions as the laboratory based acetic acid extraction. However, the method is rapid, simple to perform in field conditions and is able to identify samples containing significant amounts of analytes.

Site characterisation and monitoring are essential steps in land management. Demonstration of innovative field based techniques should increase awareness of the practical utility of these techniques to end-users, ultimately encouraging wider acceptance in industry.

CHAPTER 5

FABRICATION AND CHARACTERISATION OF DISPOSABLE GOLD ELECTRODES FOR *IN SITU* METAL ANALYSIS

5.1 INTRODUCTION

The growing awareness of environmental As and Hg pollution and their toxicity to a broad spectrum of organisms at the low microgram per litre level, has been the driving force behind the development of a rapid and cost-effective *in situ* screening sensor. The attributes of *in situ* screening have been discussed previously (Section 1.2).

Approved laboratory based methodologies such as ion chromatography coupled with either ICP-AES/MS, GFAAS, GHAA (Section 1.6) offer excellent performance and reliability, however represent considerable investment, high operational costs, skilled personnel and are unsuitable for field use. The adaptability of electrochemical methods to field analysis coupled with the ability to perform low cost speciation analysis make them an attractive alternative to standard laboratory methods.

In order to measure As and Hg electrochemically, a suitable working electrode is required. The limited anodic range of MFEs, make them unsuitable for analytes such as As which have a positive (anodic) oxidation potential. Solid electrodes, particularly Au electrodes, are widely employed for measurements at more positive potentials and several electrochemical methodologies have been applied to the determination of As or Hg.

5.1.1 Mercury (Hg^{2+})

In general, methods for the electrochemical determination of Hg involve stripping voltammetry and potentiometry using glassy carbon (GC), solid Au and Pt, Au fibres and Au plated supports such as carbon paste SPE, GC, and Pt disks (Richter *et al.*, 2002). Au electrodes are widely used due to the high affinity for Hg, which enhances the electrochemical pre-concentration effect (Watson *et al.*, 1999).

Table 5.1 contains a selection of the reported electrochemical techniques utilising Au electrodes for the trace analysis of Hg. A US-EPA method (SW- 486 series method #7472, ASV on Au plated GCE, 1994) also exists for the determination of Hg in aqueous samples and soil extracts.

The main drawbacks encountered using these procedures include electrode memory effects, limited sensitivity and poor precision (Ferreira and Barros, 2002), with the measurement of an analytical signal for Hg on a Au surface being hindered by the variable background current behaviour (Jayaratna, 1997). Co-existing metal ions, particularly Cu also interfere with the Hg stripping response.

In the case of solid Au electrodes, the surface has to be regenerated by mechanical polishing or electrochemical cleaning which are usually time-consuming procedures requiring experience and manual skills (Svancara *et al.*, 1997). These operations complicate and restrain determinations thereby making their routine use in field analysis virtually impossible.

Table 5.1 Determination of Hg using electro-analytical techniques and Au electrodes.

Method	**Working electrode**	**LOD**	**Reference**
ASV	Flow injection cell Au plated GCE	0.14 $\mu g\ l^{-1}$	Richter *et al.*, (2002)
PSA	Heated Au Wire	80 ng l^{-1}	Wang *et al.*, (1999)
DPASV	Au plated carbon paste	1 $\mu g\ l^{-1}$	Svancara *et al.*, (1997)
LSSV	Solid Au disk	100 $\mu g\ l^{-1}$	Jayaratna (1997)
PSA	Au plated carbon SPE	0.5 $\mu g\ l^{-1}$	Wang and Tian (1993c)
SWASV	Rotating disk Au	0.4 $\mu g\ l^{-1}$	Bonfil *et al.*, (2000)
LSSV	Heated Au wire	1 $\mu g\ l^{-1}$	Flechsig *et al.*, (2000)
DPASV	Solid Au in flow system	50 $\mu g\ l^{-1}$	Gorecki & Golas (2001)

Regeneration can be simplified when employing a Au film electrode. Preparation can be performed electrochemically either via an external pre-electrolysis in a separate solution or by plating *in situ,* similar to MFE's (Section 1.7.3).

Alternatively, chemically modified electrodes (CME) have been widely applied for the ultra trace analysis of Hg. These can benefit analytical applications in several ways; acceleration of electron-transfer reactions; preferential accumulation; or selective membrane permeation (Wang and Tian, 1993c). Lower detection limits and minimisation of interferences from co-existing metal cations are often attained.

Various modifiers, including ion-exchange resins and organic/inorganic compounds that are able to pre-concentrate Hg have also been utilised. Ugo *et al.* (1998) reported on a carbon SPE modified by mixing the ink with the chelating resin Sumichelate Q10R, which contains dithiocarbamate groups able to complex Hg selectively. Detection limits reached 12pM and Cu interference was eliminated when present in a 100-fold excess.

Turyan *et al.* (2001) developed an on-line sequential-injection system using a modified GC electrode. A selective Hg reagent, Kryptofix-222, was incorporated inside a positively charged polymer poly (4-vinylpyridine) and used to accumulate Hg in the form of negatively charged chloride complexes by anion-exchange mechanisms. A detection limit of 10^{-9} M was achieved.

Other schemes have been developed by Lu *et al.* (2003), using a GCE modified with a calixarene derivative, obtained detection limits of 5 $\mu g\ l^{-1}$; Stojko *et al.* (1998) used a disposable thick film graphite-epoxy electrode modified with soluble Au salts and Nafion, achieving an LOD of 0.2 $\mu g\ l^{-1}$; Faller *et al.* (1999) modified disposable thick film graphite electrodes with a PDC/Au^{3+} complex and achieved a LOD of 5 ng l^{-1} for Hg.

Obtaining reproducible data with adequate sensitivity and selectivity requires either complicated, time-consuming surface regeneration procedures, or surface modification schemes. As such, few suitable *in situ* electrochemical methodologies have been reported for the trace analysis of Hg.

Wang *et al.* (1998c) developed a submersible stripping probe for remote monitoring in natural waters using a Au wire electrode. A detection limit of 0.3 μg l^{-1} was achieved. The stability of the probe was maintained by a 'cleaning' period which was reported to completely remove the Hg deposit, leading to a reversible and stable response. However, a study conducted by Watson *et al.* (1999) demonstrated that a percentage of Hg deposited during accumulation is retained on the electrode surface even after a stripping and electrode cleaning cycle.

Foust and Gui (1997) reported on an *in situ* method for the analysis of Hg in soils, developed and tested at the General Electric Co (NY, USA). The method involved extracting Hg from soils using KI and iodine and performing SWASV with a Au solid electrode. Quantitative data was obtained in the range of 1-10,000 mg l^{-1}. Electrode contamination was reputedly eliminated as KI and iodine strongly absorb onto the Au surface as iodine atoms producing a monolayer. This protects the electrode from contamination resulting in a reproducible surface for measurement following an electrode cleaning procedure.

Gerlach *et al.* (2001) developed an *in* situ method based on ASV and a Au plated rotating GCE. Using a 2-point standard addition, quantitative data down to 3 μg g^{-1} was achieved in soil samples. Wang and Tain (1993c) utilised methodologies similar to those used for the determination of metals such as Zn, Cd, Pb and Cu on a carbon SPE plated with a mercuric salt. The carbon SPE was pre-plated electrochemically for 20 min using a Au solution. A detection limit of 0.5 μg l^{-1} was obtained following a 4 min accumulation using PSA.

These field methodologies have demonstrated low level analysis of Hg in soils and water samples. However, the requirement for electrode cleaning procedures or generation of Au films complicates and lengthens analysis. Significant improvements in analysis time, using simplistic methodologies without the need for fully skilled personnel and the straightforward mass fabrication of reproducible electrodes, would be advantageous. This would facilitate remediation activities and site characterisation studies by minimising delays and reducing costs.

5.1.2 Arsenic

Electrochemical methods applied to the analysis of As commonly involve CSA or ASV using a pulsed waveform. ASV is based on reducing As^{3+} in a strongly acidic media and high chloride concentration at solid or wire Au electrodes and Au plated supports such as carbon paste SPEs, GCEs, and Pt electrodes.

Alternatively CSV using a HMDE can be applied when accumulation prior to the stripping step is performed in a Cu^{2+} or selenium (Se) containing solution, forming intermetallic compounds, or by adsorption of a complex of As^{3+} with pyrrolidine dithiocarbamate (Kowalska *et al.*, 2002).

As discussed in Section 1.4.5 As can exist in four oxidation states; -3, 0, +3 and +5. The trivalent inorganic forms are highly toxic and 60 times more poisonous than the metal, its pentavalent salts or organoarsenic compounds, monomethlarsonic acid (MMAA) and dimethylarsinic (DMAA) (Feeney and Kounaves, 2000b). Although As^{5+} is thermodynamically favoured, unusually high As^{3+} concentrations are often associated with significant biological activity and dissolved organic matter in the medium. Both oxidation states are potentially harmful, although As^{3+} is more toxic as it can complex with certain thiol-containing enzymes (Barra and Correia dos Santos, 2001). Therefore inorganic speciation of As is essential in assessing the contamination risk.

Since As^{5+} is electrochemically inactive, the determination of both species As^{3+} and As^{5+} can be achieved by quantifying the difference between the original content of As^{3+} and the total inorganic As following reduction of As^{5+} to As^{3+} (Savncara *et al.*, 2002). Table 5.2 contains a selection of reported electrochemical techniques utilising Au electrodes for the determination of total inorganic As. A US-EPA method (SW-

486 series method #7063), ASV on Au plated GCE, 1996) also exists for the determination of As in aqueous samples and soil extracts.

Table 5.2 Electro-analytical techniques using Au electrodes for total inorganic As

Method	**Working electrode**	**Reductant**	**Reference**
CCSA	Au plated carbon paste	□-cysteine	Svancara *et al.* (2002)
SWCSV	HMDE + Cu^{2+}	Thiosulfate	Ferreira & Barros (2002)
SWASV	HMDE + Cu^{2+}	KI/ascorbic acid	Barra *et al.* (2001)
CCSA	Au fibre	KI/HCl	Hua *et al.* (1987)
CSV	HMDE + Cu^{2+}	KI/ascorbic acid	Kowalska *et al.* (2001)
SWASV	Au UME array	Sodium sulfite	Feeney & Kounaves 2002
DPASV	Au plated GCE	SO_2 (g), N_2H_4	Sun *et al.* (1997)
ASV	Rotating Au solid disk	SO_2/N_2/ascorbic acid	Esteban *et al.* (1994)
DPASV	Au film plated GCE	Na_2SO_3	Rasul *et al.* (2002)

These methods provide a cost-effective approach to speciation compared to standard methods such as ICP-MS and GHAA. Au is the most suitable electrode material for As due to high hydrogen over-voltage and improved reversibility of the electrode reactions which results in higher and sharper stripping peaks (Billing *et al.*, 2002).

However, Au working electrodes can suffer from memory effects, limited sensitivity and poor precision. As discussed in Section 5.1.1 time-consuming surface regeneration procedures, or surface modification schemes are required to obtain reliable quantitative data. Consequently, few suitable *in situ* electrochemical methodologies have been reported for the trace analysis of As and no commercially available portable instruments that can be routinely used for rapid field screening of As exist (Feeney and Kounaves, 2002).

Huang and Dasgupta (1999) developed a portable instrument utilizing a flow cell, ASV and a renewable gold film on a Pt wire electrode to measure As^{3+} and total As. The instrument was packaged into a wheeled carrying case (60x24x42 cm). A LOD of 0.5 $\mu g\ l^{-1}$ was achieved using a t_{dep} of 80 s. An As peak depression of 50% was recorded in the presence of 20-fold excess of Cu and continued to decrease with increasing concentrations of Cu. This was attributed to the formation of the intermetallic compound Cu_3As.

Feeney and Kounaves (2002) have reported an electrochemical technique utilising micro-fabricated Au arrays (AuUMEA), applicable for use in field portable or *in situ* instrumentation. An LOD at or near to 0.1 μg l^{-1} was achieved. Interference studies showed Cu had the most deleterious effect on the As stripping response, decreasing the peak of a 100 μg l^{-1} As sample by 50% when present at 50 μg l^{-1} level. Using the method of standard addition, results obtained for the analysis of groundwater samples compared well with ICP-MS data.

These instruments have demonstrated the feasibility of trace determination of As in water samples, although the procedures are complicated, requiring surface regeneration or complex and expensive fabrication techniques. Simple, rapid and robust techniques enabling field measurements by semi-skilled personnel would be advantageous.

The requirement for rapid *in situ* screening tools for the detection of Hg and As is intensifying due to the continued contamination of drinking water throughout the world. For example, in Bangladesh it has been estimated that up to 40 million people are currently exposed to the risk of As poisoning (Tanabe *et al.*, 2001). Arsenic contamination of drinking water has also been found in many other countries including India, England and Thailand and in the United States in California (Feeney and Kounaves, 2000). Hg contamination is similarly widespread and can also be directly ingested by humans through the accumulation in fish populations.

To realise a screening tool that can provide a rapid and cost-effective semi-quantitative determination for As and Hg, an alternative Au electrode was sought. Unlike other electrodes, whose response is strongly dependent on previous electrode usage, pre-treatment and oxide film formations, single use disposal electrodes are not affected by past analyses. Screen-printing provides a convenient and cheap technology for the fabrication of one shot sensors, as discussed in Section 1.7.4.

The use of disposal Au SPEs would significantly reduce the complexity inherent in previously reported electrochemical procedures, while maintaining low fabrication and experimental costs. Susmel *et al.*, (2002) reported on a screen printed Au electrode for the detection of food pathogens. An Au conductive ink (Agmet, Reading, UK) was screen-printed onto ceramic substrates. The electrodes were fabricated in batches of ten and required curing at 900 °C for 30 min. Masawat *et al.*, (2003) reported on an electrode strip whose working electrode was modified with a sputtered Au layer applied with a SEMPREP 2 sputter gold coater (Nanotech, England). Although these methods provide single use sensors, the need for large scale production make them less attractive in terms of ease and cost of fabrication.

The novel aspect of the sensor presented here is the use of a new Au ink, which can be cured at a low temperature (130°C). As a consequence, the ceramic plates currently used in the screen printing process may be replaced with disposable, cheap, plastic substrates. Up to 100 electrodes may be printed using a single polyester sheet, as opposed to the mentioned single electrode techniques presently used. This has obvious implications regarding the feasibility of sensor mass production, due to the concomitant reduction in fabrication costs and hence in the overall cost of analysis. Furthermore, no surface modification or subsequent electrode adulteration to facilitate metal analyte depositioning is required. A patent has been submitted for this work (Appendix A1).

Studies focused on optimising the experimental conditions required for the single scan analysis of As^{3+}, As^{5+} and Hg^{2+}. Au SPEs fabricated using three types of insulating ink were evaluated and Key DPASV parameters optimised. Calibration curves and limits of detection (LOD) were established and the simultaneous analysis of As^{3+} and Hg^{2+} was evaluated. As^{5+} determination was carried out following pre-reduction using cysteine, as will be described in this chapter.

The most problematic interferent, which can severely hinder the applicability of many electrochemical techniques to the analysis of Hg and As, is from Cu^{2+}. Therefore, the extent of Cu^{2+} interference was also investigated. When analyte peak overlap is evident, multivariate calibration techniques, such as Partial Least Squares, can be used to extract individual analyte responses from the voltammetric response of aqueous mixtures of metals. Thus, a preliminary evaluation of this technique with respect to Cu^{2+} interference was also conducted. Finally, the Au SPEs were examined for their applicability in detecting other environmentally relevant metals, including Cd and Pb.

5.2 RESEARCH METHODOLOGY

5.2.1 Reagents and preparation of standard solutions

Unless otherwise stated, all reagents used were of Analar grade, with minimal impurities, purchased from BDH (Poole, England), Sigma Chemical Company (Poole, England), and Fisher Chemicals (Loughborough, England). All solutions were prepared using high purity grade water (HPLC). Glassware was soaked overnight in a 10% v/v nitric acid solution, rinsed with HPLC grade water, then oven dried at 80°C.

A 1000 mg l^{-1} As^{3+} stock solution was prepared by dissolving 0.1320 g of arsenic trioxide in 1 ml of 25% v/v sodium hydroxide (NaOH), immediately acidified with 2 ml concentrated HCl (30% w/w) and diluted to 100 ml using HPLC grade water. Standards of As^{5+}, Hg^{2+}, Cu^{2+}, Cd^{2+} and Pb^{2+} were commercially available as 1000 mg l^{-1} stock solutions. By dilution of these stock solutions, test solutions were freshly prepared in either 2 or 4 mol l^{-1} HCl (As) and 0.1 mol l^{-1} KCl (Hg^{2+}) before use. As^{3+} standards were stabilised by adding 0.001 mol l^{-1} hydrazinium chloride as recommended by Svancara *et al.*, (2002). To reduce As^{5+} to As^{3+}, 5 g of *L*-Cysteine was dissolved in 25 ml HPLC grade water, acidified with 6.25 ml HCl (32% v/v) and diluted to 100 ml using HPLC grade water. 0.1 ml of this solution was then added to the As^{5+} test solutions (10 ml) and heated for 10 min at 75 °C (while shaken occasionally) prior to analysis. A 0.1mM potassium ferricyanide ($K_3Fe(CN)_6^{3-}$) solution, prepared in 0.1 mol l^{-1} KCl was used for cyclic voltammetry investigations.

5.2.2 Fabrication of gold screen-printed electrode

The Au SPEs were produced by Du Pont (UK) Ltd (Bristol, UK) using the Cranfield specifications and design illustrated in Figure 5.1. The SPEs were fabricated using HT5 heat stabilised and surface treated polyester sheets of thickness 125 µm. Inks used include 7105 carbon electrode, 5874 Ag/AgCl electrode, 7106 E93335-92D Au electrode and 5018 insulator (Du Pont, Bristol, UK). Alternative insulating inks tested include 5036 and 7165. All inks were printed with a 77T, 45 degree mesh screen with a 20 µm emulsion (Du Pont, Bristol). SPEs were dried in a fan assisted box oven at 130 °C for 15 mins (except 5018, UV cured at 500 mJ cm^2).

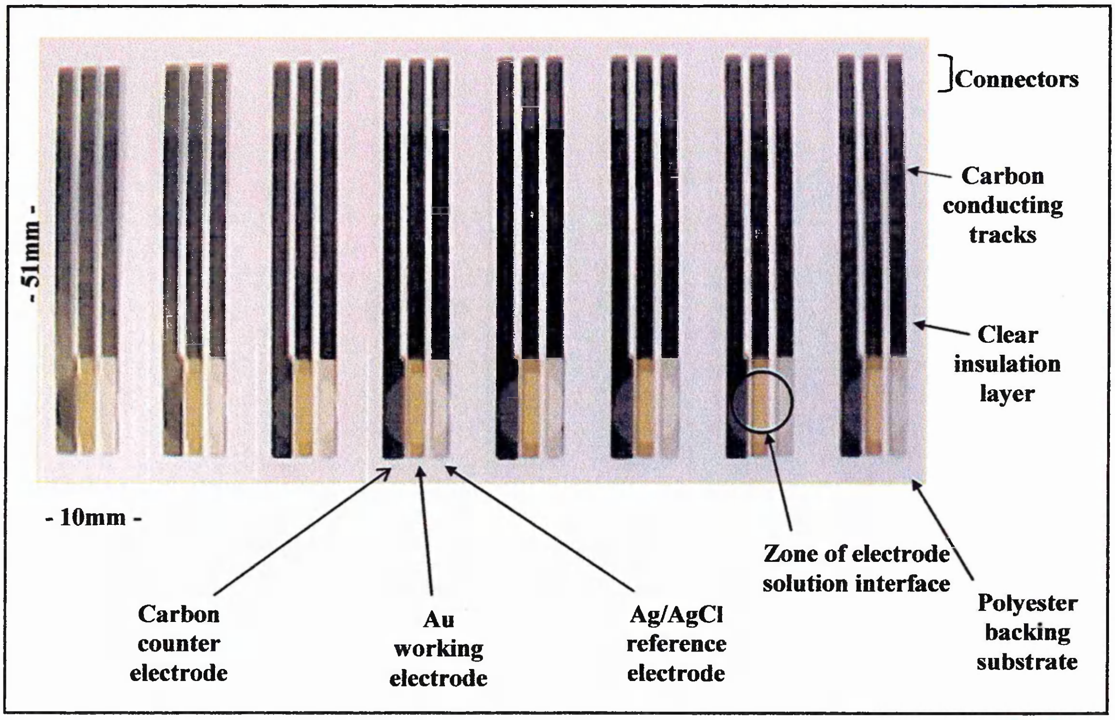


Figure 5.1 Au SPE design.

5.2.3 Electrochemical measurements

Electrochemical measurements were performed at ambient temperature, using a computer controlled potentiostat-10, Autolab Electrochemical Analyser with a general-purpose electrochemical software operating system (GPES) (Ecochemie, Utrecht, Netherlands).

The Au SPE was connected to the GPES, a 100μl sample droplet pipetted onto the working area of the SPE and DPASV analysis performed in unstirred and non-dearated solution. Varying the deposition time (t_{dep}) potential (E_{dep}) identified the optimal key stripping parameters for As^{3+}, As^{5+} and Hg^{2+} (Section 5.3).

Conventional electrodes used for comparative analysis included a circular Au disk working electrode (1.6mm diameter), Pt wire counter electrode (0.5mm diameter, 7.5cm long), and a Ag/AgCl reference electrode, purchased from BAS Technicol (Congleton, England). The Au working electrode was polished every three experiments, using a HPLC grade water slurry of 0.3 μm alumina on a disc pad and rinsed thoroughly with water.

Au SPEs fabricated using alternative insulator inks were compared using cyclic voltammetry. The potential range was set at -0.2 V to 0.5 V. The electrochemical behaviour of $K_3Fe(CN)_6^{3-}$ on the SPE was studied by varying the scan rate (v) between 10-150 mV s^{-1}. For each scan rate, 10 complete cycles were performed, in order to establish equilibrium in the system, the peak height of the anodic (oxidation peak) current of the tenth scan was taken as the equilibrated maximum peak current. The *Randles-Sevcik* equation was used to calculate the Au working electrode area.

5.2.4 Data analysis

All experimental/exploratory stripping analysis was repeated three to five times using new SPEs for each measurement. All data reported represent the mean of three replicate measurements, unless otherwise stated.

The relative standard deviation (RSD), expressed as a percentage, was used as a measure of assay precision. The correlation coefficients (r), slopes (b) and intercepts (a) of the calibration curves were obtained by employing regression and correlation analysis using Microsoft Excel.

5.3 DATA ANALYSIS AND COLLECTION

5.3.1 Characterisation of Au SPE

Preliminary cyclic voltammetry investigations using a 0.1 mol l^{-1} $K_4Fe(CN)_6$ showed clear reversible electrochemical behaviour using the new curable Au ink (Figure 5.2-5.4). All sensors exhibited the expected symmetrical shape characteristic of a reversible redox reaction, with an increase in peak currents (I_p) with increasing scan rate (v). At low v (20 mV/s), the anodic to cathodic peak separation (ΔE_p) was approximately 100 mV for sensors fabricated using A1 or A2 insulating ink, and 130 mV using the sensor fabricated using A3 insulating ink. The Au disk electrode yielded a ΔE_p of 70 mV. This suggests the electrochemical reversibility at the Au SPE is less affected by the presence of the polymer binder and other additives used for the printing process, relative to the carbon based SPEs evaluated in Section 2.3.1, where a ΔE_p of approximately 200 mV was observed.

Using the slopes obtained from plots of anodic Ip versus square root of scan rate ($v^{1/2}$) for 0.1mM ferrocyanide, the *Randles-Sevcik* equation was applied and the active surface areas were calculated as; 0.1255, 0.1287 and 0.1203 cm^2 for the Au SPEs fabricated using A1, A2 and A3 type insulating ink respectively. Comparing these values to the overall geometric area of the working electrode of 0.14cm^2 (0.7 x 0.2 cm), suggests *ca.* 85-90% of the printed Au surface is actively used for deposition. This is in contrast to the carbon SPEs investigated in Section 2.3.1 where the calculated active area was approximately 19% of the geometric area. This was attributed to low carbon content and the presence of additional components in the ink. This suggests the Au ink has a high content of Au and may contain less or a different polymeric binder which has minimum effect on redox behaviour of the Au SPE. The Au SPEs were also fabricated using base substrates and insulating inks different to that used for the fabrication of the carbon SPEs.

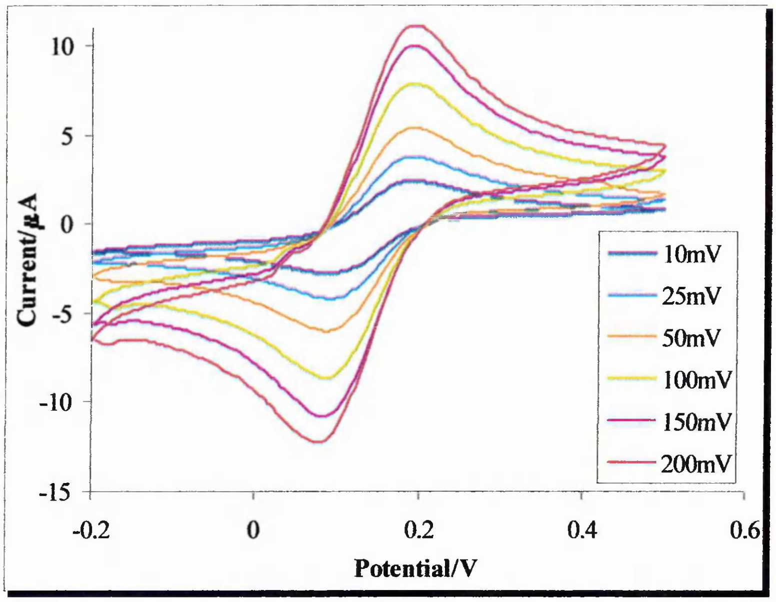


Figure 5.2 Cyclic voltammograms of a 0.1mM $K_3Fe(CN)_6^{3-}$ on Au SPE fabricated using Type A1 insulating ink. Initial potential +0.5V; switching potential -0.2V, scan rate (v) 10-200 mV s^{-1}.

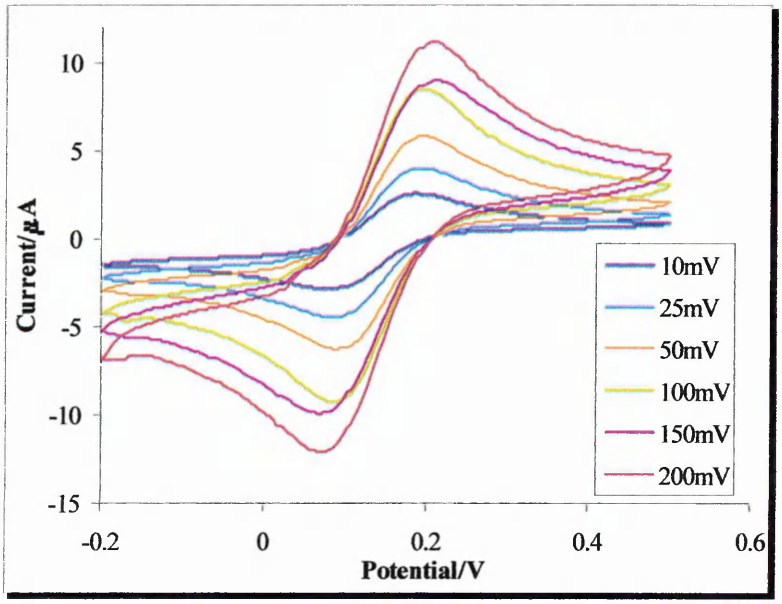


Figure 5.3 Cyclic voltammograms of a 0.1mM $K_3Fe(CN)_6^{3-}$ on Au SPE fabricated using type A2 insulating ink. Initial potential +0.5V; switching potential -0.2V, scan rate (v) 10-200 mV s^{-1}.

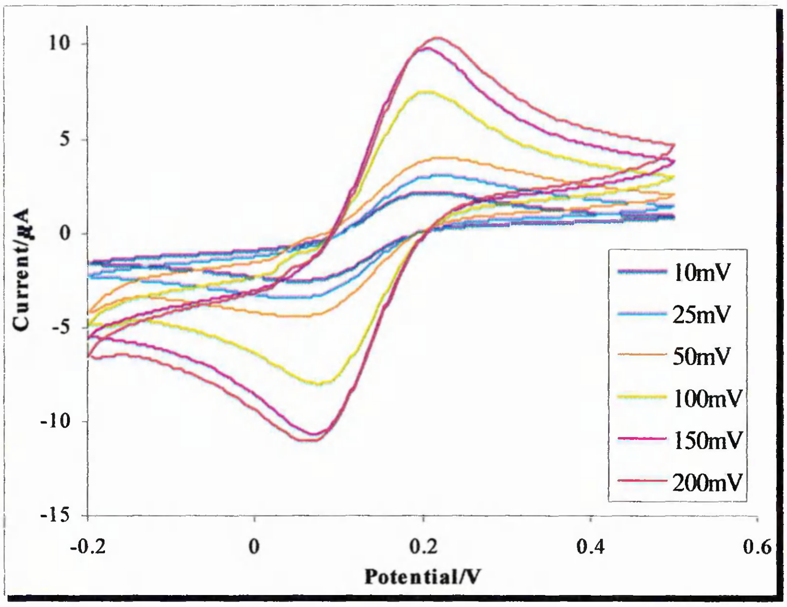


Figure 5.4 Cyclic voltammograms of a 0.1mM $K_3Fe(CN)_6^{3-}$ on Au SPE fabricated using type A3 insulating ink. Initial potential +0.5V; switching potential -0.2V, scan rate (v) 10-150mV s^{-1}.

5.3.2 DPASV analysis of Hg^{2+}

Following Hg depositioning, the electrochemical dissolution of Hg from the Au electrode is strongly influenced by the supporting electrolyte (Bonfil *et al.*, 2000). Initial investigations focused on the optimum supporting electrolyte for DPASV analysis. Gil and Ostapczuk (1994) suggested determination of Hg^{2+} at an Au electrode should involve electrolytes that contain a suitable mineral acid and a complexing agent capable of reducing the strong interaction that can occur between Au and Hg. The recommended electrolyte was a mixture of 0.05 mol l^{-1} H_2SO_4 and 0.02 mol l^{-1} KCl, with the chloride ions as complexant. However, Svancara *et al.* (1997) found a mixture of HNO_3 and KCl produced the optimum signal:noise ratios using carbon pastes electrodes as supports for the Au film.

To minimise hazards associated with very strong mineral acids (H_2SO_4) and to reduce the amount of reagents required for field analysis of a range of analytes (HNO_3 and KCl used in the assay for Cd, Pb, Cu on carbon SPEs) KCl and HNO_3 were selected as the preferred supporting electrolytes. Figure 5.5 shows stripping voltammograms for 100μg l^{-1} Hg^{2+} and mercury-free solutions at different concentrations of KCl.

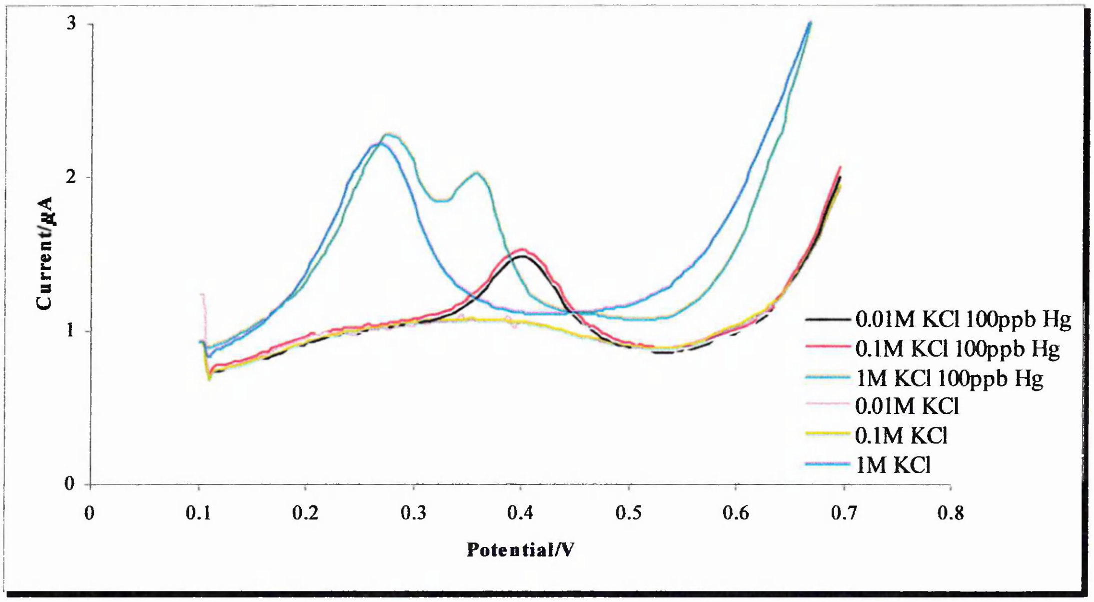


Figure 5.5 Stripping voltammograms of 100 μg l^{-1} Hg^{2+} and blanks in 0.01M, 0.1M, 1M KCl, 1% w/w HNO_3; t_{dep} 30 s; E_{dep} +0.1V; E_{scan}:+0.1V ⇒ +0.7V at 10 mV s^{-1}.

Similar Hg stripping responses at *ca.*+0.4V were observed using 0.01 mol l^{-1} and 0.1 mol l^{-1} KCl. Significant background currents characteristic of the Au surface were evident (Wang and Tian, 1993c). Chloride ions are known to interact strongly with both Au surfaces and Hg species and this is reflected in the high background currents observed (Li *et al.,* 1998). The Au ink also contains additives such as polymeric binders. This contributes to the baseline, similar to that seen using the carbon SPEs discussed in Section 2.3.4.

The Hg peak height increased and shifted to a more cathodic potential in the presence of 1 mol l^{-1} KCl. This is due to the enhanced complexation of Hg and chloride acting as a driving force for oxidation and hence lowering the oxidative potential (Jayaratna, 1997). The background voltammograms containing 1 mol l^{-1} KCl displayed a large interfering peak at approximately +0.25V, which overlapped with the Hg peak.

A possible source of this oxidative peak could be complex formation between Au and a large excess of chloride ions producing auric chloride ion ($AuCl_4$) (Sun *et al.*, 1997). Svancara *et al.* (1997) reported 'a certain drawback of Au electrodes appears to be their considerable sensitivity towards electrolytic oxidation during anodic scanning. In comparison with other electrodes (Pt, carbon) the surface of Au-based electrodes are oxidised more easily. The oxidation gives rise to an increased background signal and other undesirable responses which may result in various complications during electrochemical measurements'.

Ugo *et al.* (2001) also reported 'Au is preferred as it allows the efficient faradaic pre-concentration of metallic Hg as an amalgam. In spite of the high sensitivity and relatively short pre-concentration times, the use of Au electrodes suffers from some limitations, particularly for the analysis of chloride containing samples; in fact during ASV Au itself can be oxidised to give soluble Au^{3+} chloride complexes.

The same experiments were performed using a solid Au disk electrode and are illustrated in Figure 5.6. In all cases the oxidation peaks of Hg^{2+} and background currents shifted to more anodic (positive) potentials by *ca.*1 V. The Hg^{2+} peak in 0.01 mol l^{-1} KCl was similar in size/shape to that using the Au SPE. In 0.1 mol l^{-1} KCl the Hg^{2+} signal was greater and more defined than the signal obtained at the Au SPE. The Hg^{2+} peak was completely distorted at a KCl concentration of 1 mol l^{-1}. Similar characteristics were observed for blank KCl electrolytes using both electrode types. The sharp oxidative peak observed in the presence of 1 mol l^{-1} KCl is evident, although the background current in the region of Hg^{2+} oxidation is more pronounced using the solid Au electrode. This indicates the baseline features exhibited by the Au SPE are inherent to Au electrodes in general, rather than a consequence of the Au SPE itself.

Considering the adequate discrimination between the background and Hg^{2+} currents obtained using the Au SPEs (Figure 5.5), a 0.1 mol l^{-1} KCl was selected for subsequent single DPASV analysis of Hg^{2+}. This would also serve to stabilise the

Ag/AgCl reference electrode and account for any chloride ions present in the sample matrix.

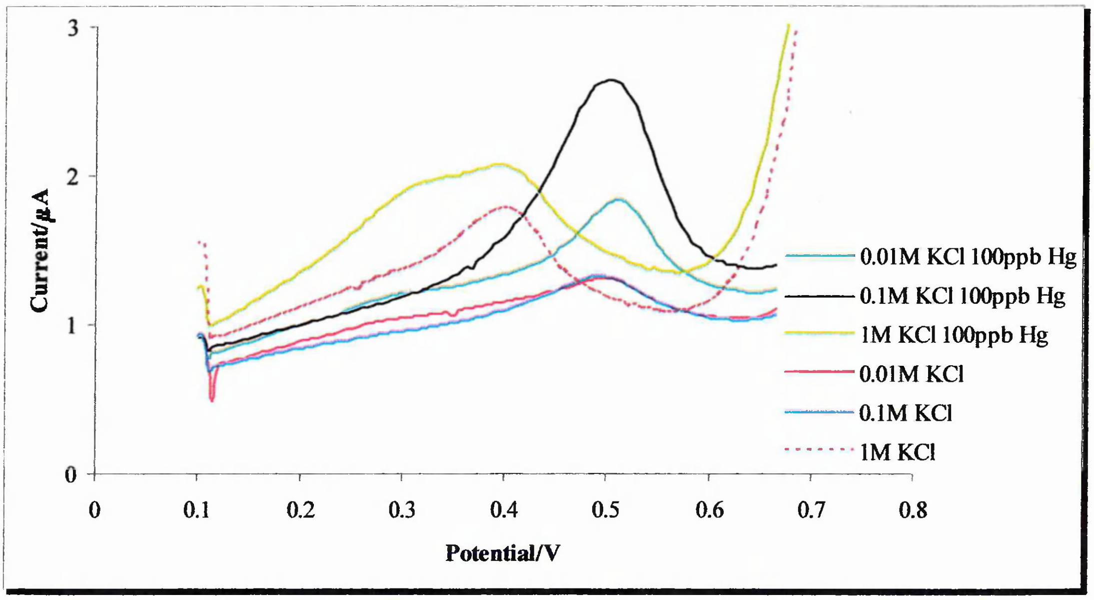


Figure 5.6 Stripping voltammograms of 100 µg l^{-1} Hg^{2+} and blanks in 1% w/w HNO_3 and 0.01M, 0.1M, 1M KCl, at a solid Au disk (vs. Ag/AgCl) t_{dep} 30 s; E_{dep} 0.1V; E_{scan}:0.1v ⇒ 0.7v @ 10mV s^{-1}.

To improve the sensitivity and background characteristics, optimisation of key DPASV parameters for the analysis of Hg^{2+} using Au SPE was performed. For all experiments a 0.1 mol l^{-1} KCl and 1% w/w HNO_3 (pH = *ca.* 1.5) supporting electrolyte was used.

Optimisation of DPASV parameters for measurement of Hg using Au SPEs.

Deposition potential - E_{dep}

Figure 5.7 illustrates the effect of the E_{dep} on the maximum peak current of a 100µg l^{-1} Hg^{2+} solution. The Hg^{2+} I_p and background were greater at more negative potentials and sensor reproducibility was lower. Upon increasing the E_{dep} from -0.1V to 0.2V, the Hg^{2+} I_p and background currents decreased and reproducibility improved. At potentials more positive than +0.2V no response was observed (approaching the Hg^{2+}

peak potential). A potential of +0.2V was considered the most suitable for the pre-concentration and subsequent stripping of Hg^{2+} with respect to sensitivity, background characteristics and minimisation of interferences from co-existing metals, particularly Cu.

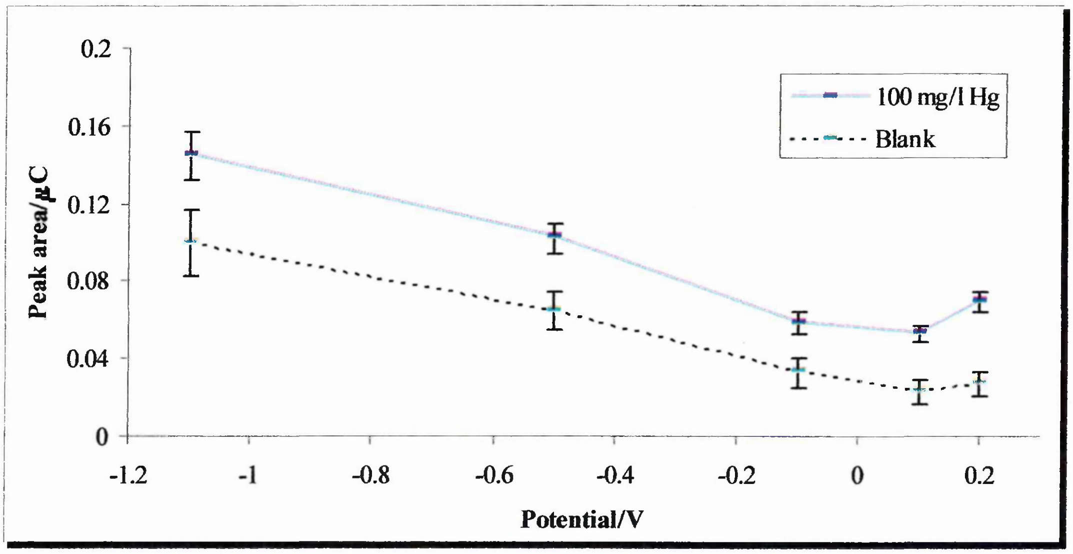


Figure 5.7 Effect of E_{dep} on the magnitude of stripping peak currents at Au SPE for 100µg l^{-1} Hg^{2+} in 0.1M KCl and 1% v/v HNO_3; t_{dep} 30 sec.

Deposition time -t_{dep}

Figure 5.8 shows the effect of t_{dep} on the I_p for a solution containing 100µg l^{-1} Hg^{2+} following deposition at +0.2V. The Hg^{2+} maximum peak current exhibits a linear dependence upon the t_{dep} over the interval of 0-180s, reflecting a proportional increase in the amount of Hg ions being reduced at the Au surface. After 180s, no significant change in the response was seen, which may be due to saturation of the Au film by deposited Hg.

The demands of decentralised operation dictate that the preferred t_{dep} would be no longer than that required to obtain a well-defined stripping peak. Unduly long plating times lead to progressively more complicated voltammograms due to the oxidation of Hg species deposited on the Au surface and the formation of Hg-Au intermetallic compounds (Ugo *et al.*, 2001).

The stripping response obtained after a 30s deposition leads to rapid detection at microgram per litre concentrations. This offers a rapid screening capability allowing 120 analyses per hour on-site following sample preparation. For samples containing higher Hg^{2+} concentrations a smaller t_{dep} time would be necessary.

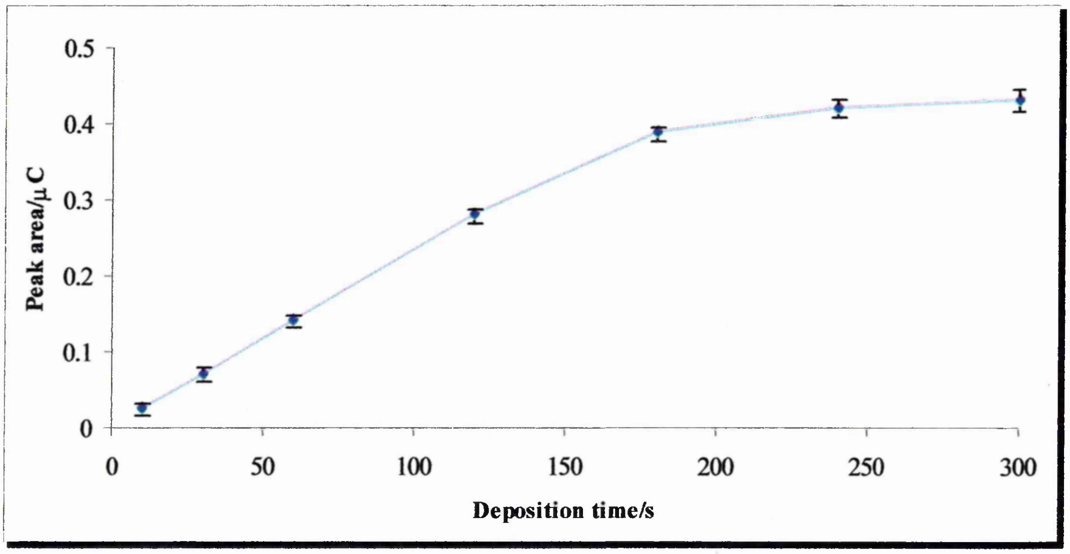


Figure 5.8 Effect of t_{dep} on the magnitude of stripping peak currents at Au SPE for 100μg l^{-1} Hg^{2+}, in 0.1M KCl and 1% v/v HNO_3; E_{dep} +0.2 V.

Following optimisation of assay parameters, improvements were sought by considering alternative insulator inks. As previously discussed (Chapter 2) components used in the fabrication of SPE can affect the redox activity of a particular reaction and also the overall analytical performance of the SPE. Figure 5.9 shows stripping voltammograms of a 100 μg l^{-1} Hg^{2+} and corresponding background traces using three types of insulator ink (A1 type used thus far).

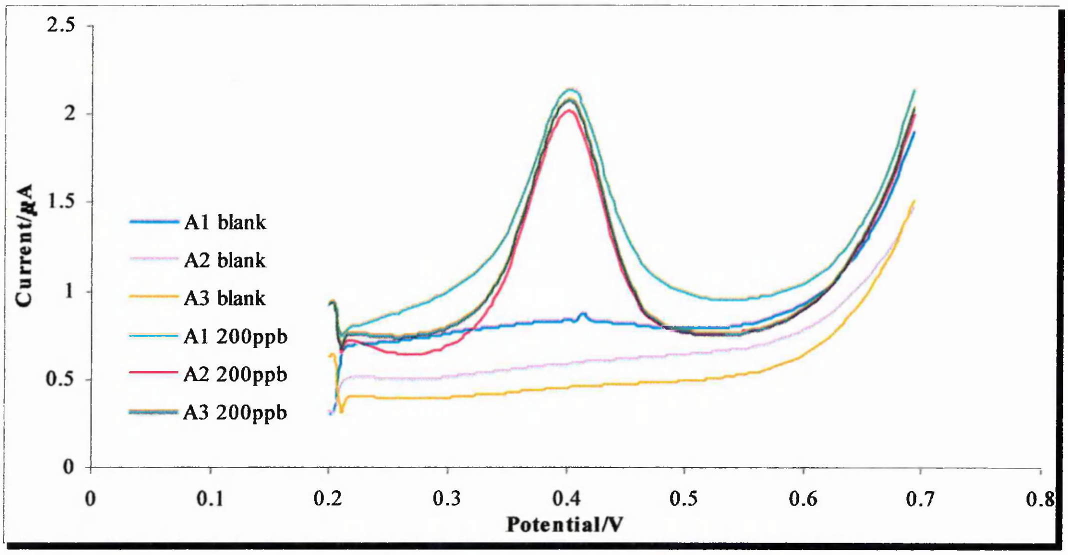


Figure 5.9 Stripping voltammograms of 100 µg l^{-1} Hg^{2+} and 0.1 mol l^{-1} KCl and 1% v/v HNO_3 using Au SPE fabricated using different insulating inks; t_{dep} 30 s; E_{dep} +0.2V; E_{scan}:+0.2v ⇒ +0.7v @ 10mV s^{-1}.

The Au SPE fabricated using A2 type ink resulted in sharper, less broad stripping peaks and adequate baseline characteristics compared with the A1 and A3 inks. The reproducibility using this Au SPE was estimated from a series of 10 successive measurements of a 10 and 100 µg l^{-1} Hg^{2+} solution. Mean I_p values of 0.05µC and 0.0044µC with RSD of 8.4% and 9.1% for 100 and 10 µg l^{-1} Hg^{2+} respectively was achieved using a deposition time of 30s.

Under the optimised conditions; 30s t_{dep}, +0.2V E_{dep} in a 0.1 mol l^{-1} KCl and 1% v/v HNO_3 using Au SPE fabricated with A2 type insulating ink, calibration plots of the Hg^{2+} stripping response versus its concentration were established. Fourteen concentration increments from 0-2000 µg l^{-1} resulted in a linear calibration plot (Figure 5.10) with a correlation coefficient of 0.9971.

However, the peaks became increasingly broad and less well-defined at higher concentrations. A possible cause for this could be due to amalgam formation when higher levels of Hg^{2+} are available for reduction (this would also occur using a prolonged t_{dep}). Bonfil *et al.*, (2000) suggested 'structural changes of the Au surface occur caused by amalgam formation. This, however only applies when bulk deposition of Hg takes place. In ASV, the fractional surface coverage during the deposition step is typically less than 1%. Under such conditions some metal ions form a uniform adlayer, a process referred to as underpotential deposition (UPD).' At higher concentrations an amalgam forms and structural changes affect the peak.

Linearity was maintained in the concentration range of 0-400 μg l^{-1} yielding an r^2 value of 0.9989 (Figure 5.11). An LOD, calculated according to the IUPAC convention (*S/N* = 3) of the blank measurement was calculated as 2.7 μg l^{-1} following 30 s depositioning.

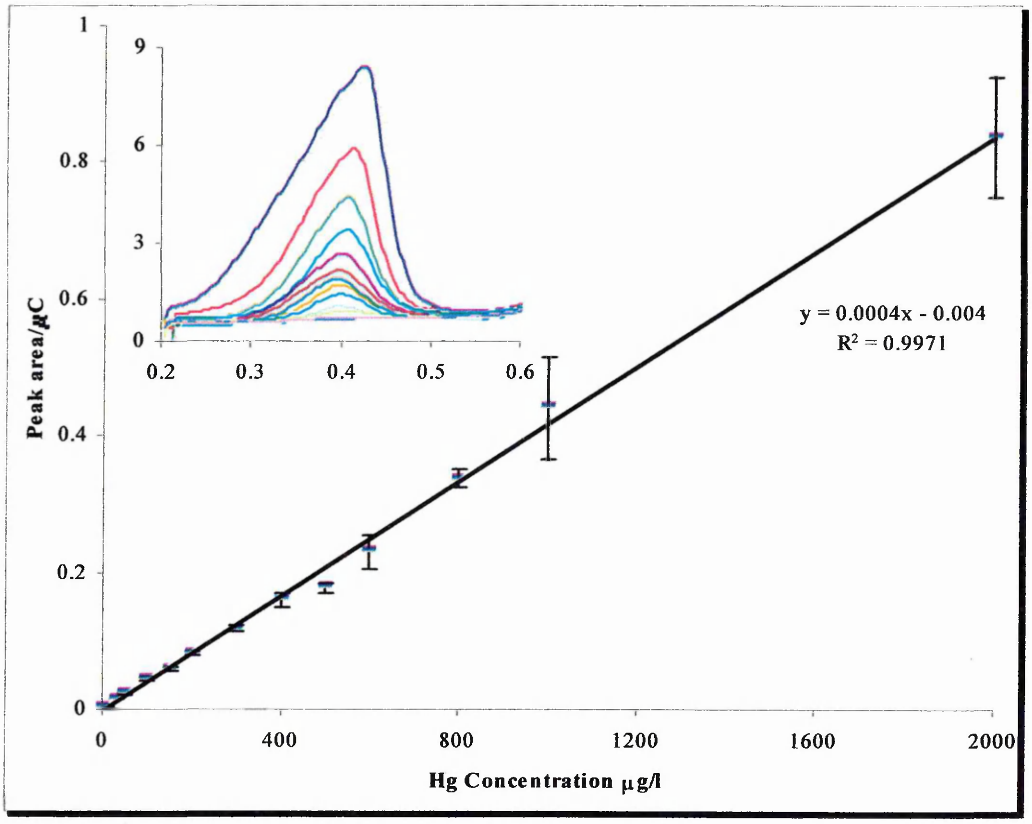


Figure 5.10 Calibration curve and corresponding stripping voltammograms for Hg^{2+} using Au SPEs. Experimental conditions are as defined in Figure 5.9.

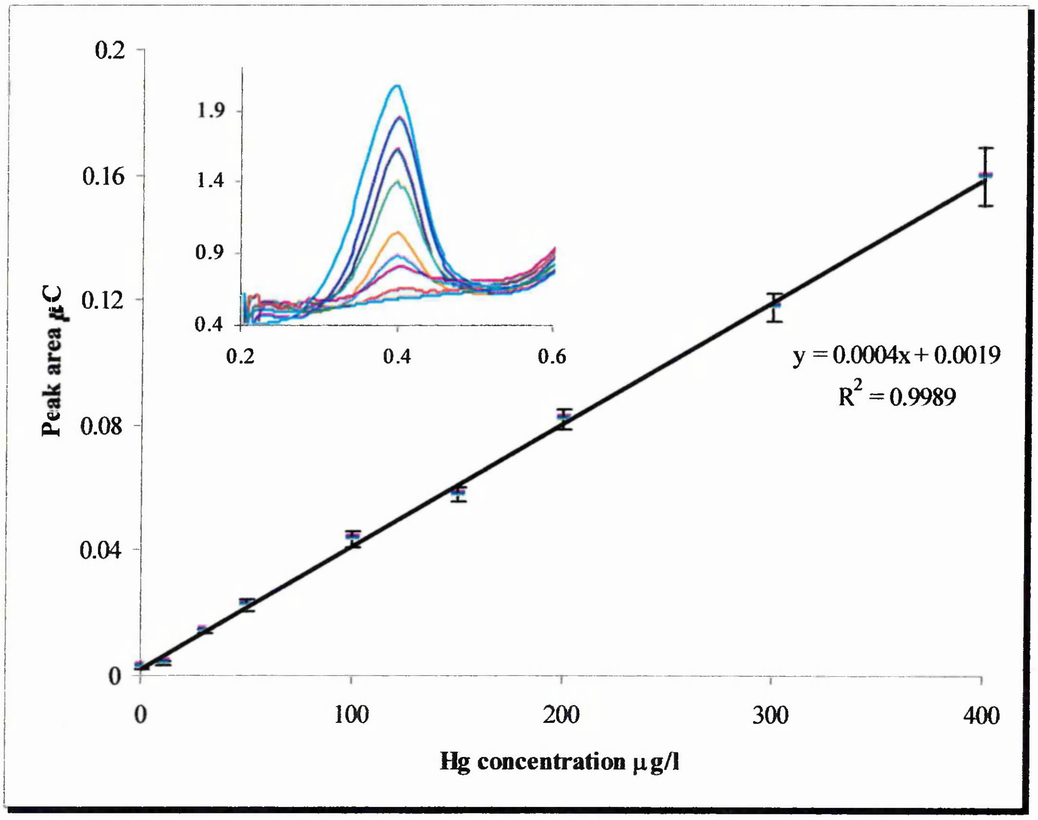


Figure 5.11 Calibration curve for Hg^{2+} using Au SPE's in the range of 0-400 μg l^{-1}. Experimental conditions are as defined in Figure 5.9.

To achieve a lower LOD, the t_{dep} was increased to 120 s. Figure 5.12 Shows linearity was maintained in the range 0-10μg l^{-1}, yielding an r^2 value of 0.9989. An LOD, calculated according to the IUPAC convention (*S/N* = 3) of the blank measurement was calculated as 0.15 μg l^{-1}.

The use of Au SPE has provided a low cost technique for the detection of Hg. The experimental protocol is simple to perform, allowing semi-skilled personnel the ability to rapidly measure Hg in field conditions.

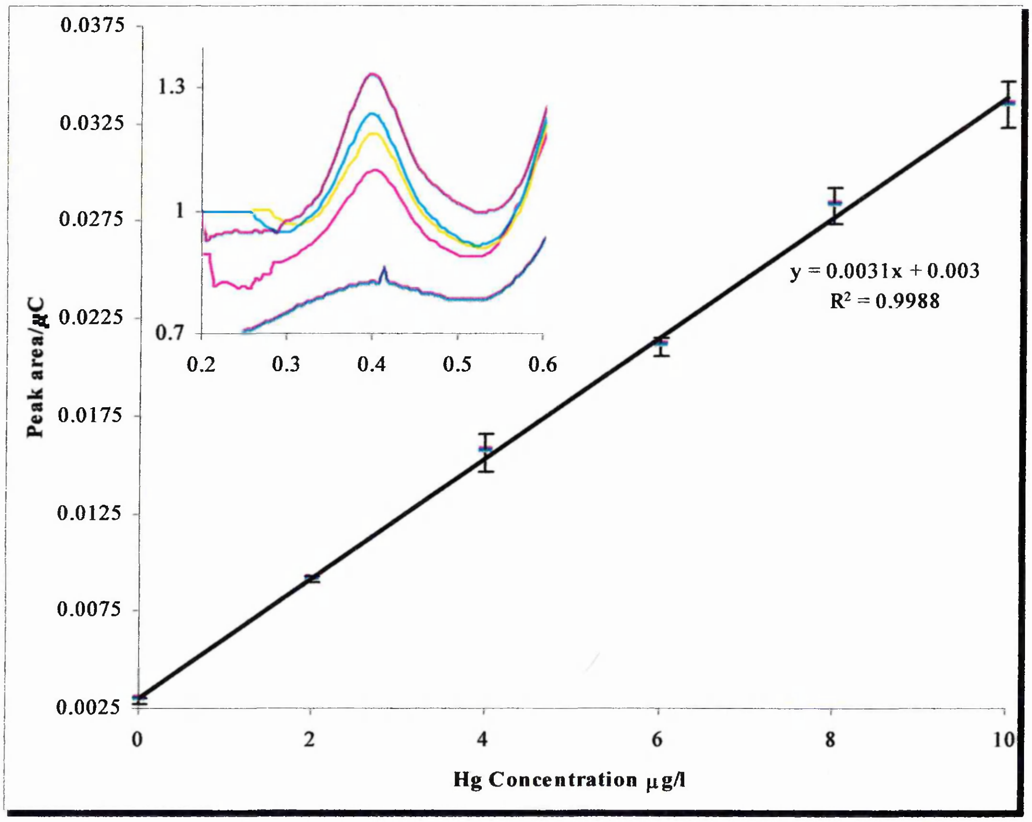


Figure 5.12 Calibration curve for Hg^{2+} on Au SPEs using a t_{dep} of 120 s. All other experimental conditions are as defined in Figure 5.9.

Interference from Copper

The applicability to real sample analysis may be complicated due to the presence of co-existing metals. The most common interference in the voltammetric determination of Hg using an Au working electrode is Cu due to its possible co-deposition (Faller *et al.*, 1999). Figure 5.13 shows stripping voltammograms of single and mixed test solutions of 100 µg l^{-1} Hg^{2+} and Cu^{2+}. A stripping peak for Cu^{2+} was observed at *ca.* +0.2V using a E_{dep} of +0.1V. A less well-defined Hg^{2+} stripping response (as opposed to that observed using a E_{dep} of +0.2V) was obtained when simultaneous analysis of a mixed solution containing equal amounts of Hg^{2+} and Cu^{2+} was performed, due to the co-deposition of Cu^{2+} and Hg^{2+} and the close proximity of their oxidation potentials.

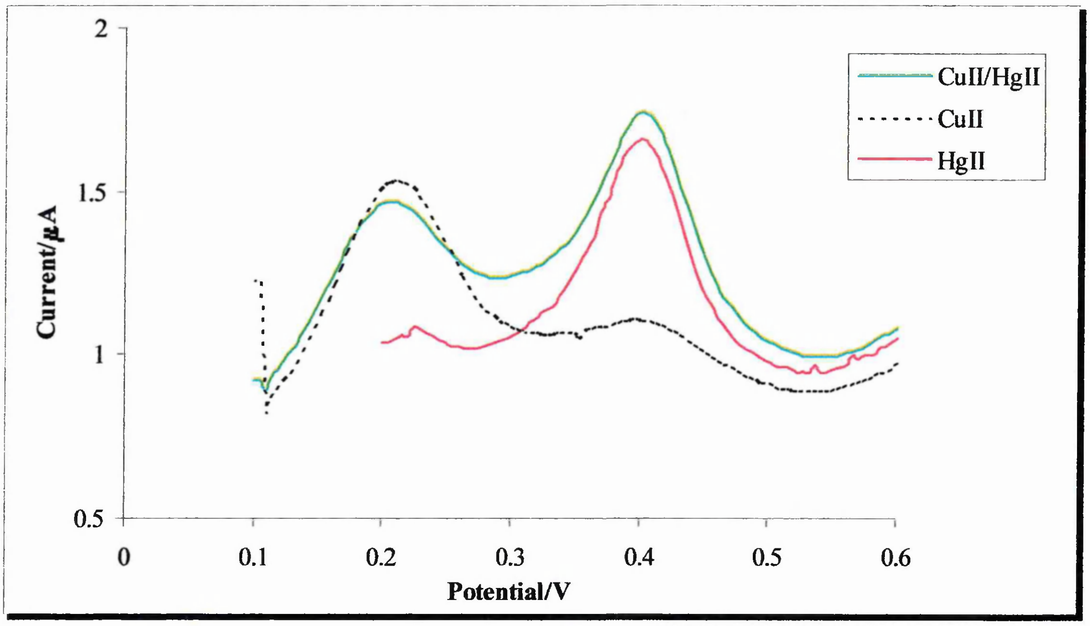


Figure 5.13 Stripping voltammograms of single and mixed test solutions of 100 µg l^{-1} Hg^{2+} and Cu^{2+} in 0.1 mol l^{-1} KCl 1% v/v HNO_3 t_{dep} 30 s; E_{dep} +0.1V or +0.2V; E_{scan}:+0.1V (+0.2V) ⇒ 0.7v @ 10mV s^{-1}.

To eliminate the Cu^{2+} interference, experiments were continued using the optimised E_{dep} of +0.2V. Figure 5.14 shows stripping voltammograms of a 50 µg l^{-1} Hg^{2+} solution containing 0, 1, 10 and 100 mg l^{-1} Cu^{2+}. No significant change in the Hg^{2+} stripping peak shape or oxidation potential was observed in the presence of a 200-fold excess of Cu^{2+} (10 mg l^{-1} Cu^{2+}). Although the Hg^{2+} stripping response became distorted in the presence of a 2000-fold excess of Cu^{2+}, the calculated peak area was not significantly different. Using the GPES operating software calculated Hg^{2+} stripping peaks areas (µC) were 3.62, 3.64, 3.31 and 3.60 x 10^{-8} for solutions containing, 0, 1, 10 and 100 mg l^{-1} Cu^{2+} respectively (Mean=3.54 x 10^{-8}; SD=1.531 x 10^{-9}; RSD=4.3).

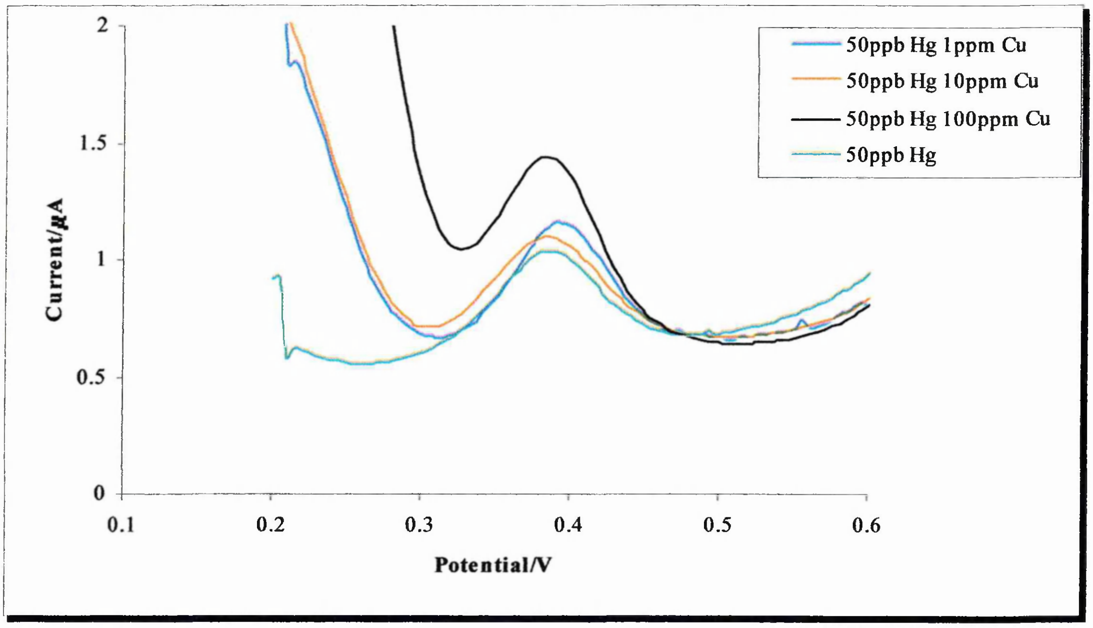


Figure 5.14 The affect of Cu^{2+} (1, 10, 100 mg l^{-1}) on the Hg^{2+} stripping response (50µg l^{-1}) using a E_{dep} of +0.2V; in 0.1 mol l^{-1} KCl 1% v/v HNO_3 t_{dep} 30 s; E_{scan}:+0.2V⇒ 0.7v @ 10mV s^{-1}.

These investigations indicate Hg^{2+} determination in the presence of Cu^{2+} is achievable using a positive E_{dep} of +0.2V at the Au SPEs. When a large excess of Cu^{2+} is present (i.e. in heavily contaminated soils), either a standard addition method or a factor analysis multivariate calibration technique such as Partial Least Squares (Section 5.3.3) may be implemented.

5.3.3 DPASV analysis of Arsenite

To obtain maximum response and optimal background characteristics for measurement of As^{3+} by DPASV, stripping responses of a 500 µg l^{-1} As^{3+} in KCl/HNO_3 and HCl supporting electrolytes were compared. The voltammograms in Figure 5.15 show no significant As^{3+} response was observed using KCl/HNO_3 or HCl below 2 mol l^{-1}. Myers and Osteryoung (1973) studied a number of supporting electrolytes and found HCl produced the best sensitivity and the narrowest peak, suggesting the charge-transfer reaction is fastest in HCl. Saban and Darling (1999) reported 'since the $As^{3+}\longleftrightarrow As(s)$ is a quasi-reversible reaction, a 2 M HCl supporting electrolyte can be used to enhance the electron transfer rate of the reaction'.

A stripping peak was obtained at an oxidative potential of *ca.* +0.16 V using 2 mol l^{-1} HCl. This shifted to more anodic potentials with increased HCl concentrations, with a peak potential of *ca.* +0.24 at 6 mol l^{-1}.

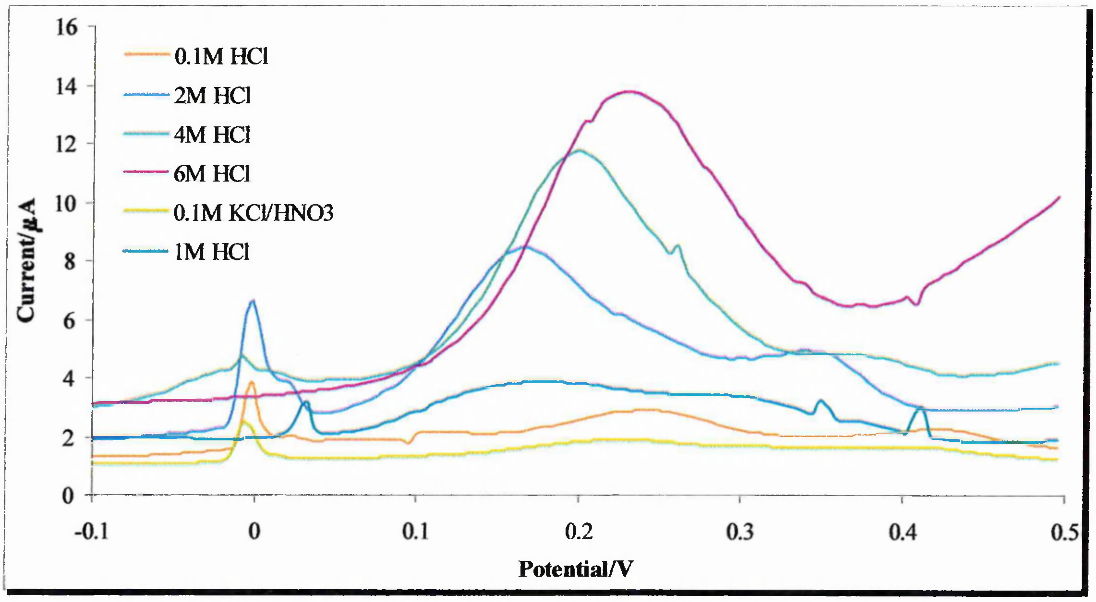


Figure 5.15: Effect of different HCl concentrations on the stripping current of 500µg l^{-1} As^{3+}; E_{dep} -0.3V; t_{dep} 30 sec; E_{scan}:-0.3V ⇒ 0.5V at 20mV s^{-1}; interval time 0.5secs.

To explore the redox behaviour of As^{3+} further the actual species taking part in the electrochemical process was considered. Billing (2002) reported, in an acidic solution, As^{3+} exists as the meta-arsenious acid ($HAsO_2$) and the electrochemical process is as follows:

$$HAsO_2 + 3H^{3+} \rightarrow As + 2H_2O \qquad (1)$$

$$E^0 = +240 \text{ mV}$$

However, the As^{3+} I_p also increased with higher HCl concentrations. According to Arnold and Johnson (1967), the As^{3+} species present in aqueous solutions containing

HCl at increasing concentrations are found to be consistent with the existence of all of the following species:

$$H_3ASO_3, As(OH)_2^-, As(OH)_2Cl, As(OH)Cl_2, AsCl_3 \qquad (2)$$

Since $As(OH)_2Cl$, $As(OH)Cl_2$ and $AsCl_3$ are thought to be the actual species which take part in the electron-transfer reaction on the electrode (Meites, 1954), the deposition efficiency of As^{3+} becomes higher as the amount of these species increases with increasing chloride concentration. In addition, it is possible that more chloride ions tightly bound in the double layer around the electrode, acting as a bridge between the As ion and the electrode (Sun *et al.,* 1997). This results in a more reversible redox reaction, producing increases in peak current and shifts in peak potential.

Huang and Dasgupta (1999) also suggested the arsenic oxidation and reduction are reversible in higher concentration of HCl, due to the formation of $AsCl_3$ rather than AsH_3. It was found the Arsenic stripping peak gets narrower and increases with increasing HCl concentrations from 0.5-6M.

Feeney and Kounaves (2000b) investigated this phenomenon after finding that increasing the chloride concentration by addition of HCl resulted in a significant increase and cathodic shift of the stripping peak. The increase was attributed to an increase in chloride concentration, or a decrease in pH. Since pH does not significantly change when chloride concentration is increased from 0.5M to 2M, the chloride concentration was thought to be the major influence. It was concluded that in HCl, the peaks were more Gaussian shaped, indicative of faster electron kinetics. In a chloride media at concentrations greater than 0.1M, chloride ions strongly complex As to form $AsCl_3$. A low pH and a chloride concentration greater than 0.5M were found to be optimal.

This implies that at low Cl^- concentration As hydrides ($HAsO_2$ H_3ASO_3 AsH_3) are the electrochemical species being deposited at the electrode surface. However, as the chloride ion concentration increases, the dominant species become $As(OH)_2Cl$,

$As(OH)Cl_2$ and $AsCl_3$. This was investigated using a 500μg l^{-1} As^{3+} in 2 mol l^{-1} sulphuric acid (H_2SO_4) supporting electrolyte. No stripping response was observed (Figure 5.16), suggesting chloride ions present in HCl are the controlling factor on the electrochemical response for As^{3+} at the Au SPE.

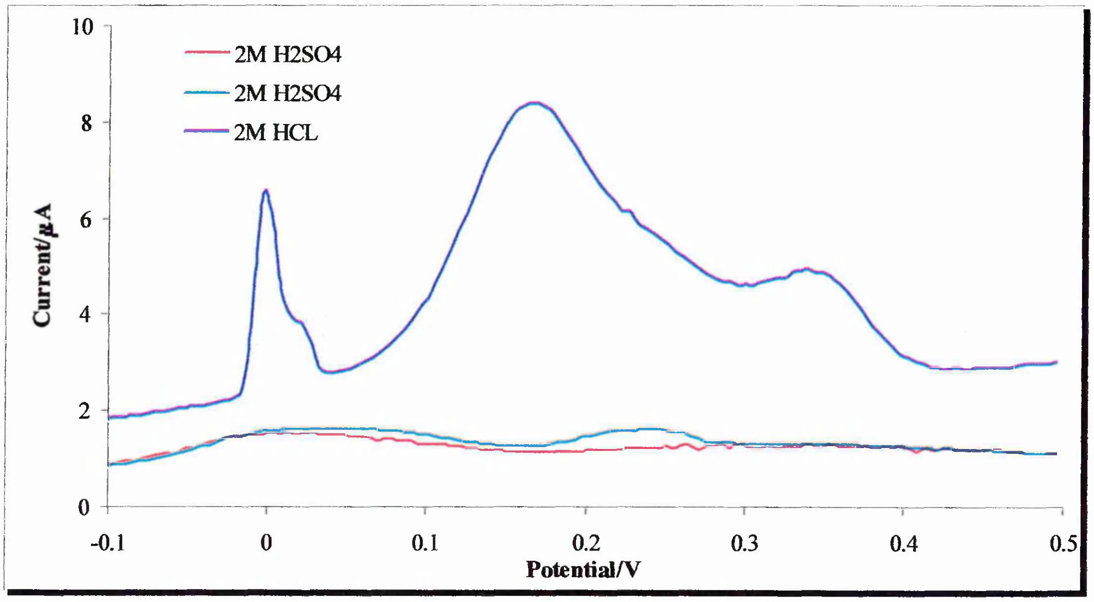


Figure 5.16 Stripping response of 500μg l^{-1} As^{3+} in HCl and H_2SO_4; E_{dep} -0.3V; t_{dep} 30 sec; E_{scan}:-0.3V ⇒ 0.5V at 10mV s^{-1}; interval time 0.5secs.

Background voltammograms displayed in Figure 5.17, show additional redox processes occurring at the electrode surface. These are similar to those exhibited under experimental conditions for the analysis of Hg^{2+} and have been discussed in Section 5.3.2. The peak evident at approximately 0V in Figure 5.15, 5.16 and 5.17 may be a consequence of interaction between Au and Cl^- ions, discussed in Section 5.3.2, as it was not observed using a H_2SO_4 supporting electrolyte (Figure 5.16).

However, the voltammograms displayed in Figure 5.18 show clear discrimination between a blank and 500 μg l^{-1} As^{3+} solution in 2 and 4 mol l^{-1} HCl. The background peak at *ca.* 0.25 V slightly overlaps the tail of the As^{3+} peak in 2 mol l^{-1} supporting electrolyte. This was eliminated in 4 mol l^{-1} HCl, although oxidation peaks at *ca.* 0 V were observed. To improve these voltammograms, key DPASV parameters were optimised.

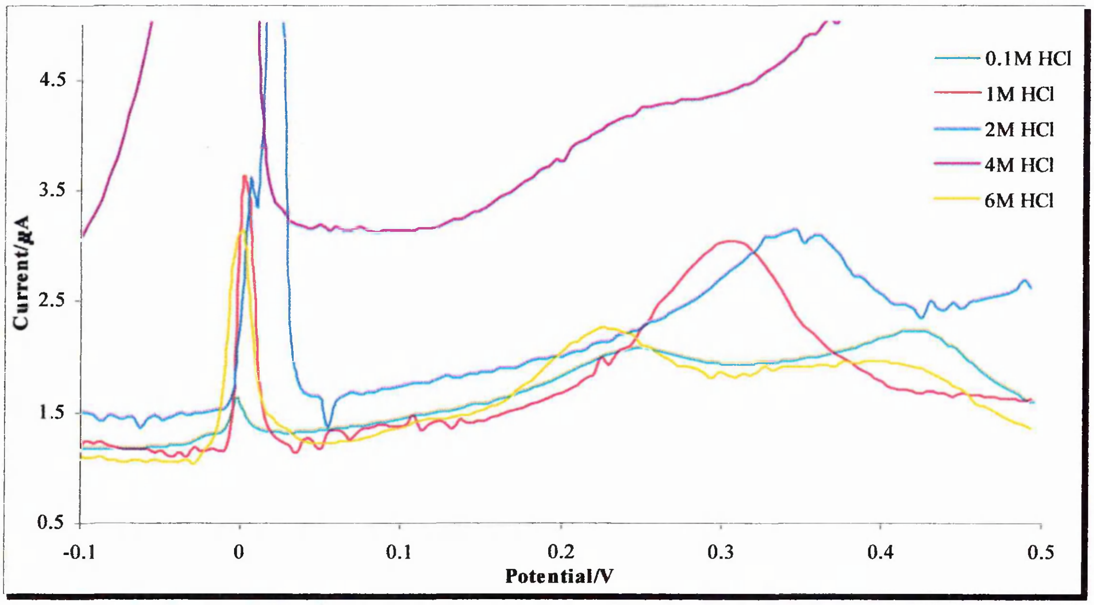


Figure 5.17 Stripping voltammograms of HCl supporting electrolyte on Au SPEs at varying concentrations; E_{dep} -0.3V; t_{dep} 30 sec; E_{scan}:-0.3V ⇒ 0.5V at 10mV s^{-1}; interval time 0.5secs.

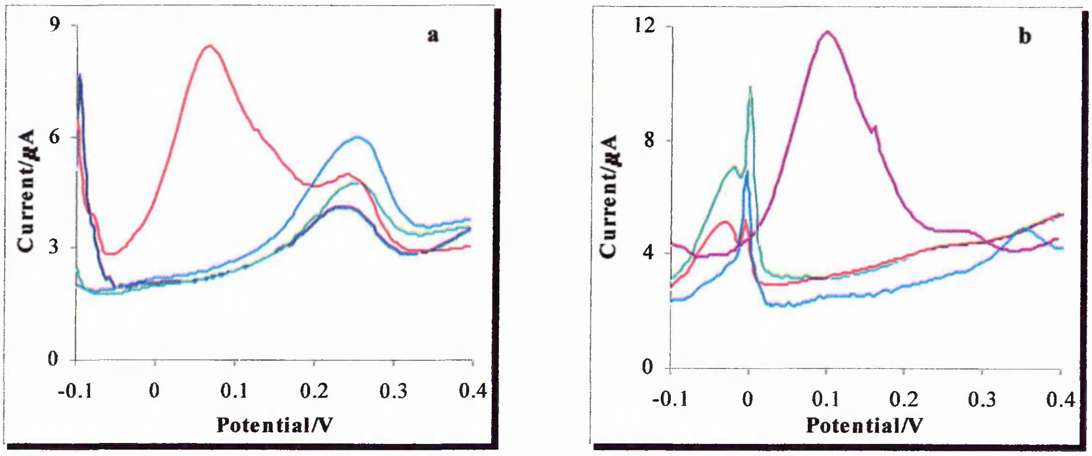


Figure 5.18: Stripping voltammograms on Au SPEs of blank and 500μg l^{-1} As^{3+} in a) 2 mol l^{-1} HCl b) 4 mol l^{-1} HCl; Experimental conditions are as defined in Figure 5.17.

Optimisation of DPASV parameters for measurement of As^{3+} using Au SPEs

Deposition potential - E_{dep}

Experimental data was collected by applying E_{dep} in the range of -0.5V-0.1V to a solution containing 500μg l^{-1} As^{3+}. Each point is the mean of 5 replicate measurements.

Figure 5.19 shows the dependence of the As^{3+} stripping response on the E_{dep} in 2 and 4 mol l^{-1} HCl. The maximum response was seen at 0 V for 4 mol l^{-1} and –0.2V using 2 mol l^{-1} HCl electrolyte. At greater cathodic (negative) potentials, the I_p decreased as the reduction of As^{3+} starts to compete with the reduction of water and the associated production of hydrogen, further blocking the available working electrode surface and decreasing the current (Feeney and Kounaves, 2000b). A more anodic E_{dep} also results in lower I_p values until no significant accumulation of As^{3+} occurs on the electrode surface. A less negative potential is required to accumulate As^{3+} using a 4 mol l^{-1} HCl as the deposition efficiency of As^{3+} becomes higher with increasing chloride concentration, discussed at the beginning of this section.

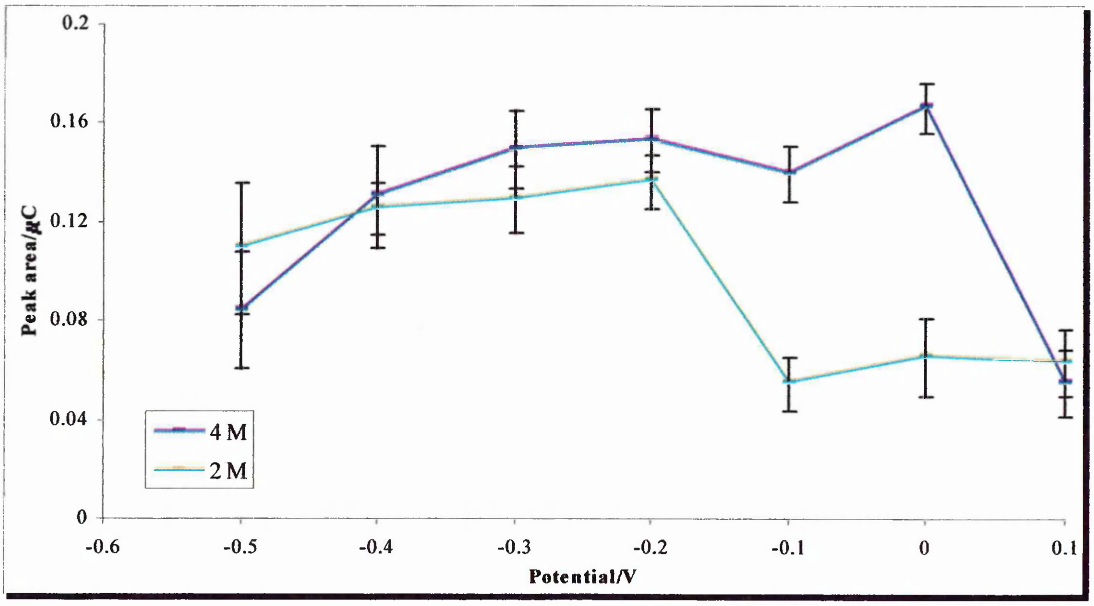


Figure 5.19 Effect of E_{dep} on the magnitude of stripping peak current of 500μg l^{-1} As^{3+}, in 2 and 4 mol l^{-1} HCl; all other experimental parameters are as defined in Figure 5.17.

Deposition time - t_{dep}

Unlike Hg, which forms an amalgam, As does not readily dissolve significantly at the Au film. Extensive deposition of As on the film changes the film resistance and can alter the deposition rate (Haung and Dasgupta, 1999). Therefore the effect of the t_{dep} on the stripping response was investigated using a 500 μg l^{-1} As^{3+} test solution in 2 and 4 mol l^{-1} HCl electrolyte. Figure 5.20 shows the optimum t_{dep} at both HCl concentrations was 30 s, with only a slight increase at 60 s in 2 mol l^{-1} HCl.

Since elemental arsenic is a poor conductor of electricity, the I_p of As^{3+} was found to be limited when the active surface of the electrode was fully covered with elemental arsenic, which is in accordance with the work of Sun *et al.* (1997). The I_p reached a limiting height, indicating the electrode surface saturated with As at a t_{dep} greater than 30s.

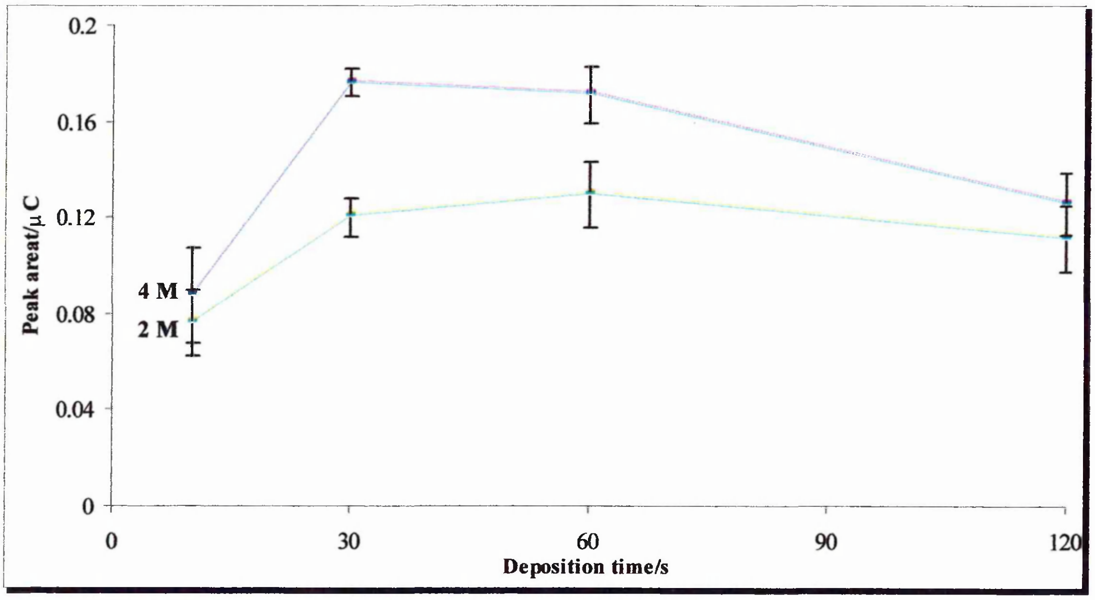


Figure 5.20 Effect of t_{dep} on the magnitude of stripping peak current of 500μg l^{-1} As $^{3+}$, in 2 and 4 mol l^{-1} HCl on Au SPEs; E_{dep} -0.2V (2 mol l^{-1} HCl) 0 V (4 mol l^{-1} HCl); end potential 0.5V; scan rate 20mV s^{-1}; interval time 0.5secs.

This agrees with findings of Feeney and Kounaves (2000b) who reported 'after the first layer covers the electrode surface, the resistance of the monolayer is sufficient to prevent further electron transfer at the applied potential. For a given t_{dep}, the relationship between the t_{dep} and the I_p remains linear until the surface deposition exceeds that of a monolayer. For As, once the monolayer is formed, there is a rigid levelling and eventual decrease in the stripping current due to the insulating properties of As^0.

Background characteristics were examined using the optimised t_{dep} and E_{dep}. Figure 5.21 illustrates previously observed interferences from noisy peaks at *ca.* 0.25 V in 2 mol l^{-1} HCl (Figure 5.18a) and *ca.* 0 V using 4 mol l^{-1} (Figure 5.18b) have been resolved. Although using a 4 mol l^{-1} HCl supporting electrolyte results in better sensitivity and narrower As^{3+} stripping peaks, operating in 2 mol l^{-1} provides adequate discrimination between the background current and As^{3+} response when As^{3+} is present at higher levels.

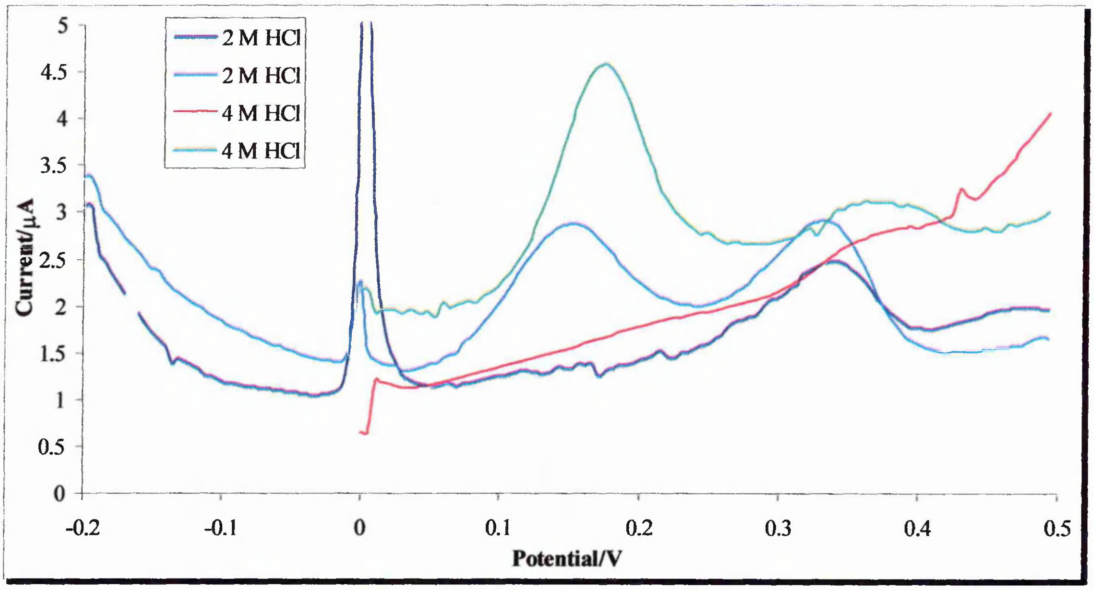


Figure 5.21 Voltammograms of blank and 500µg l^{-1} As^{3+} in 2 (E_{dep} -0.2V) and 4 mol l^{-1} (E_{dep} 0V); t_{dep} 30 sec; E_{scan}:-0.3V ⇒ 0.5V @ 20mV s^{-1}; interval time 0.5secs.

The reproducibility of the As^{3+} stripping response was evaluated from repetitive measurements (n=10) of a 200 and 500 µg l^{-1} As^{3+} solution in 2 and 4 mol l^{-1} HCl. A RSD of 9.11% and 10.1% for 200 µg l^{-1} and 500 µg l^{-1} As^{3+} in 4 mol l^{-1} HCl and 12.95% and 11.5% using 2 mol l^{-1} HCl were obtained under optimised experimental conditions. The RSD values reflect the reproducibility of the DPASV method, the Au electrode-to-electrode precision and the stability of the As^{3+} species in solution.

The stability of As^{3+} at trace levels can be affected due to its oxidation by atmospheric oxygen in solution with a high Cl^- concentration and low pH (Sun *et al.*, 1997). Svancara *et al.* (2002) analysed As^{3+} with and without hydrazinium chloride as a stabiliser. The data proved the instability of As^{3+} at trace concentration (ppb). Noticeable decreases were observed in samples not spiked with a hydrazine salt serving as a stabilizing agent.

Sun *et al.* (1997) also reported a continuous decrease in the I_p during a series measurement of As^{3+} in the same samples. After addition of the antioxidant hydrazinium chloride, the reproducibility was apparently improved; however background currents were elevated as more concentrated hydrazinium chloride was added. A concentration of 1 mmol l^{-1} hydrazinium chloride was found to be optimal.

In contrast Kopanica and Novotny (1998) found when As^{3+} was stored in an acidic supporting electrolyte (1 mol l^{-1} H_2SO_4 and 0.1 mol l^{-1} HCl), the results of ASV determination showed no change of the As^{3+} concentration during 8 h even in the absence of hydrazine.

The reproducibility study was performed with the addition of 1 mmol l^{-1} hydrazinium chloride to the As^{3+} test solutions. A slight improvement in the RSD using 2 mol l^{-1} supporting electrolyte was observed, however no change in the reproducibility was evident at 4 mol l^{-1} over an 8 hr period. No significant adverse affect on the background and stripping response of As^{3+} were displayed using test solutions containing hydrazinium chloride. Since stability may deteriorate when operating in field conditions with 'real' sample matrices, hydrazinium chloride was included in all subsequent investigations.

Under optimised conditions, calibration curves of the As^{3+} stripping response versus concentration in 2 and 4 mol l^{-1} supporting electrolyte were established. Figure 5.22

shows the sensor assay displayed a linear correlation between peak area and analyte concentration up to 800μg l^{-1} in 4 mol l^{-1} HCl (correlation coefficient, $r^2 = 0.99398$). Above this concentration saturation occurs (Figure 5.23). A RSD of ≤10% was obtained (n=6) at each As^{3+} concentration. The LOD, calculated according to the IUPAC convention (*S/N* = 3) of the blank measurement was calculated as 1 μg l^{-1}. The voltammograms illustrated in Figure 5.24 show well-defined stripping peaks for As^{3+} at each concentration increment.

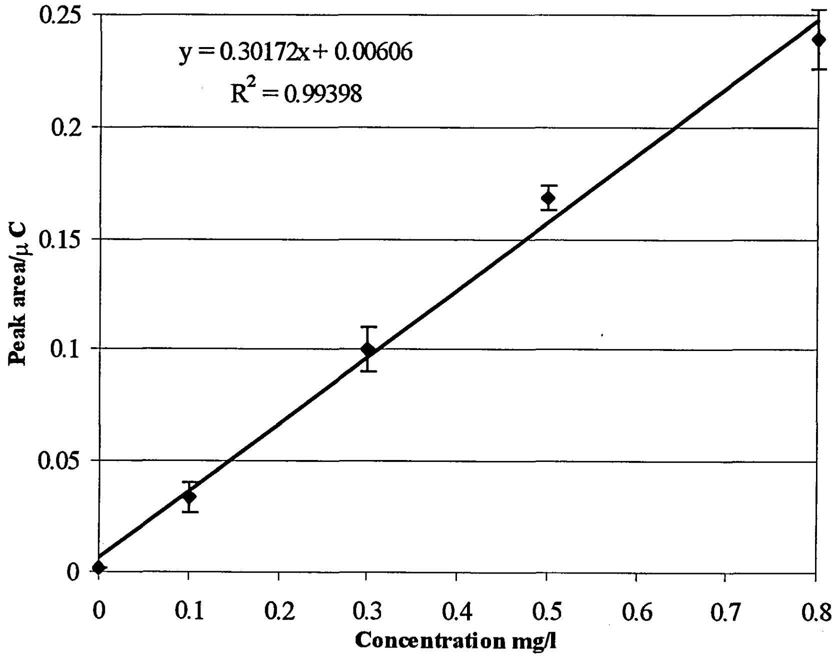


Figure 5.22 Calibration curve for As^{3+} on Au SPEs; in 4 mol l^{-1} HCl, 1 mmol l^{-1} hydrazinium chloride; DPASV parameters: E_{dep} 0V; t_{dep} 30 s; E_{scan}:0V ⇒ 0.5V @ 20mV s^{-1}; interval time 0.5secs.

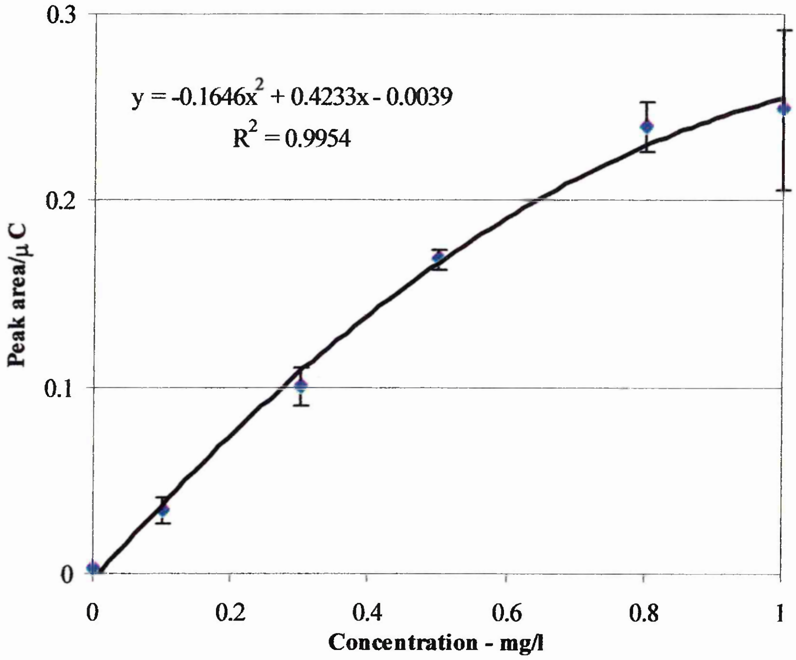


Figure 5.23 Calibration curve As^{3+}on Au SPEs; in 4 mol l^{-1} HCl, 1mmol l^{-1} hydrazinium chloride, showing non linearity at higher As^{3+} concentrations. Experimental conditions are as defined in Figure 5.22.

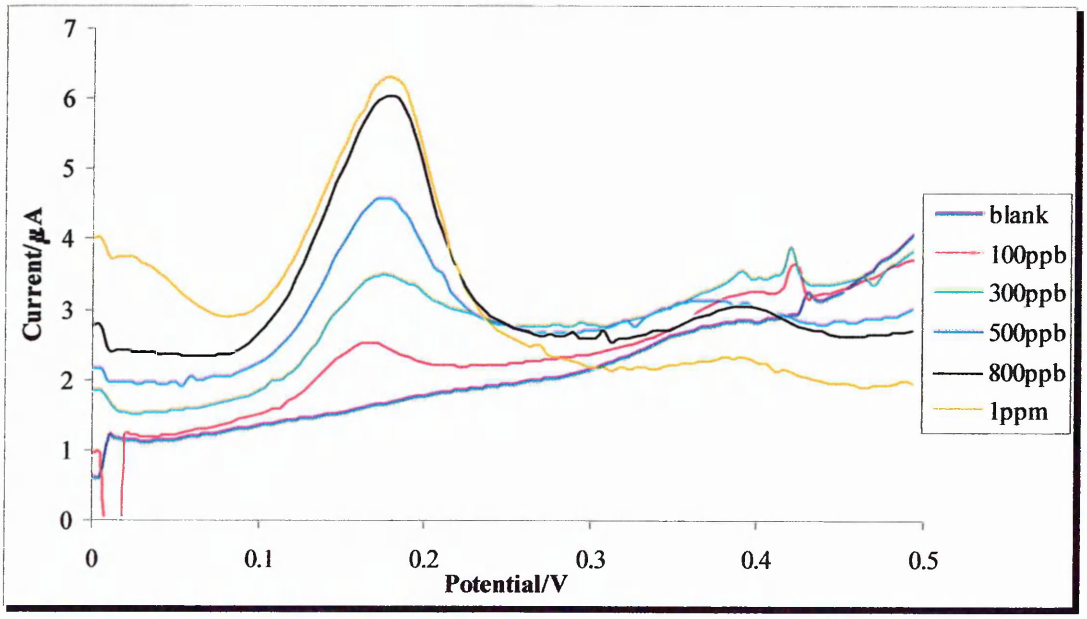


Figure 5.24 Stripping voltammograms of As^{3+} test solutions at Au SPEs in 4 mol l^{-1} HCl; 1 mmol l^{-1} hydrazinium chloride. Experimental conditions are as defined in Figure 5.22.

In 2 mol l^{-1} HCl, the sensor assay yielded a linear calibration plot up to 1 mg l^{-1} As^{3+} with an r^2 value of 0.9930 (Figure 5.25). Increasing stripping peaks were obtained up to 5 mg l^{-1} As^{3+}, however a non-linear relationship (Figure 5.26) was apparent, with the As^{3+} peaks becoming increasingly broad and distorted. A RSD of ≤12% was obtained (n=6) at each As^{3+} concentration in the linear range. The LOD, calculated according to the IUPAC convention (*S/N* = 3) of the blank measurement was estimated as 48 µg l^{-1}. Voltammograms displayed in Figure 5.27 illustrate the stripping peaks are less well-defined in 2 mol l^{-1} HCl, but are adequate when measuring higher As^{3+} levels.

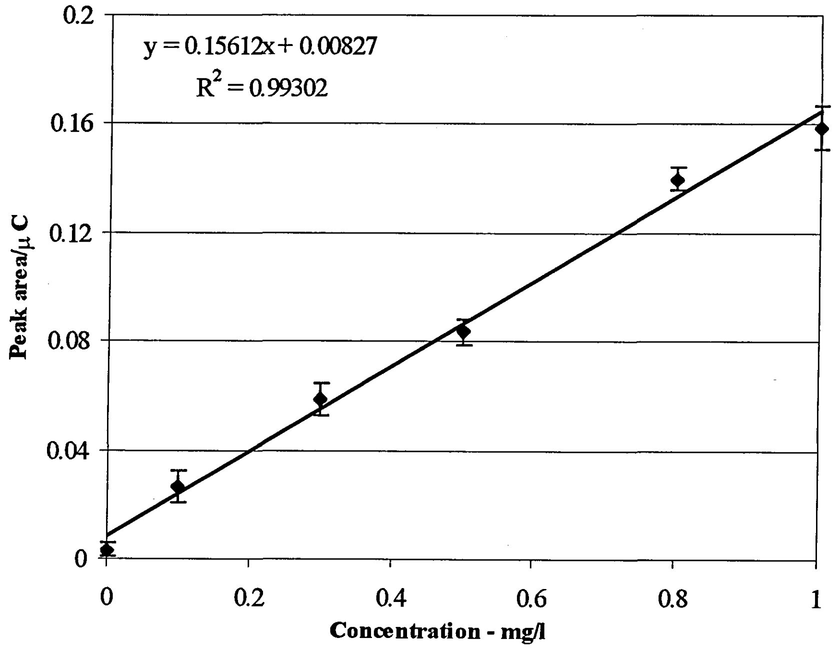


Figure 5.25: Calibration curve for As^{3+} at Au SPEs in 2 mol l^{-1} HCl; 1 mmol l^{-1} hydrazinium chloride; DPASV parameters: E_{dep} -0.2V; t_{dep} 30 s; E_{scan}:-0.3V ⇒ 0.5V at 20mV s^{-1}; interval time 0.5secs.

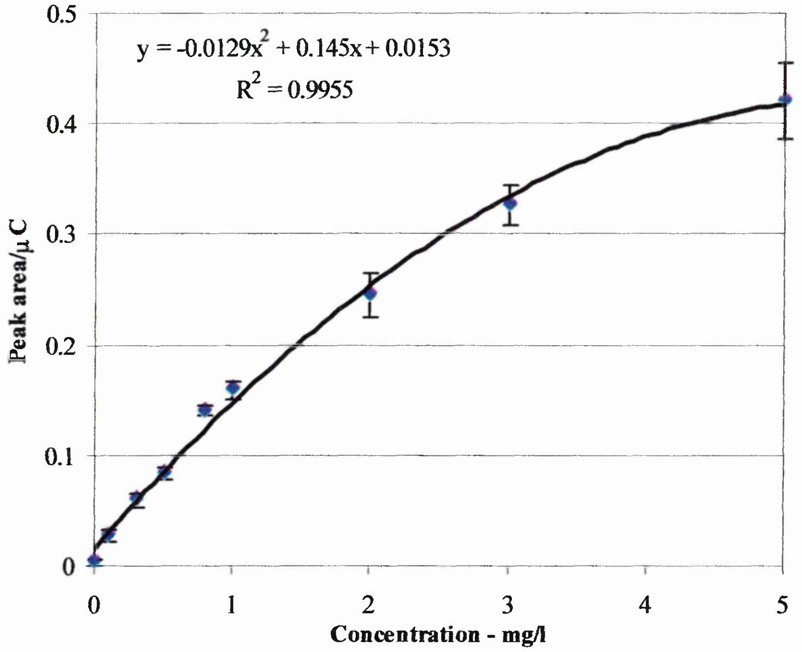


Figure 5.26 Calibration curve for As^{3+} at Au SPEs in 2 mol l^{-1} HCl; 1 mmol l^{-1} hydrazinium chloride, showing non linearity at higher As^{3+} concentrations. Experimental conditions are as defined in Figure 5.25

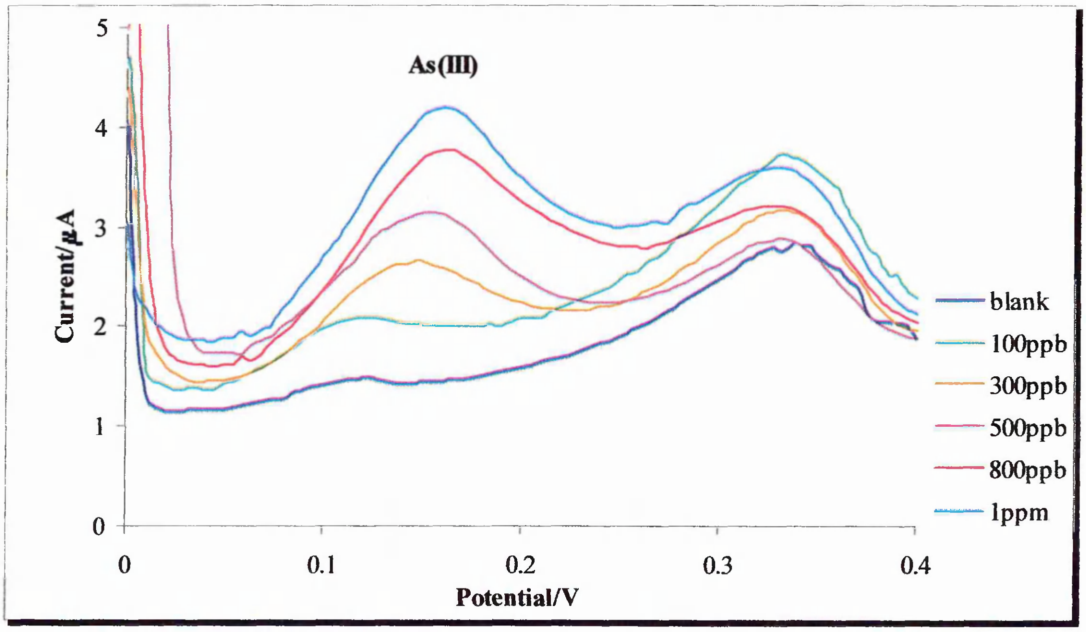


Figure 5.27 Stripping voltammograms of As^{3+} test solutions at Au SPEs; in 2 mol l^{-1} HCl; 1 mmol l^{-1} hydrazinium chloride. Experimental conditions are as defined in Figure 5.25.

Interference from copper and mercury

Studies were performed to examine the effect of interferents upon the stripping signal of As^{3+}. The most common interferents in the voltammetric determination of As using a Au working electrode are Hg^{2+} and Cu^{2+} (Chi Hua *et al.*, 1987). This results from close oxidation potentials relative to As, and the formation of intermetallic compounds at the electrode surface.

To determine the stripping peak potentials of Hg^{2+} and Cu^{2+} single element standards of 500 µg l^{-1} were prepared in 4 mol l^{-1} HCl and DPASV performed using optimised conditions for the determination of As^{3+}. Figure 5.28 shows Hg^{2+} and Cu^{2+} stripped at a potential of *ca.* +0.33V and +0.38V respectively using the Au SPE.

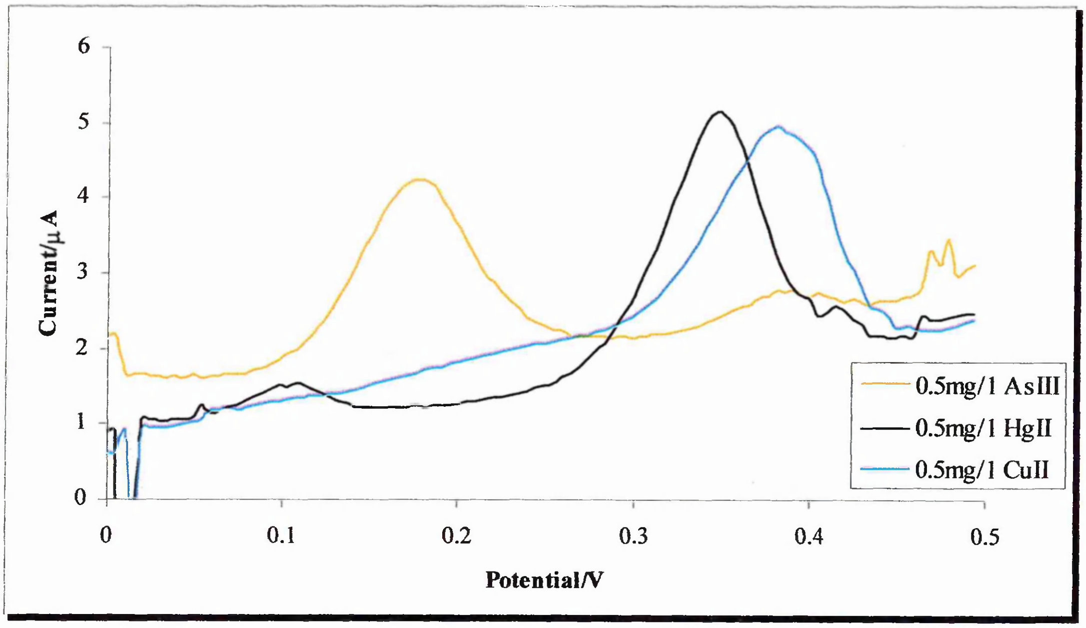


Figure 5.28 Stripping voltammograms of 500 µg l^{-1} As^{3+}, Hg^{2+}, Cu^{2+} in 4 mol l^{-1} HCl; 1 mmol l^{-1} hydrazinium chloride; DPASV parameters: E_{dep} 0V; t_{dep} 30 s; E_{scan}:0V ⇒ 0.5V at 10mV s^{-1}; interval time 0.5secs.

Analysis using mixed test solutions containing 500 µg l^{-1} As^{3+}, Hg^{2+} and Cu^{2+}, illustrated in Figure 5.29 showed when Hg^{2+} and Cu^{2+} were present at the same concentration as As^{3+}, no significant change in the calculated peak area for As^{3+} was evident and the relationship between As^{3+} concentration and stripping response was not affected. However, in the presence of Cu^{2+} the As^{3+} peak shifted potential and became broader.

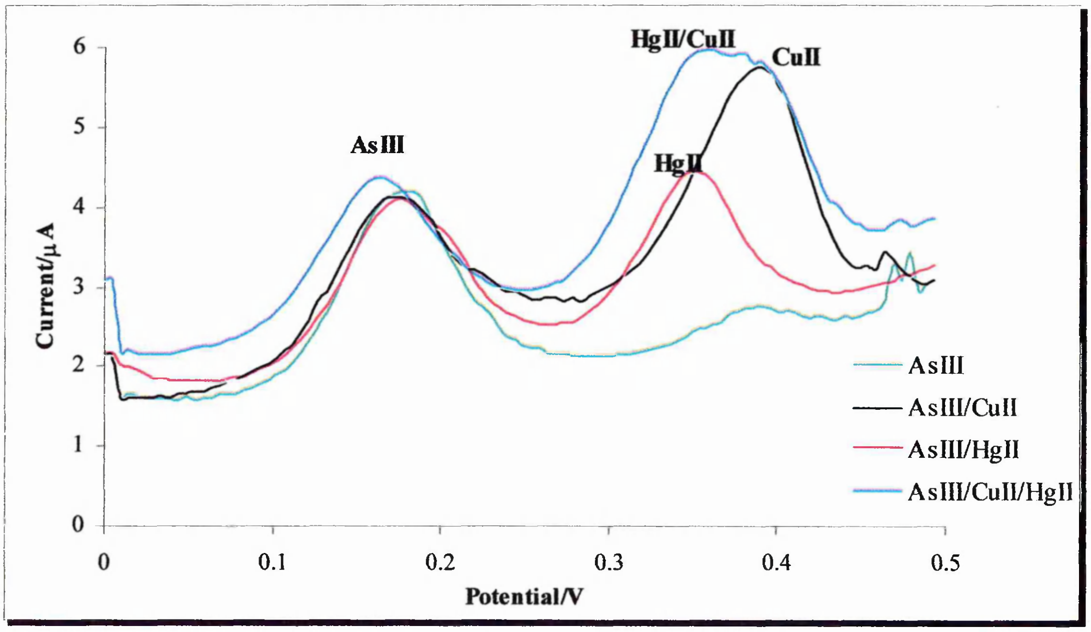


Figure 5.29 Stripping voltammograms of mixed solutions of 500 μg l^{-1} As^{3+}, Cu^{2+} and Hg^{2+} in 4 mol l^{-1} HCl. Experimental conditions are as defined in Figure 5.28.

The situation was slightly different when the solutions were prepared in 2 mol l^{-1} HCl as supporting electrolyte (Figure 5.30). The Cu^{2+} and Hg^{2+} stripping potentials changed order, with Cu^{2+} being stripped more negatively than Hg^{2+} at *ca.* +0.32V. This was also evident when DPASV of Hg^{2+} was performed in 0.1 mol l^{-1} KCl (Section 5.3.2), where the stripping peak for Hg^{2+} peak was more positive than the Cu^{2+} stripping potential. The observed cathodic shift in the stripping peak potential of Hg^{2+} in higher HCl concentrations is due to the greater degree of complexation of Hg^{2+} relative to Cu^{2+} and has served to, at least partially, de-convolute the As^{3+} and Cu^{2+} stripping peaks.

Huang and Dasgupta (1999) also reported this affect and concluded 'at high HCl concentrations Hg^{2+} forms an even stronger association with chloride relative to Cu^{2+} and As^{3+}. Consequently the Hg^{2+} stripping peak appears after the Cu^{2+} peak in 0.5 mol l^{-1} HCl, while the situation is reversed in 6 mol l^{-1} HCl'.

Since the As^{3+} and Cu^{2+} stripping peaks are in closer proximity using 2 mol l^{-1}, the As^{3+} has been severely depressed by the presence of Cu^{2+}. Characteristic broadening of the As^{3+} and Cu^{2+} stripping peaks are due to two overlapping signals where the rising current response indicates the re-oxidation of an intermetallic compound (Svancara *et al.*, 2002). Further investigations were continued using 4 mol l^{-1} HCl.

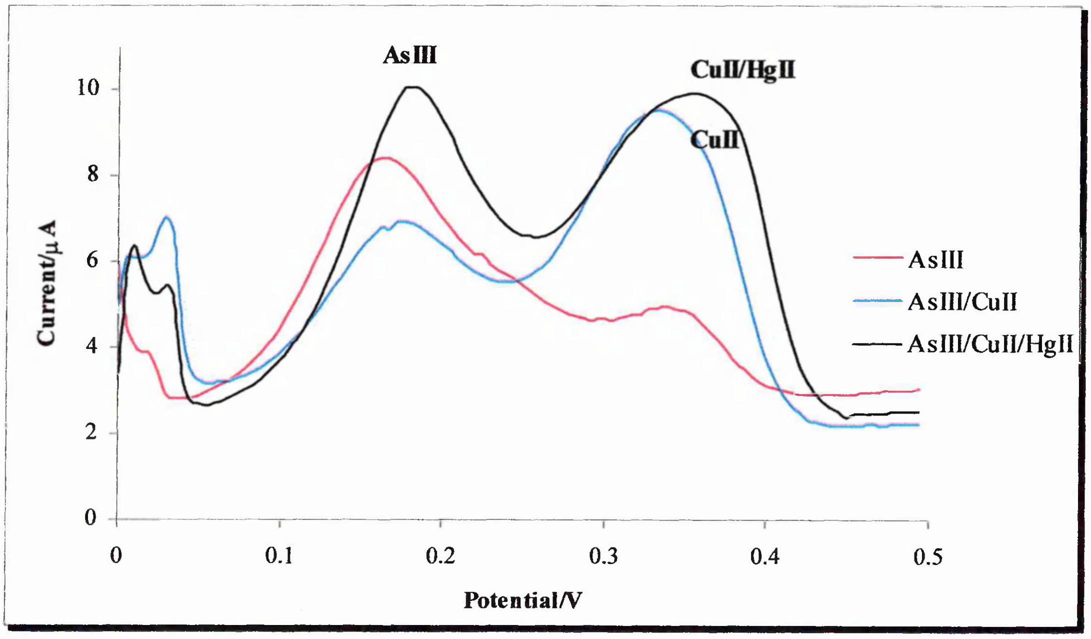


Figure 5.30 Stripping voltammograms of 500 μg l^{-1} As^{3+}, Hg^{2+}, Cu^{2+} in 2 mol l^{-1} HCl; 1 mmol l^{-1} hydrazinium chloride. All other experimental parameters are as defined in Figure 5.28.

The affect of Hg^{2+} on the stripping response of As^{3+} was evaluated further using a 4 mol l^{-1} HCl supporting electrolyte. Calibration plots for Hg^{2+} and As^{3+} using mixed test solutions, analysed under optimised As^{3+} stripping parameters were obtained, illustrated in Figure 5.31. The corresponding stripping voltammograms are displayed in Figure 5.32.

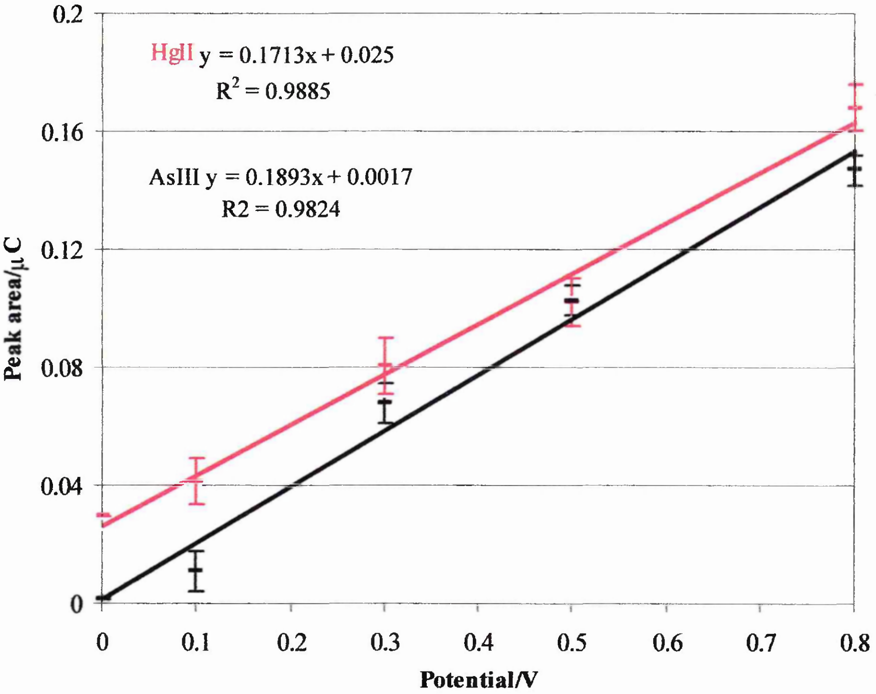


Figure 5.31 Calibration curve for As^{3+} and Hg^{2+} measured simultaneously on Au SPEs; in 4 mol l^{-1} HCl, 1 mmol l^{-1} hydrazinium chloride; DPASV parameters: E_{dep} 0V; t_{dep} 30 s; E_{scan}:0V $\Rightarrow$ 0.5V at 10mV s^{-1}; interval time 0.5secs.

No adverse effect on the calibration curve for As^{3+} in the presence of an equal molar concentration of Hg^{2+} ions was observed, although the stripping peaks were less well-defined. Chi Hua *et al.* (1987), using a Au fibre electrode, constant current stripping analysis (CCSA) and a 5 mol l^{-1} HCl electrolyte, also found Hg^{2+} was stripped at a potential 0.35 V more positive of As^{3+} (0.16 V) with no interference evident at low levels. This was possible as Hg^{2+} competes for sites on the Au surface without forming inter-metallic compounds (Feeney and Kounaves, 2000b). This demonstrates the applicability of the sensor to simultaneous determinations of As^{3+} and Hg^{2+}.

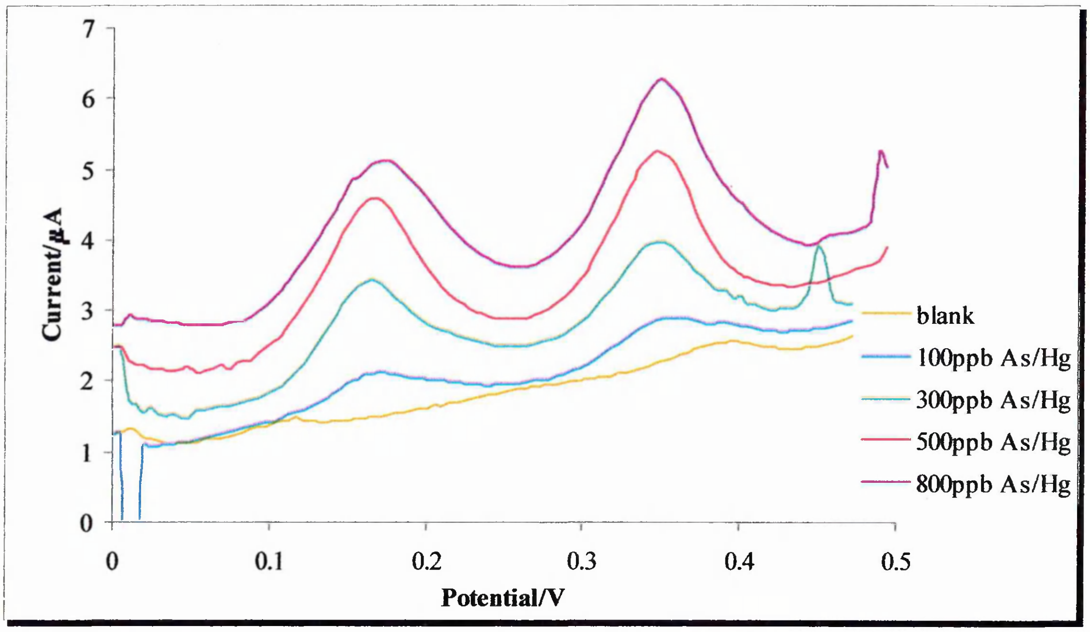


Figure 5.32 Stripping voltammograms of As^{3+} and Hg^{2+} test solutions at Au SPEs in 4 mol l^{-1} HCl; 1 mmol l^{-1} hydrazinium chloride. Experimental conditions are as defined in Figure 5.31.

When Hg^{2+} was present in concentrations above As^{3+} a decrease in the As^{3+} I_p was observed, although the stripping peaks of As^{3+} and Hg^{2+} were well separated. This can be attributed to Hg^{2+} competing for depositioning sites on the Au surface. Hg is known to have a strong interaction with Au and there would presumably be a stronger affinity for Hg^{2+} depositioning onto Au in comparison to As^{3+} (Feeney and Kounaves, 2000b). Since a large excess of Hg^{2+} relative to As^{3+} is not common in most environmental samples, this may not limit the applicability of the sensor to real sample analysis.

Upon increasing the concentration of Cu^{2+}, the affect to the As^{3+} stripping response was much more problematic, as displayed in Figure 5.33. The addition of 200 and 500 µg l^{-1} Cu^{2}, resulted in an As^{3+} calculated I_p decrease of approximately 30 and 62% respectively. The As^{3+} peaks became increasingly distorted, with peak overlap with Cu^{2+} evident.

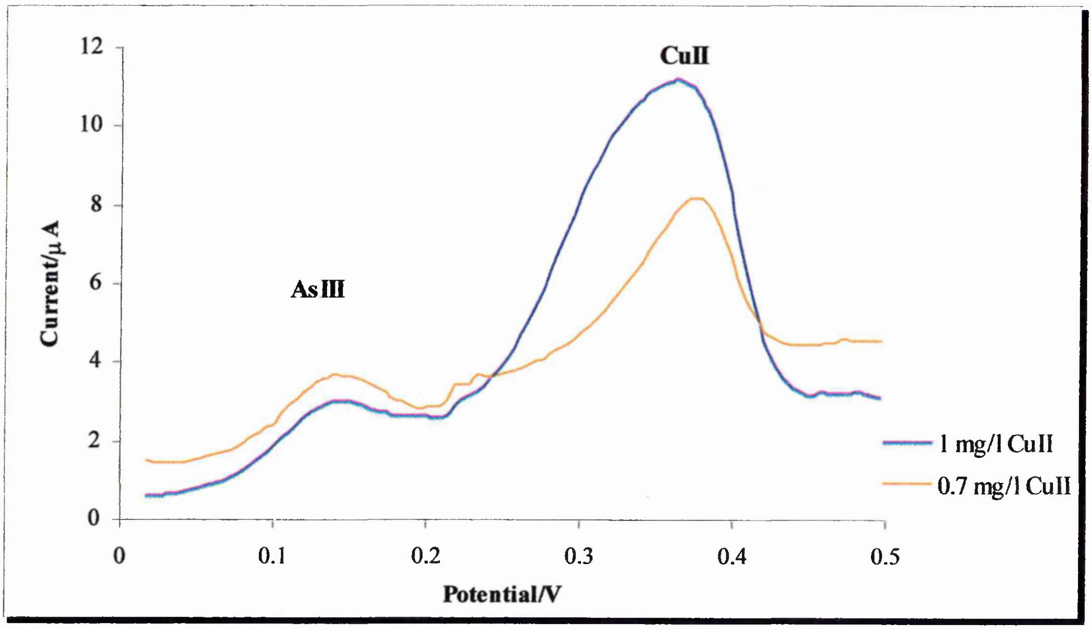


Figure 5.33. Effect of Cu^{2+} on the stripping response of 500 μg l^{-1} As^{3+} test solution at Au SPEs in 4 mol l^{-1} HCl; 1 mmol l^{-1} hydrazinium chloride. Experimental conditions are as defined in Figure 5.31.

Several studies, using alternative Au working electrodes and procedures, found Cu to be the most problematic interference in the determination of As^{3+} using voltammetric techniques, as is discussed below.

Huang and Dasgupta (1999) found the As^{3+} stripping response decreased by 50% when Cu was present in a 20-fold excess using a Au plated GCE, DPASV and a 4.5 mol l^{-1} HCl electrolyte. This was attributed to the deposition of the intermetallic compound, Cu_3As.

Svancara *et al.* (2002) used a Au plated carbon paste electrode and CCSA and found the As^{3+} stripping response decreased in the presence of Cu, which was particularly evident at a ratio of Cu-As >5:1. Similarly, Billing *et al.* (2002) using a Au plated GCE and DPASV found Cu interfered even when present at similar levels to that of As^{3+}. If a 10-fold excess of Cu was present, the As peak was merely a shoulder on the Cu peak. It was concluded 'apart from their oxidation peaks being close together, Cu and As combine strongly if Cu is present in slight excess and greatly reduces the As response'.

Since Cu is generally present at much higher concentrations than As and Hg, this interference presents a significant problem in the applicability of the sensor to real samples analysis. Possible solutions include complexing the Cu with a ligand that is stable and selective for only Cu or as suggested by Svancara *et al.* (2002) by the inclusion of an additional step such as ion-exchange separation in order to eliminate Cu effectively from the sample matrix prior to analysis. Alternatively, chemometric treatment of data is a promising approach without the need for additional analytical procedures. Therefore it was decided to conduct a preliminary evaluation of this technique with respect to DPASV on Au SPEs.

Chemometric data treatment - Partial Least Squares

Otto (1999) defines chemometrics as "the chemical discipline that uses mathematical and statistical methods to (a) design or select optimal measurement procedures and experiments and (b) to provide maximum chemical information by analysing chemical data".

This field includes linear regression, multivariate analysis e.g. pattern recognition, and artificial neural networks (ANN) (Cladera *et al.,* 1997). Factor analysis is a key part of chemometrics. Factors, or principal components, are weighted linear combinations of the original variables, and are effectively new, abstract axes that span the data set, and by retaining only those factors, noise may be removed from the data set. The remaining principal components can be exploited for qualitative identification or classification of samples in pattern recognition or for quantification using principal components regression (PCR) or partial least square (PLS) algorithms (Cladera *et al.*, 1997).

These methods are able to identify and quantify samples and their components without the time-consuming chemical separation and identification methods that are generally heavily reliant on expensive instrumentation (Schreyer and Mikkelsen, 2000).

Multivariate data reduction techniques to elucidate latent information from the data which cannot easily be identified using a univariate approach (standard addition), has been increasingly applied in voltammetric studies; Fernandez *et al.* (2001); Ni and Jin (1999); Antunes *et al.* (2001); Diaz-Cruz *et al.* (2001); Bessant and Saini (2000); Richards *et al.* (2002/2003). Multivariate calibration techniques such as PLS and PCR have been used to construct a calibration model between the concentration of the analyte of interest and the factors of the data matrix, offering a simple more robust calibration method than that obtained using the conventional analysis. A recent review covering multivariate data analysis in electroanalytical chemistry has been published by Richards *et al.* (2002a).

The main difference between PLS and PCR is that the modelling structure of PLS finds factors (called latent variables or LV's) that describe both the variation in the dependent variables (*y*-block) as well as the measured variables (*x*-block). Thus, any additional error in the *y*-block (concentration) is taken into account, producing a more robust model with greater predictive powers than the calibration method constructed using PCR, which only models variation in the *x*-block. Therefore, the PLS algorithm was selected to extract quantitative data for As^{3+} and Hg^{2+} from the voltammetric responses of mixed solutions containing As^{3+}, Hg^{2+} and Cu^{2+}.

The application of PLS involves a calibration step where the relationship between the signal and component concentration is deduced from a set of reference samples, followed by a prediction step in which the results of the calibration are used to determine the component concentrations for the sample signal. In the modelling process the optimum number of factors (latent variables) is selected. This will depend on the number of independently varying chemical species present, as well as other sources of systematic variation, such as the presence of randomly varying baselines, detector noise, interaction between components, and changes in the shape of the component peaks due to sample matrix interference's. Because of this, with adequate design of the calibration matrix the above mentioned influences can be modelled (Cabanillas *et al.,* 1995).

To simplify the preparation and avoid correlation between the different calibration standards, the calibration set was prepared using a three-level full factorial design with a central point (Antunes *et al.,* 2001). Five repetitions of the central point were used to measure precision. The composition of the training set is summarised in Table 5.3. 31 test solutions containing 0, 400, 800 μg l^{-1} of As^{3}, Hg^{2+}, Cu^{2+} were measured to construct a 31x3 *x*-block matrix of response variables; where 31 is the number of rows, which corresponds to the experimental trails, and 3 is the number of columns, which corresponds to the factors; with the corresponding concentration data contained in the *y*-block matrix. The analysis was performed using the optimised DPASV parameters for As^{3+}, as defined in Figure 5.32.

Using Matlab version 6.0 and the PLS Toolbox 2.0, the voltammograms were arranged in a data matrix of currents and the PLS algorithm was applied to analyse electrochemical data and to calculate the concentration of As and Hg in the test solutions (Appendix A6). The optimum number of latent variables was selected using a cross-validation method. This entails leaving out one sample from the calibration set at a time, building a calibration model with the remaining samples and then estimating the concentration of the left out sample. This process is repeated until each sample had been left out once. The predicted concentration is compared with the known concentration and the root mean square error of cross-validation (RMSECV) is calculated (Antunes *et al.*, 2001). A minimum value of RMSECV indicates the optimal number of latent variables and as shown in Figure 5.34, the optimal for As and Hg is 6.

Table 5.3 Composition of the PLS calibration set.

Test solution	*x*1 – As^{3+}	*x*2 – Cu^{2+}	*x*3 – Hg^{2+}
1	0	0	0
2	400	0	0
3	800	0	0
4	400	400	0
5	800	400	0
6	0	400	0
7	0	800	0
8	400	800	0
9	800	800	0
10	0	0	400
11	0	0	800
12	0	400	400
13	0	400	800
14	0	800	400
15	0	800	800
16	400	0	400
17	400	0	800
18	800	0	400
19	800	0	800
Central 1	400	400	400
Central 2	400	400	400
Central 3	400	400	400
Central 4	400	400	400
Central 5	400	400	400
25	800	400	400
26	800	400	800
27	800	800	400
28	800	800	800
29	400	400	800
30	400	800	400
31	400	800	800

The mean predicted concentration for the 400 μg l^{-1} central calibration test solution was 404.56 and 399.0397 with a standard deviation of 23.03 and 22.72 for As^{3+} and Hg^{2+} respectively. A RSD of 5.69% was obtained for both As^{3+} and Hg^{2+}.

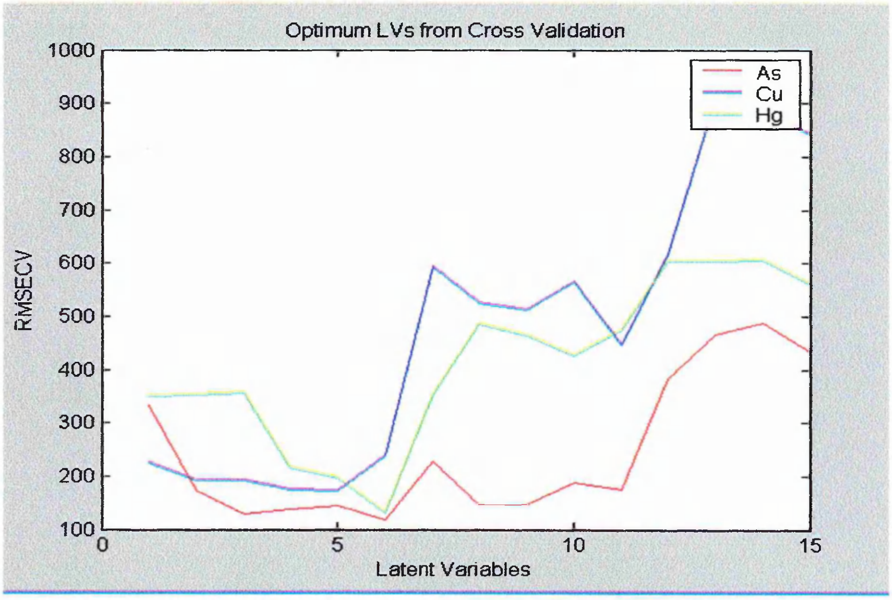


Figure 5.34 Optimum numbers of latent variables following cross-validation of calibration set.

To further assess the predictive ability of the PLS calibration model, a set of 20 validation samples were prepared containing 0, 300, 400, 600 and 800 µg l^{-1} As^{3+}, Hg^{2+} and Cu^{2+}, as detailed in Table 5.4. The voltammograms of these solutions were recorded using the same experimental conditions as the calibration set.

Table 5.4 Composition of the validation set.

Test solution	**$x1$ – As^{3+}**	**$x2$ – Cu^{2+}**	**$x3$ – Hg^{2+}**
1	300	300	300
2	600	600	600
3	300	300	600
4	300	600	300
5	300	600	600
6	600	300	300
7	600	600	300
8	600	300	600
9	0	0	0
10	800	800	800
11	400	400	400
12	800	0	0
13	400	0	0
14	0	0	800
15	0	0	400
16	0	800	0
17	0	400	0
18	400	0	400
19	800	0	800
20	400	0	800

The predicted concentrations of As^{3+} and Hg^{2+} for the validation samples using the PLS calibration model with 6 latent variables *vs*. the actual concentrations are shown in Figure 5.35a/b. The highest and lowest predicted value for As^{3+} and Hg^{2+} at each concentration is contained in Table 5.5. The predicted values for As^{3+} fall close to the actual concentrations, even in the presence of an excess of Cu^{2+}. For example, sample 5 of the validation set contains 300 μg l^{-1} As^{3+} and 600 μg l^{-1} Cu^{2+} and Hg^{2+}. The predicted As^{3+} value was 244 μg l^{-1}. Although some disparity is evident, the results illustrated in Figure 5.35a are within acceptable limits considering the degree of distortion and suppression of the As^{3+} response by the Cu^{2+} stripping peak shown in Figure 5.33.

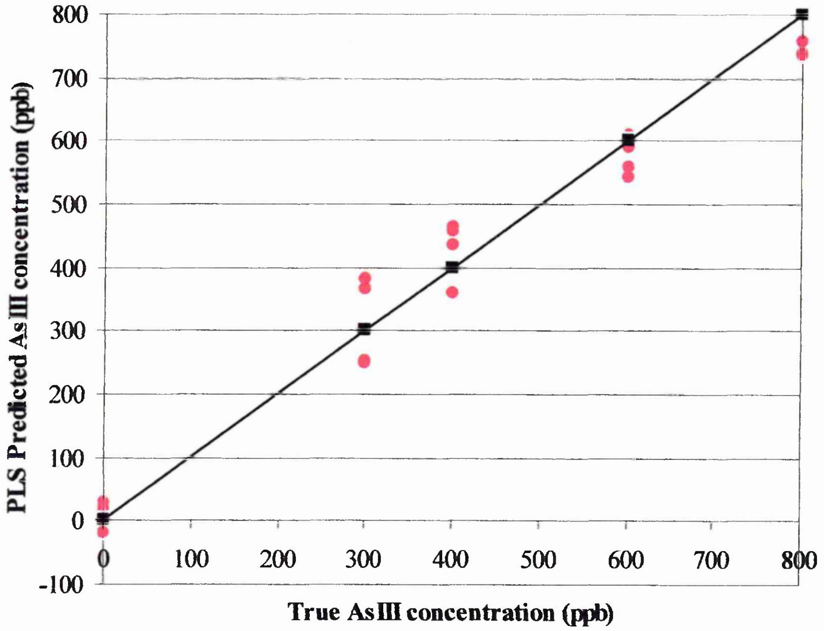


Figure 5.35a Predicted concentrations of As^{3+} for the validation set using the PLS calibration model with 5 latent variables. The line represents the ideal response.

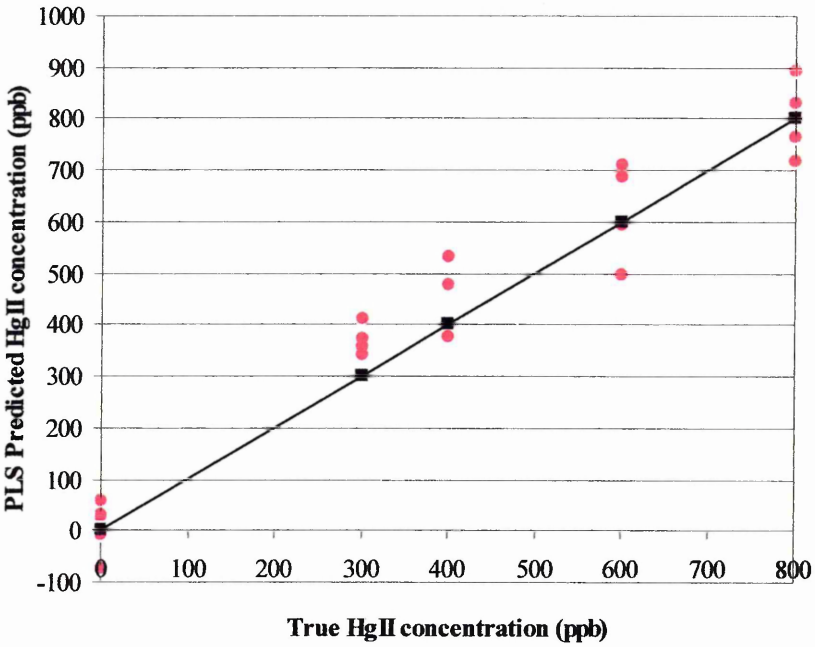


Figure 5.35b Predicted concentrations of Hg^{2+} for the validation set using the PLS calibration model with 5 latent variables. The line represents the ideal response.

Table 5.5 The highest and lowest predicted values for As^{3+} and Hg^{2+} ($\mu g\ l^{-1}$).

	As^{3+} predicted		**Hg^{2+} predicted**	
Actual concentration	**highest**	**lowest**	**Highest**	**Lowest**
0	25 (16)	0 (15)	52 (17)	0 (9)
300	376 (1)	244 (5)	406 (1)	334 (4)
400	460 (20)	353 (18)	524 (11)	371 (18)
600	605 (6)	536 (8)	706 (3)	491 (5)
800	753 (10)	730 (12)	888 (14)	711 (10)

Figures in parentheses – sample number; values below zero were expressed as zero.

The predicted concentrations obtained for Hg^{2+} were generally poorer than those obtained for As^{3+}. However, the experiment was conducted using optimised conditions for As^{3+}. The use of a 4 mol l^{-1} supporting electrolyte has shifted the oxidation potential of Cu^{2+} more anodically; relative to the oxidation potential using 0.1 mol l^{-1} KCl (Figure 5.13); such that the Cu^{2+} and Hg^{2+} stripping peaks completely overlap (Figure 5.29). This shows the PLS calibration to be a robust method, able to provide semi-quantitative data under the 'worse case scenario' (i.e. severe peak overlap and non-ideal experimental conditions).

This preliminary investigation has demonstrated the applicability of the PLS algorithm in solving the problems associated with overlapping voltammetric peaks when measuring solutions containing metals with similar oxidation potentials. Further work would be necessary to improve the prediction for As^{3+} and Hg^{2+}, for example, by using appropriate data pre-treatment techniques (i.e. scaling method employed, smoothing and advanced data filtering, and detection of outliers). Further enhancement would include the incorporation of other matrix effects into the PLS calibration model, by constructing the calibration model using spiked soil samples. The prediction for Hg^{2+} would be greatly improved by constructing the calibration model under optimised conditions for the single analysis of Hg. Comparison with alternative calibration methods, particularly artificial neural networks (ANN), would also be advantageous. Several studies reported in Richards *et al.* (2002a) found that ANN provided improved prediction accuracies over PLS, although construction of an optimised ANN calibration model was more time-consuming. The chemometric approach would make sufficient advances in the applicability of utilising DPASV coupled to Au SPE's for measuring As and Hg in 'real' samples in field conditions.

5.3.4 Preliminary investigations for the analysis of Arsenate (As^{5+})

Under oxidising and aerated conditions, the predominant form of arsenic in water and soil is As^{5+}, while under reducing and waterlogged conditions the As^{3+} species is normally predominant. In natural waters and depending on the environment the ratio of As^{5+} and As^{3+} may vary between 0.1:1 and 10:1 (Barra and Correia dos Santos, 2001). Therefore the analysis of both As^{3+} and As^{5+} is necessary in order to properly assess the extent of As contamination.

For the speciation measurement of As^{5+}, a pre-reduction step, to reduce As^{5+} to As^{3+}, henceforth referred to as $As^{5+}{}_{RED}$, is necessary. To perform this under site conditions, a simple and rapid method is required. Several reducing agents have been reported (as shown previously in Table 5.2), although KI with or without ascorbic acid has been most frequently used (Dasgupta *et al.*, 2002). This requires very strong acidity, typically 5-6 mol l^{-1} HCl; the reduction is driven by the formation of $AsCl_3$. As^{5+} can be determined electrochemically without prior chemical reduction by stripping voltammetry in strongly acidic solutions using high negative potentials (Greulach and Henze, 1995). However these suffer from problems associated with large hydrogen and chlorine generation at the electrode surface.

L-cysteine has been used as a reducing agent in standard laboratory based methods such as HGAA (Le *et al.*, 1994) and ICP-AES (Feng and Cao, 1994). Chen *et al.* (1992) determined As^{5+} by HG/ICP-AES following reduction using cysteine in 1 mol l^{-1} HCl. As^{5+} was completely reduced within 5 min when heated. It was concluded 'compared with other systems tested *L*-cysteine is an excellent reagent for the reduction of As^{5+} to As^{3+}'.

Consequently, cysteine was evaluated for the pre-reduction of As^{5+} using a 500 μg l^{-1} As^{5+} in 1, 2, and 4 mol l^{-1} HCl supporting electrolyte. The test solutions were measured every 30 min for 2 hr, then every hour for a further 4 hr. No stripping response was observed over this period. The investigations were repeated using higher and lower cysteine concentrations. No change in response was observed over this time scale. The test samples were then stored for 2 days. Upon repeat analysis a slight stripping response for $As^{5+}{}_{RED}$ was achieved in 1 mol l^{-1} HCl.

To increase the reaction kinetics, further samples of 500 μg l^{-1} As^{5+} containing cysteine (0.1 ml 20% v/v cysteine), in 1, 2, 4 mol l^{-1} HCl, were heated for 10 min at 75°C. Figure 5.36 shows a significant stripping response for $As^{5+}{}_{RED}$ was achieved in 1 mol l^{-1} HCl. A slight response was observed using 2 mol l^{-1} HCl, however this was distorted by the presence of a sharp interfering peak. No response was seen using 4 mol l^{-1} HCl. The voltammogram of a solution containing As^{3+} shows the oxidative potential is unchanged when analysing $As^{5+}{}_{RED}$.

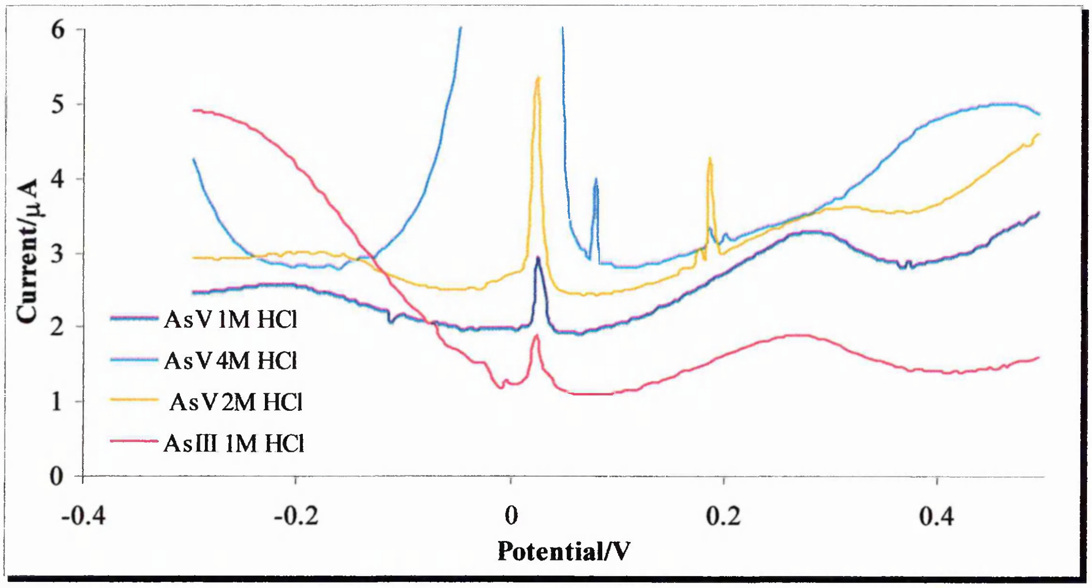


Figure 5.36 500 μg l^{-1} As^{5+}_{RED} in 1, 2, 4 mol l^{-1} HCl, 20% cysteine, following 10 min heating at 75°C; E_{dep} -0.4 V; t_{dep} 30 sec; E_{scan}:-0.4V ⇒ 0.5V @ 10mV s^{-1}; interval time 0.5secs.

The effect of HCl concentration on the stripping response of As^{5+}_{RED} shown in Figure 5.36 is in contrast to that of As^{3+}, where an increase in the stripping response was observed with increasing HCl concentration (Figure 5.15). Svancara *et al.* (2002) reported the reduction as:

$$H_2AsO_4^- + 2SH\text{-}Ch_2\text{-}CH(\text{-}NH_2)\text{-}COOH \rightarrow$$

(cysteine)

$$H_2AsO_3^- + HOOC\text{-}CH(\text{-}NH_2)\text{-}CH_2\text{-}S\text{-}S\text{-}CH_2\text{-}CH(\text{-}NH_2)\text{-}COOH \text{ (cystine)} + H_2O$$

This suggests $H_2AsO_3^-$ is the species involved in the reaction following reduction from As^{5+} to As^{5+}_{RED}. Since the stripping response for As^{3+} improved with increased HCl concentrations presumably due to greater formation of $As(OH)_2Cl$, $As(OH)Cl_2$, and $AsCl_3$ species, the use of higher HCl concentrations did not result in increased stripping responses due to the $H_2AsO_3^-$ species. Indeed, higher HCl concentrations had the reverse effect preventing the effective reduction of H_2AsO_4 to $H_2AsO_3^-$ and hence subsequent measurement of As^{5+}_{RED}.

To verify these findings, further analysis of a 500 µg l^{-1} As^{5+} in 1 and 2 mol l^{-1} HCl was performed. Again, no response was observed in 2 mol l^{-1} HCl following 10 min heating. However, when heating was continued for a further 10 min, a more defined stripping response for $As^{5+}{}_{RED}$ was achieved.

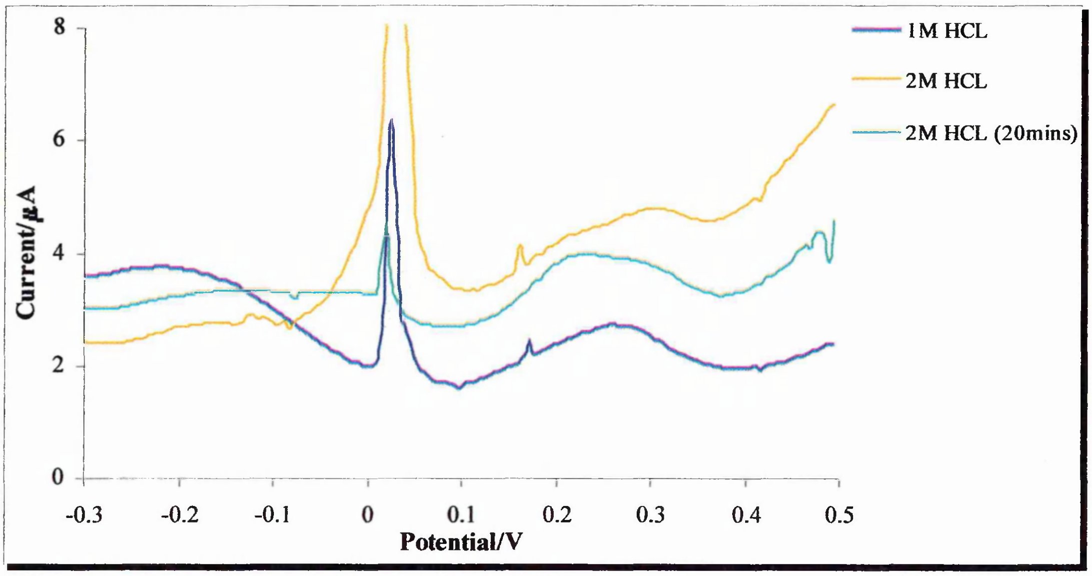


Figure 5.37 500 µg l^{-1} $As^{5+}{}_{RED}$ in 1, 2 mol l^{-1} HCl, 20% cysteine, following 10 and 20 min heating at 75°C; E_{dep} -0.4 V; t_{dep} 30 sec; E_{scan}:-0.4V ⇒ 0.5V @ 10mV s^{-1}; interval time 0.5secs.

This indicates that increases in reaction times allow sufficient $H_2ASO_3^-$ to be generated, even in the presence of higher chloride ion content. Further solutions of 1 mg l^{-1} As^{5+} in 1 and 2 mol l^{-1} HCl were prepared and then reduced (20 min; 2 mol l^{-1} HCl). From these a 400 µg $l^{-1}$$As^{5+}{}_{RED}$ solution in 1, 2 and 4 mol l^{-1} HCl solutions were prepared. Figure 5.38 shows an adequate stripping response can be achieved from these stock solutions following dilution using a 1 mol l^{-1}. When dilution is carried out using higher molar concentrations of HCl, the $As^{5+}{}_{RED}$ stripping response is completely lost or severely distorted stripping peaks are obtained. When reduction is performed in 2 mol l^{-1}, as shown in Figure 5.39, all diluted test solutions exhibited distorted stripping responses.

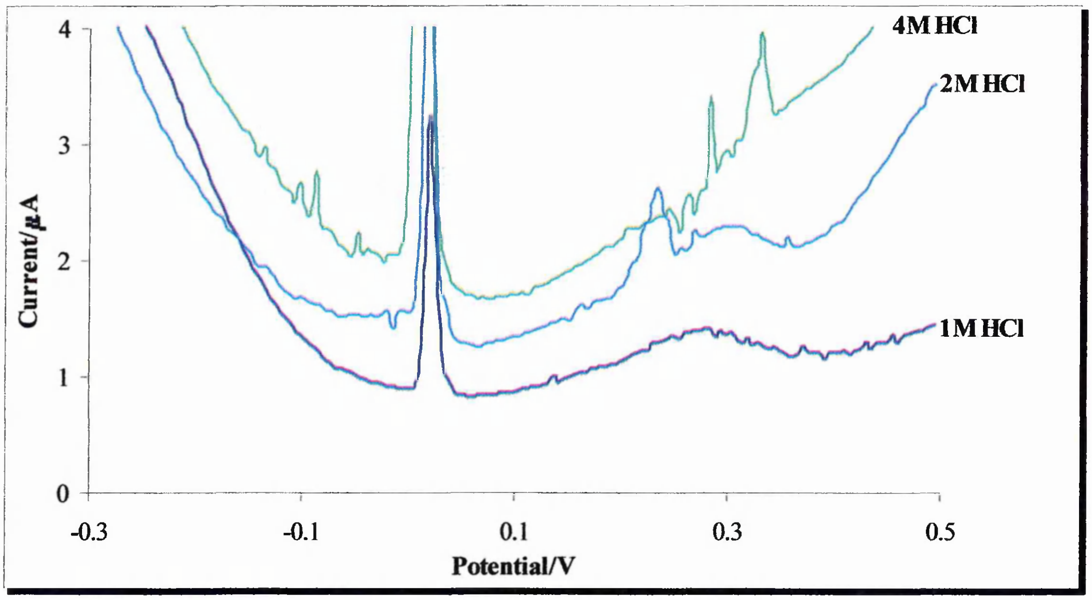


Figure 5.38 Stripping voltammograms of 400 µg l^{-1} As$^{5+}_{RED}$ in 1, 2, 4 mol l^{-1} HCl. Reduction was performed in 1 mol l^{-1} HCl; 20 % cysteine for 10 min. All other experimental parameters are as defined in Figure 5.37.

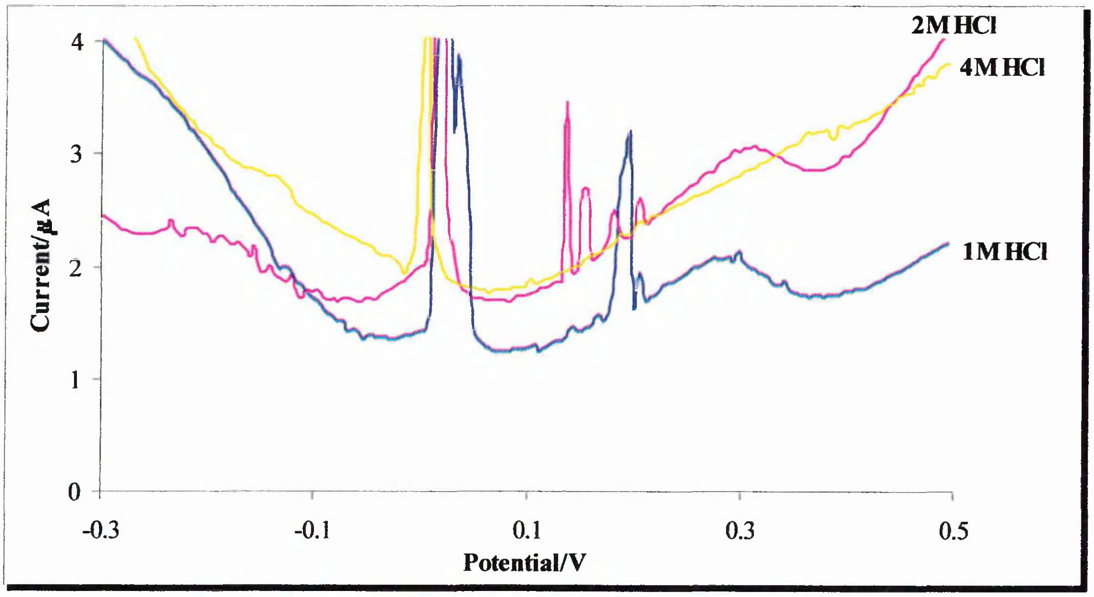


Figure 5.39 Stripping voltammograms of 400 µg l^{-1} As$^{5+}_{RED}$ in 1, 2, 4 mol l^{-1} HCl. Reduction was performed in 2 mol l^{-1} HCl; 20 % cysteine for 20 min. All other experimental parameters are as defined in Figure 5.37.

This suggests effective reduction of As^{5+} using cysteine is only possible at low HCl concentrations (1 mol l^{-1}). The presence of high chloride, although necessary to achieve optimal deposition and subsequent stripping of As^{3+}, is not required for the analysis of As^{5+}.

The different electrochemical behaviour of As^{5+}_{RED} also became evident upon optimisation of key DPASV parameters. A E_{dep} of -0.3V, as opposed to 0V for As^{3+} was required to sufficiently reduce an equivalent quantity of As^{5+} (Figure 5.40), although even at these potentials the stripping response did not equal that of As^{3+}.

As illustrated in Figure 5.41, an increase in the stripping response with increasing t_{dep} was observed, which is in contrast to As^{3+} (Figure 5.17). Svancara *et al.* (2002) also found while As^{3+} yields a maximum stripping current at low t_{dep}, and further prolongation of the depositioning time leads to a decrease in stripping response, the $As5+_{RED}$ assay could be performed using a longer t_{dep} with a near linear response between signal magnitude and deposition time. This was attributed to cysteine becoming entrapped onto the electrode surface during accumulation, modifying the electrical double layer, thus hindering the redox transformation of the arsenic species Svancara *et al.* (2002). Hence the presence of cysteine at high concentrations may lead to a prolongation of the optimal accumulation time as well as to a broadening of the peaks obtained.

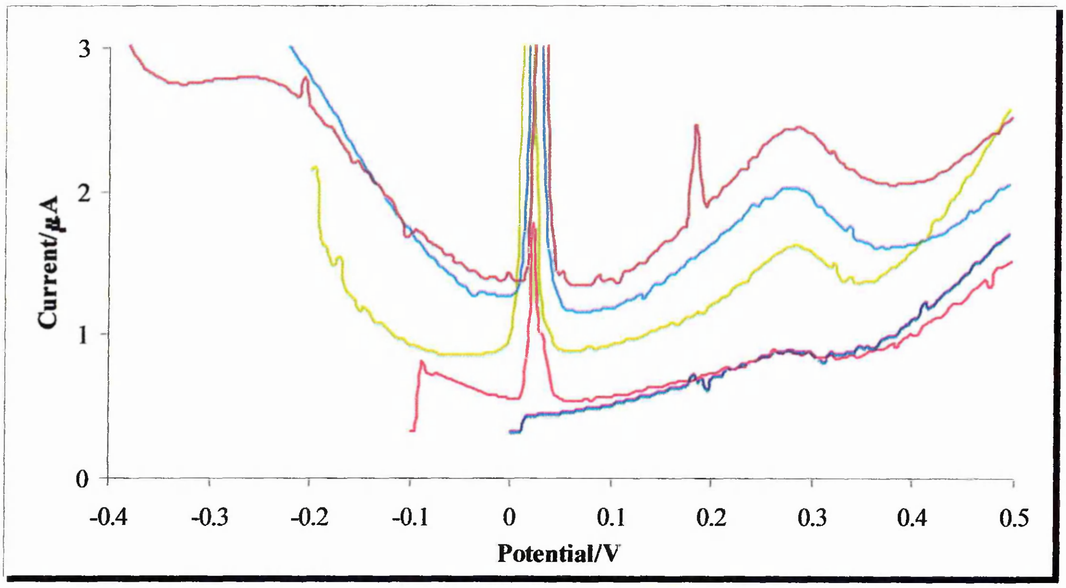


Figure 5.40 Effect of E_{dep} on the stripping response of a 500 µg l^{-1} $As^{5+}{}_{RED}$; in 1 mol l^{-1} HCl, 20% cysteine, following 10 min heating at 75°C; t_{dep} 30 sec; E_{scan}:-0.4V ⇒ 0.5V @ 10mV s^{-1}; interval time 0.5secs.

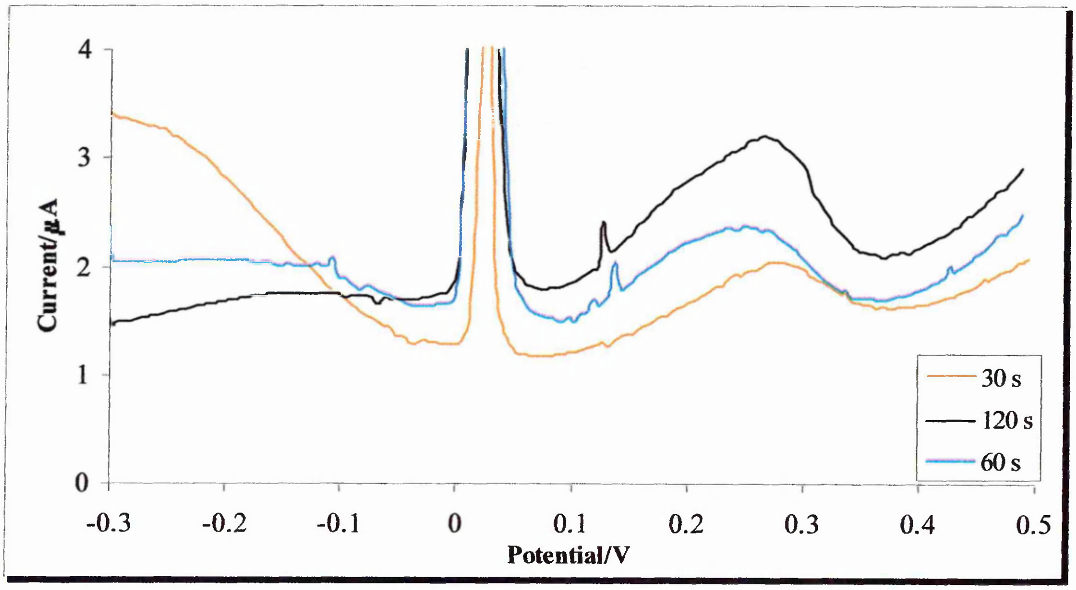


Figure 5.41 Effect of t_{dep} on the stripping response of a 500 µg l^{-1} $As^{5+}{}_{RED}$; in 1mol l^{-1} HCl, 20% cysteine, following 10 min heating at 75°C; E_{dep} -0.3V; E_{scan}:-0.3V ⇒ 0.5V @ 10mV s^{-1}; interval time 0.5secs.

However, the appearance of the 'noisy' peak, also evident when high HCl concentrations or negative potentials were used, would be disadvantageous in the determination of As^{5+}_{RED}. The origin of this peak is difficult to fully define, although since it did not appear during analysis of As^{3+}, it may be attributed to an increase in the deposition of excess cysteine or its reaction products.

Therefore, preparation and analysis of two separate samples would be necessary to assess total As content. However, since the two forms are predominant under different environmental conditions, as discussed in Section 1.4.5, initial site investigations would indicate which species are likely to be present and the analysis performed accordingly.

The efficiency of the reduction of As^{5+} to As^{5+}_{RED} in 1 mol l^{-1} HCl was evaluated by comparing the results obtained for 5 solutions containing the same concentration of total As (500 µg l^{-1}) but made up with different ratios of $As^{3+/5+}$. The data displayed in Figure 5.42 show the reduction of As^{5+} is quantitative and the presence of As^{3+} does not have an adverse effect on the As^{5+}_{RED} quantitative response.

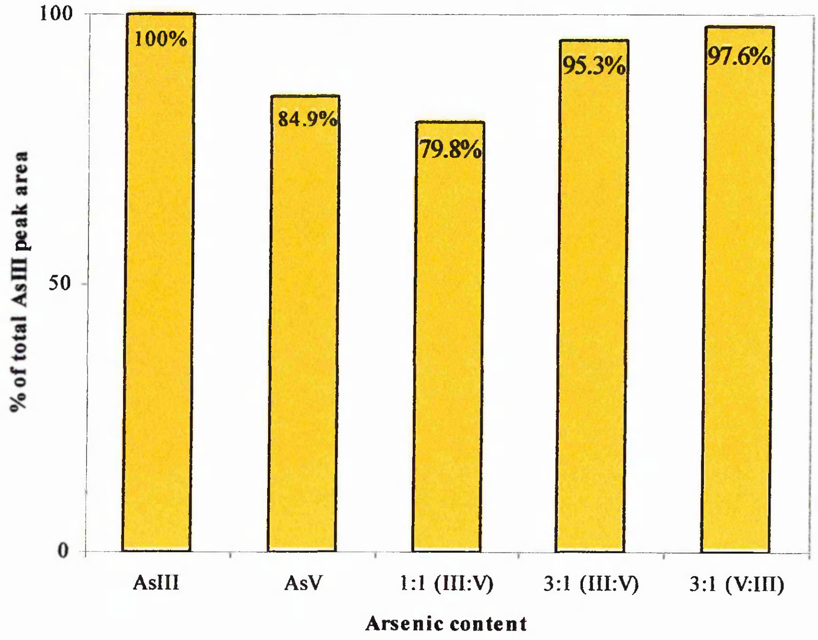


Figure 5.42 Efficiency of cysteine reduction on a 500 µg l^{-1} As solution containing different ratios of As^{5+}/As^{3+}.

The reduction procedure was simple and rapid (10 min) to perform and by using a commercially available portable heating system, could easily be carried out *in situ.* Following reduction of $As^{5+} \rightarrow As^{5+}_{RED}$ a calibration plot of the As^{5+}_{RED} stripping response versus its concentration in the range of 0-800 µg l^{-1} yielded an R^2 value of 0.9852 (Figure 5.43). A RSD of ≤15% was obtained (n=6) at each concentration increment. The LOD, calculated according to the IUPAC convention (*S/N* = 3) of the blank measurement was calculated as 42 µg l^{-1}.

The voltammograms illustrated in Figure 5.44 show a different shape of signal was obtained when analysing the pre-reduced As^{5+}_{RED} than the original trivalent As^{3+}. The stripping peaks obtained were broader and shifted to more positive potentials.

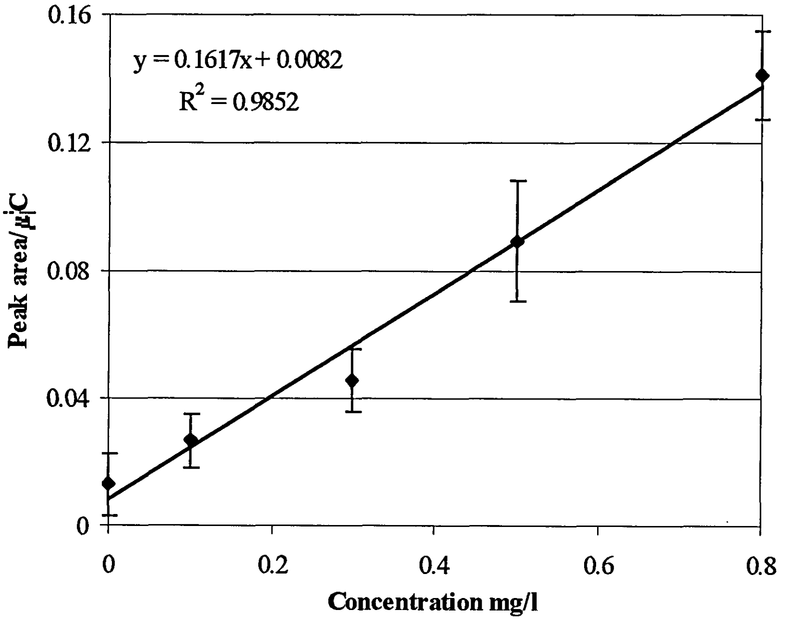


Figure 5.43 Calibration plot for As^{5+}_{RED} on Au SPEs in the range of 0-800 µg l^{-1}; in 1 mol l^{-1} HCl, 20% cysteine; 10 min heating at 75°C; E_{dep} -0.3V; t_{dep} 30 sec; E_{scan}:- 0.3V ⇒ 0.5V @ 10mV s^{-1}; interval time 0.5secs.

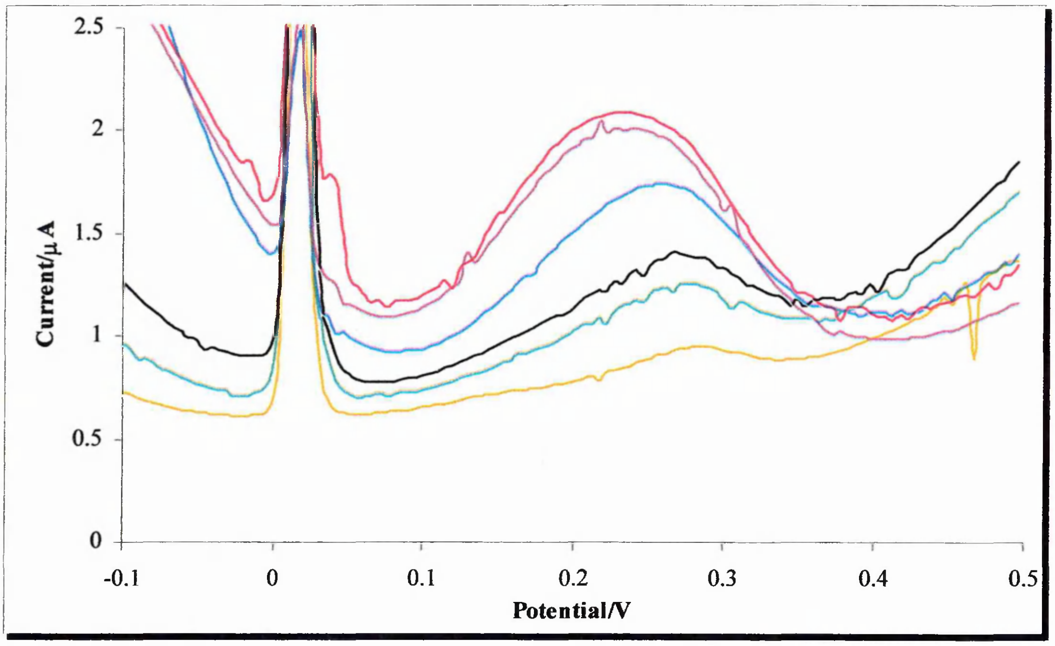


Figure 5.44 Stripping voltammograms for 0, 100, 300, 500, 800, 1000 As^{5+}_{RED} µg l^{-1}. All experimental conditions are as defined in Figure 5.43.

These preliminary findings have demonstrated the applicability of the Au SPEs to determining the less toxic As^{5+} when speciation studies are necessary. Further investigations are needed to evaluate interference from co-existing metal ions.

5.3.5 Preliminary investigation for the detection of Cd^{2+} Pb^{2+} and Cu^{2+}

Whilst an *in situ* electrochemical sensor capable of detecting Cd^{2+}, Pb^{2+} and Cu^{2+} has been developed and successfully demonstrated as part of this study, a preliminary evaluation of the applicability of measuring these metals using the Au SPEs was undertaken. The developed *in situ* electrochemical sensor (Chapter 2) utilised a Hg-film working electrode, which, as reviewed in Section 1.7.3 and Section 2.1 has many attributes, particularly for field-based analysis and is widely applied for the analysis of metals.

However, the use of Hg as an analytical tool has seen recent debate due its toxic nature (Section 2.1). Masawat *et al.* (2002) reported 'because of the toxicity of Hg, future regulations and occupational health considerations may severely restrict the use of Hg as an electrode material. Hence, mercury-free analysis is a highly desirable alternative to existing techniques'. No legal restrictions have thus far been noted by the present author.

To solve this relatively new problem, alternative Hg-free approaches have been investigated, detailed in Section 2.1. These generally involve surface adulteration or modification schemes, which complicate analyses and can result in lengthy and complex electrode fabrication methodologies. In this instance, the metals are deposited directly onto the bare Au SPE, providing a greatly simplified Hg-free procedure.

As a preliminary study, 100 μl of a solution containing 200 μg l^{-1} Pb^{2+} in 0.1 mol l^{-1} KCl and 1% v/v HNO_3 supporting electrolyte was placed onto the working area of the Au SPE and DPASV analysis performed. Lead depositioning was carried out using a negative E_{dep} of -0.8V for 165 s and 30 s. A stripping response for Pb was observed at approximately -0.2V, shown in Figure 5.45 (Pb was oxidised at a E_{dep} of approximately -0.7V at the Hg modified carbon SPE, using a E_{dep} of -1.1V and a t_{dep} of 165 s). A t_{dep} of 30 s provided an adequate Pb^{2+} stripping response and background currents. A t_{dep} of 165 s did not significantly increase the stripping response for Pb^{2+} and resulted in increased background currents. This has provided an electrochemical method with greatly simplified experimental protocols, for detecting Pb^{2+} in the microgram per litre range.

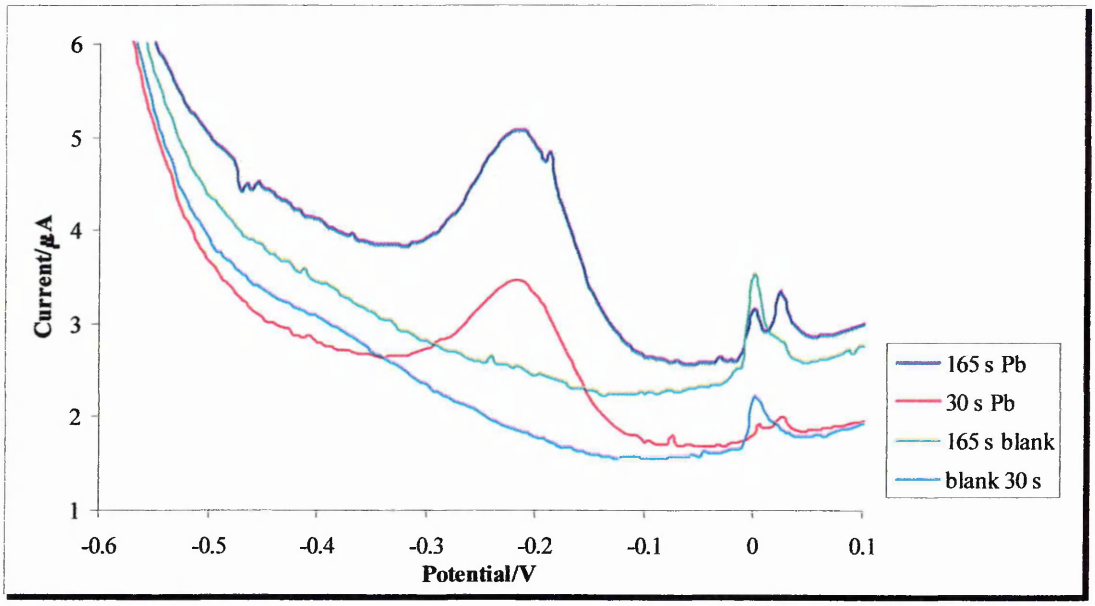


Figure 5.45 Stripping voltammograms at Au SPEs of a 200 µg l^{-1} Pb in 0.1 mol l^{-1} KCl; 1% v/v HNO_3; E_{dep} -0.8V; t_{dep} 165 and 30 s; E_{scan}:-0.8V ⇒ 0.1V @ 10mV s^{-1}; interval time 0.5secs.

Figure 5.46 shows stripping voltammograms for a solution containing 1 mg l^{-1} Cd^{2+} in 0.1 mol l^{-1} KCl and 1% v/v HNO_3 supporting electrolyte. Cadmium depositioning was carried out at a E_{dep} of -0.8V for 165 s and 30 s. Although a response was observed at approximately -0.25V, the stripping peaks were poorly defined and became broader upon increasing the t_{dep} to 165 s, indicating the redox reaction for Cd^{2+} at the Au SPE is less reversible than that achieved for Pb^{2+}. However, this situation may improve following optimisation of experimental protocols.

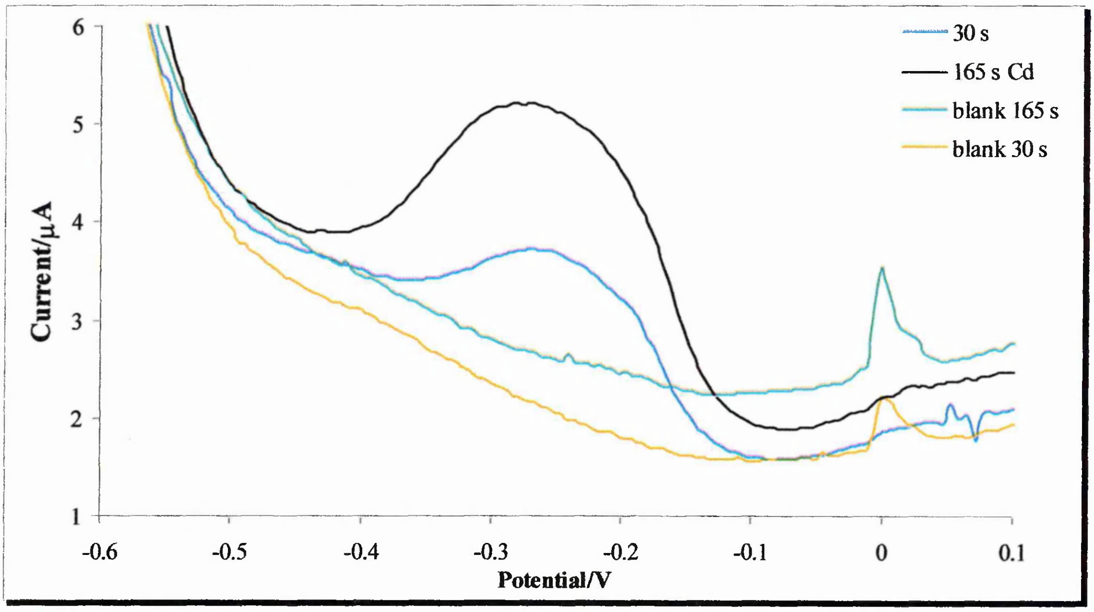


Figure 5.46 Stripping voltammograms at Au SPEs of a 1 mg l^{-1} Cd in 0.1 mol l^{-1} KCl; 1% v/v HNO_3; E_{dep} -0.8V; t_{dep} 165 and 30 s; E_{scan}:-0.8V $\Rightarrow$ 0.1V @ 10mV s^{-1}; interval time 0.5secs.

The oxidation potential for Cd^{2+} and Pb^{2+} are in close proximity, shown in Figure 5.47. In most cases, Cd^{2+} is present in much lower concentrations than Pb^{2+}, hence the peak overlap would not be a limitation in measuring Pb^{2+} in samples containing Cd^{2+}. However, the voltammetric peaks may be resolved by adjusting the supporting electrolyte and optimising assay parameters. Alternatively, as previously shown (Section 5.3.3), it may be possible to use factor analysis multivariate calibration techniques to extract quantitative data from complicated stripping voltammograms.

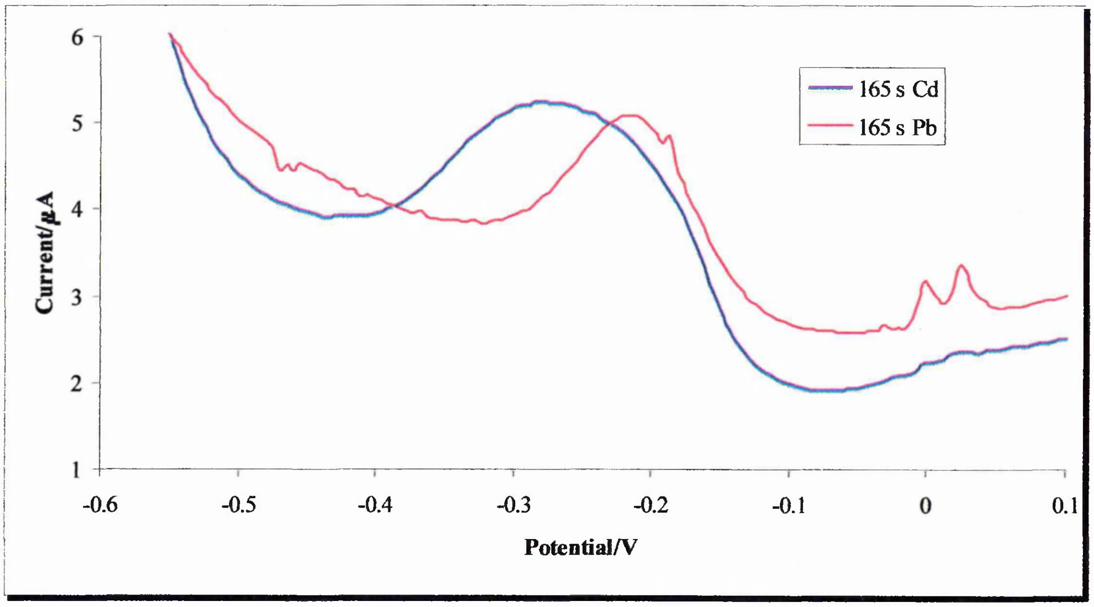


Figure 5.47 Stripping voltammograms at Au SPEs of a 1 mg l^{-1} Cd and 200 µg l^{-1} Pb in 0.1 mol l^{-1} KCl; 1% v/v HNO_3; E_{dep} -0.8V; t_{dep} 165; E_{scan}:-0.8V ⇒ 0.1V @ 10mV s^{-1}; interval time 0.5secs.

The Au SPEs were next evaluated for the detection of Cu^{2+}. DPASV was performed using solutions containing 100, 500, 800 and 1000 µg l^{-1} Cu^{2+} in 2 mol l^{-1} HCl supporting electrolyte. Depositioning was carried out using a E_{dep} of 0V for 30 s. A stripping response was observed at approximately 0.25V. A positive correlation between stripping response and Cu^{2+} concentration was observed.

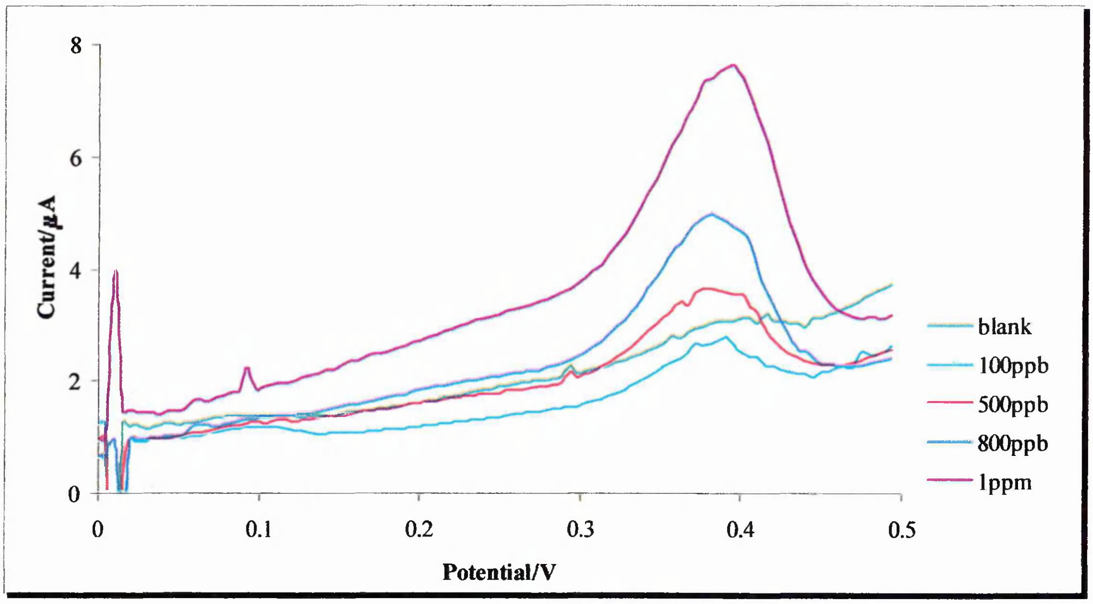


Figure 5.48 Stripping voltammograms at Au SPEs of a solution containing 100, 500, 800, 1000 µg l^{-1} Cu^{2+} in 2 mol l^{-1} HCl; E_{dep} 0V; t_{dep} 30; E_{scan}:0V ⇒ 0.7V @ 10mV s^{-1}; interval time 0.5secs.

Conclusion

These preliminary findings have demonstrated the applicability of the Au SPE to detect a range of environmentally important metals using greatly simplified experimental protocols. The Au SPE exhibits a wide potential window, with stripping responses observed for these analytes at both negative (Pb^{2+} and Cd^{2+}) and positive (Cu^{2+}) potential regions. More importantly, because Hg is omitted from the electrochemical assay, significant environmental and safety benefits are realised. Further work, including optimisation of DPASV parameters and supporting electrolyte would serve to improve experimental conditions, thus provide greater sensitivity and better defined stripping peaks.

5.4 CONCLUSION AND FUTURE WORK

As and Hg contamination has serious environmental consequences. The purpose of an *in situ* screening sensor is to provide rapid real time information regarding the extent of contamination. This allows a rapid response to environmental disasters, as well as cost-effective initial site assessments and remediation monitoring.

Consequently, a heavy metal electrochemical sensor has been developed which is capable of providing semi-quantitative determinations, for screening and identifying samples containing analyte levels above or below legal threshold values. The realisation of such a tool has been previously hindered due to the often complex nature of the electrochemical procedures required for the analysis of As and Hg.

By obtaining a new low temperature curable screen-printable Au ink, single use Au SPE can be fabricated in large quantities using simple and low cost screen printing technology. This replaces existing schemes which require substrates that can undergo curing temperatures in excess of 900 °C. This has benefits in terms of cost of the overall analysis and ease of fabrication. The use of disposable Au SPE also significantly reduces the complexity of electrochemical procedures since no expensive, time consuming and complex electrode cleaning procedures are necessary. No papers or reports have been previously published regarding truly disposable Au electrodes.

A rapid and simple DPASV procedure using Au SPE was developed for analysis of As^{3+}, Hg^{2+}, and As^{5+}. The following main conclusions have been drawn:

- Screen printed electrodes fabricated using a new curable Au ink are suitable for the electrochemical detection of As^{3+}, Hg^{2+}, and $As^{5+.}$ Although background stripping currents characteristic of other Au electrodes were evident, adequate background characteristics were obtainable.

- Under optimised stripping conditions, an LOD of 2.7 μg l^{-1} and 0.15 μg l^{-1} following a 30 s and 120 s accumulations was achieved for Hg^{2+}. A dynamic range of 0-2000 μg l^{-1} and a RSD of less than 9% (n=10) was achieved. Using a E_{dep} of +0.2V no significant change in the Hg^{2+} stripping response was observed in the presence of a 200-fold excess of Cu^{2+} (10 mg l^{-1} Cu^{2+}). Although the Hg^{2+} stripping response became distorted in the presence of a 2000-fold excess of Cu^{2+}, the calculated peak area was not significantly different.

- The supporting electrolyte has a significant effect on the stripping response of As^{3+}. A 2 mol l^{-1} HCl was required to achieve effective deposition and subsequent stripping of As^{3+}. A 4 mol l^{-1} HCl supporting electrolyte produced the best signal-to-background characteristics. Using optimised DPASV key parameters an LOD of 1 μg l^{-1} was obtained in 4 mol l^{-1} HCl.

- Simultaneous detection of As^{3+} and Hg^{2+}, using optimised stripping parameters for As^{3+}, was demonstrated. No significant adverse effect on the stripping response for As^{3+} was evident when Hg^{2+} was present at similar levels.

- The use of Au SPEs for ASV measurements of key heavy metals, coupled to multivariate calibration techniques such as PLS, is a potentially useful method for measuring mixed metal samples.

- As^{5+} was effectively reduced over a short (10 min) reduction time using cysteine. A stripping response for As^{5+}_{RED} was obtained in 1 mol l^{-1} HCl supporting electrolyte. Sensitivity did not equal that of As^{3+}, with a LOD of 42 μg l^{-1} following a 30 s accumulation period.

- Preliminary investigations have indicated the applicability of the Au SPE for measuring Cu^{2+}, Cd^{2+} and Pb^{2+}. Using a negative E_{dep} (-0.8V) a stripping response was observed at *ca.* -0.2V and -0.25V for Pb^{2+} and Cd^{2+} respectively. A stripping response for Cu^{2+} was observed at *ca.* +0.4V following depositioning at 0V for 30 s.

This work is the first example of a truly disposable sensor for measurement of metals with stripping peak potentials more positive than Hg without the need for coating the electrode surface with metal entrapment/binding agent or a MFE. The data obtained during this study are promising and a patent has been filed. Due to the novelty of this research, the following recommendations are made for future work:

- Development and optimisation of multivariate calibration techniques to obtain quantitative data when measuring mixed metal samples.

- Evaluate effect of other co-existing metals on the stripping responses of As^{3+}, Hg^{2+} and As^{5+}_{RED}, due to the possibility of overlapping stripping peaks or the formation of intermetallic compounds.

- Development and optimisation of electrochemical assays for single and simultaneous measurement of Pb^{2+}, Cd^{2+} and Cu^{2+} using Au SPEs.

- Analysis of 'real' water and soil samples, including evaluation of field based soil extraction protocols.

CHAPTER 6
CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSIONS

The study comprised of four main parts. Firstly an *in situ* electrochemical screening sensor suitable for the simultaneous detection of Cd^{2+}, Pb^{2+} and Cu^{2+} in soil and water samples was developed. Secondly, to address the lack of existing field based soil extraction protocol, an alternative simple *in situ* soil extraction procedure was developed and evaluated using a soil certified reference material and a range of 'real' soil samples. Thirdly, field demonstration, using portable instrumentation, was undertaken at a technical workshop organised by the European Commission funded EU Thematic Network on 'Sensors for Monitoring Water Pollution from Contaminated Land, Landfills and Sediment (SENSPOL). Finally, a greatly simplified electrochemical assay, utilising disposable SPEs fabricated with a new curable Au ink, was developed for the detection of Hg and As.

The main conclusions from this project are summarised below:

***In situ* electrochemical screening sensor for detection of Cd^{2+}, Pb^{2+} and Cu^{2+}**

(1) Screen-printing technology provided a reproducible method for the fabrication of low cost disposable electrodes, negating the use of conventional 'bulky' electrochemical cells which are unsuitable for *in situ* analysis. The use of disposal electrodes also negates problems associated with electrode fouling and cleaning, simplifying experimental protocols.

(2) The carbon ink used to fabricate the SPEs strongly affected the stripping performance of the fully optimised sensor, including sensitivity, reproducibility and background noise. Acheson Electrodag 423 SS was selected as the most appropriate carbon ink for the development of the electrochemical sensor, based on these criteria.

(3) The combination of disposable SPE and DPASV, together with simple experimental protocols, has provided an *in situ* electrochemical screening sensor capable of simultaneous detection of Cd, Pb and Cu, obtaining detection limits of 9.1 μg l^{-1}, 1.4 μg l^{-1} and 32 μg l^{-1} respectively, with a RSD of less than 8%.

***In situ* soil extraction procedure and soil sample analysis**

(4) Primary considerations during development of the *in situ* soil extraction procedure were speed, simplicity and extraction stability. A 1 mol l^{-1} aqua regia extractant and a rapid sample agitation method were selected to meet this requirement. Using a 3 min sonication resulted in improved accuracy and precision compared to a 3 min manual shake. Recoveries of 64%, 52% and 57% and RSD of 7%, 5% and 8% (n=10) for Cd, Pb and Cu, respectively, were obtained using a soil CRM. The recovery values were a consequence of extraction efficiency, although a degree of analyte stripping peak depression, due to sample matrix effects, was also observed. However, semi-quantitative data, adequate for most screening applications, was obtained by using a simple direct calibration method, without the need for more complicated methods, such as electrode polymer modification or standard addition.

(5) Comparative analysis of 82 soil samples showed good correlation with ICP-MS analysis. Although the *in situ* analysis appeared less effective than laboratory methods, which provide increased accuracy and precision, the combined sample extraction-DPASV *in situ* method successfully classified soils according to their metal content. The choice between the less accurate and precise but reduced cost and improved logistics offered by the *in situ* screening extraction + sensor and the higher cost, improved accuracy and higher precision offered by the centralised inflexible laboratory method will depend on the nature of the study at the time.

In many monitoring and assessment studies, the analyst is most concerned with rapid site screening, in which situation; the data suggests the *in situ* extraction + ASV method to be a valid option. The novel site characterisation data analysis tool (FASA), developed by partners within the PURE programme, was able to map the spatial distribution of contamination using data obtained by the *in situ* screening extraction + sensor, offering a more effective tool for rapid site characterisation.

Field demonstration using soil extraction procedure and soil sample analysis

(6) The *in situ* electrochemical screening sensor successfully demonstrated simultaneous analysis of Cd, Pb and Cu, in a range of soil types, extractant solutions and across a range of concentrations. Toxic 'hotspots' were located, with samples taken from contaminated areas i.e. dump and workshop areas, identified as containing high contaminant levels, whilst those taken from surrounding areas where either no history of mining was evident or where toxic floods had not previously occurred were recorded as having low contaminant levels.

The method was rapid and simple to perform in field conditions, using battery operated portable instrumentation. Pre-screening offers immediate decision-making potential usually hindered by central laboratory sample analysis, whilst reducing the cost/amount of samples ultimately being sent to a central laboratory for more in-depth analysis.

Gold electrodes for *in situ* metal analysis

(7) The novel low temperature curable screen-printable Au ink provided a means of producing single use Au SPEs in large quantities using simple and low cost screen printing technology. This is an improvement in the state-of-the-art as existing schemes require substrates that can undergo curing temperatures in excess of 900 °C. The use of disposable Au SPE significantly reduces the complexity of electrochemical assay as no expensive and time consuming electrode cleaning procedures are necessary. From searches conducted by the present author, no references were identified in which Au SPEs had been used.

(8) No adulteration of the electrode surface with Hg or other metal entrapment/binding agents was required. Hence, the electrochemical measurement of As and Hg was greatly simplified, providing a suitable method for field based analysis. This has previously been hindered due to the often complex nature of the

electrochemical procedures required for the analysis of As and Hg and is an advance on the current start of art.

(9) The rapid and simple DPASV procedure using Au SPE for the single analysis of Hg^{2+} allowed convenient quantification in the micrograms per litre range following a very short depositioning (LOD of 2.7 µg l^{-1} and 0.15 µg l^{-1} following a 30 s and 120 s accumulations). A dynamic range of 0-2000 µg l^{-1} and a RSD of less than 9% (n=10) was achieved. By using a positive E_{dep} of +0.2V no significant change in the Hg^{2+} stripping response was observed in the presence of a 2000-fold excess of Cu^{2+} (10 mg l^{-1} Cu^{2+}).

(10) The electrochemical assay for As^{3+} required a 2 mol l^{-1} HCl supporting electrolyte to achieve effective deposition and subsequent stripping of As^{3+}. A 4 mol l^{-1} HCl supporting electrolyte produced the best signal-to-background characteristics, with a limit of detection of 1 µg l^{-1}. Simultaneous detection of As^{3+} and Hg^{2+}, using optimised stripping parameters for As^{3+}, was demonstrated, with no significant adverse effects on the stripping responses evident.

(11) For speciation studies, As^{5+} was effectively reduced over a short (10 min heating at 75°C) reduction time using cysteine. A stripping response for As^{5+}_{RED} was obtained in 1 mol l^{-1} HCl supporting electrolyte, with an LOD of 42 µg l^{-1} following a 30 s depositioning period.

(12) Preliminary investigations indicated the Au SPE as suitable for measuring Cu^{2+}, Cd^{2+} and Pb^{2+} without the need for surface modification schemes such as the formation of a MFE. In this instance, the metals were deposited directly onto the bare Au SPE, providing a greatly simplified Hg-free procedure. Since Hg was omitted from the electrochemical assay, significant environmental and safety benefits were realised.

The Au SPE exhibited a wide potential window, with stripping responses observed for these analytes at both negative (Pb^{2+} and Cd^{2+}) and positive (Cu^{2+}) potential regions.

Optimisation of DPASV parameters and supporting electrolyte would serve to improve experimental conditions and provide greater sensitivity and better defined stripping peaks.

(13) The use of Au SPEs for ASV measurements of key heavy metals, coupled to multivariate calibration techniques such as PLS, is a potentially useful method for measuring mixed metal samples.

Effective environmental monitoring, remediation and control depend upon chemical analysis. Rapid field-based screening tools can facilitate this dependence in a number of ways: (1) by substantially increasing the depth of information available at the risk assessment Phase 1 stage (Part IIA, EPA Act 1990). This is used to create a conceptual model of a particular site and, based on the available information, will determine whether a Phase 2 risk assessment, including laboratory analysis, is required. At present, chemical analysis at the Phase 1 stage is considered superfluous due to the complex and costly nature of laboratory analysis; (2) as a tool to assess whether water treatment plants meet regulations. If the process is faulty, it can be shut-down immediately, preventing further contamination; (3) redevelopment delays, due to unexpected site conditions, can be minimised if a decision as to whether soil can be re-used or requires removal or 'clean-up' can be made immediately; (4) continual assessment to determine the efficiency of a remediation process can be facilitated more effectively using field based analysis. Transportation of samples to an off-site laboratory often results in drastic changes to the composition of the sample (i.e. biological activity).

The benefits of rapid real-time data are invaluable, and it could be argued that these benefits far outweigh issues regarding the level of precision and accuracy obtained. The site manager is concerned with determining whether a certain action is required, not the level of accuracy obtained compared to standard methods. In addition, changes that can occur to samples during transportation and the possibility of contamination (Section 1.5) can bring into question what the level of accuracy obtained at a centralised laboratory may actually represent. Does this data really relate to what is happening on-site? Therefore, it is vital for the field of analytical chemistry to continually research innovative field-based techniques. These issues have been addressed within this study with the development of a new, highly simplified methodology for measuring important toxic contaminants. Finally, practical concerns, upon which the implementation of the technology will ultimately depend, have been confronted.

6.2 RECOMMENDATIONS FOR FUTURE WORK

The following recommendations are made for future work:

(1) The development of a range of *in situ* soil extraction procedures, aimed at different metal fractions (i.e. soluble bioavailable) recovered by a water shake, should be carried out. This would expand the applicability of the *in situ* electrochemical screening sensor to a range of environmental soil studies, similar to laboratory based sequential extraction schemes, such as the well known Tessier method. A user friendly field-based extraction 'kit' containing all the necessary reagents and equipment would be a convenient way of providing data to meet the specific requirements of the end-user.

(2) Development and optimisation of electrochemical assays for single and simultaneous measurement of Pb^{2+}, Cd^{2+} and Cu^{2+} using the Au SPEs should be completed. This would provide an alternative Hg-free electrochemical method for these metals offering important benefits to end-users.

(3) The Au SPEs have exhibited stripping responses for As^{3+}, Hg^{2+}, Cd^{2+}, Pb^{2+} and Cu^{2+}. The development of one electrochemical assay, for the simultaneous analysis of all five metals, would have significant economic benefits to the end-user and would be a valuable tool for *in situ* pollution studies. The implementation of suitable multivariate calibration techniques would be a viable option to de-convolute overlapping stripping peaks for measuring mixed metal samples.

(4) To examine sample matrix effects upon the stripping responses of As^{3+}, Hg^{2+}, Cd^{2+}, Pb^{2+}, As^{5+} and Cu^{2+}, analysis of a range of 'real' water and soil samples should be undertaken. The efficiency of the developed *in situ* extraction procedures to extract As and Hg from the sample matrix, should also be evaluated.

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APPENDIX A1

PATENT FOR GOLD SCREEN PRINTED ELECTRODES

Method and apparatus for trace level determination of metals

Technical Field

This invention relates to a method and apparatus for elemental analysis, particularly the electrochemical detection and trace level quantification of metals and metal-like species. It particularly relates to a method of and apparatus for electrochemical stripping analysis.

Background Art

The classical electrochemical method for detection of metals and metal-like species in solution is stripping analysis, which can be of two fundamental types, amperometric or potentiometric. Examples of these techniques include, but are not limited to, anodic stripping voltammetry (ASV) and potentiometric stripping analysis (PSA). Examples of species which can be detected using these techniques may include; antimony, arsenic, bismuth, cadmium, copper, gallium, germanium, gold, indium, lead, mercury, silver, thallium, tin, zinc and chromium. Such species may be referred to as metals hereafter.

Both ASV and PSA involve the preliminary electrochemical deposition of the analyte metals onto a working electrode surface. Inherent in this process is a pre-concentration effect, at, or in the immediate vicinity of, the electrode surface region. In both ASV and PSA, electrochemical deposition is a reductive process and is achieved by poising the working electrode potential such that a cathodic (reducing) current is effected between the working electrode and analyte species. After reductive deposition the metals are re-oxidised; this process results in their re-solution and is consequently called "stripping" - the metals being stripped back into solution from the electrode. Monitoring and quantifying the stripping process is the way in which the metals are identified and analysed; the way in which the stripping is effected is the basis for the difference between ASV and PSA.

In ASV, after metal deposition, the potential is moved in a positive direction and the current is monitored. Each deposited metal is oxidised (stripped) at a characteristic potential (the stripping potential) and the value of this potential can be used to identify the metal. At the stripping potential, a current change can be recorded (the stripping current) which is an anodic current concomitant with the metal oxidation and proportional to its concentration. Consequently, by measuring stripping potentials and stripping currents, metals can be identified and quantified. If the potential is *swept* to a more positive potential, the metals are stripped sequentially and a series of stripping current peaks are obtained, each at a metal-specific stripping potential. The precise value of the stripping potentials depends upon the assay conditions and the type of electrode used.

PSA differs from ASV in that, after reductive deposition, the metals are allowed to re-oxidise either electrochemically or by way of chemical oxidants and the concomitant change in electrode potential is monitored. A typical result is a plot of (falling) potential against time (or some derivative thereof) in which the metals are characterised by periods in which the potential decay is slowed or arrested. The potential at which each such event occurs is characteristic of a particular metal (stripping potential) and the duration of the event (transition time) is proportional to the metal concentration.

A mercury electrode is conventionally used for both types of metal stripping analysis. The mercury electrode can be pre-formed from metallic mercury, as is the case with the classical mercury hanging drop electrode, or it can be formed *in situ,* during the deposition phase of the assay, by electrochemical reduction of soluble mercury ions at the surface of an electrode of some other material (such as carbon). Because the mercury is deposited at the same time as the analyte metals, this procedure is called co-deposition and patents exist for two different ways of doing this on screen-printed carbon electrodes (US 5292423 and WO 94/28405). In the first of these, the analyte sample is mixed with a solution of soluble mercury ions before the assay is begun. In the second, a dry layer containing a soluble mercury salt is coated across the surface of the electrode and is hydrated by the analyte sample when it is introduced.

Several types of electrode have been described for stripping voltammetry; these include gold, platinum, glassy carbon and wax-impregnated graphite, carbon paste and screen-printed carbon. The carbon-based electrodes can be electroplated with gold for the same purpose.

Examination of the prior art indicates that solid metal electrodes have been well utilised for the trace determination of metals by voltammetric or potentiometric means. Furthermore, prior art exists relating to the use of screen-printed carbon electrodes, utilised in conjunction with mercuric films or electroplated gold for the measurement of trace metals. In such cases, it is evident that the electrodes must either be pre-dosed with materials to provide the requisite metal deposition surface, or appropriate reagents must be added to the measurement solution to allow the real time *in situ* creation of the deposition surface in conjunction with the metals deposition process. Such methods have clear drawbacks in that mercury containing materials are required, posing a potential environmental issue and also a user-toxicity danger which renders the method less amenable to decentralised field-based usage. Additionally, the use of carbon working electrodes in conjunction with mercury containing materials, restricts the applicability of the method with respect to the determination of, for example, mercury itself and also other metals, such as arsenic, that would be stripped back into solution at potentials more electropositive than elemental mercury, at a given carbon working electrode.

The relatively limited potential window offered by the carbon electrode, utilised in conjunction with mercury films for metal deposition, prevents the measurement of mercury and metals that undergo stripping at more positive potentials than mercury. Thus, the method becomes limited with respect to the total number of different metals it can be used to detect. Screen-printed electrode assemblies that are not reliant on metal deposition films such as elemental mercury, therefore offer the possibility of wider potential windows and hence the determination of a wider number of metal species. Such screen-printed assemblies therefore may serve as useful screening tools for the semi-quantitative evaluation of the total metal content of a sample.

To date, the prior art does not identify any screen-printed electrode assemblies capable of direct metal analyte deposition onto a screen-printed electrode surface. Such an invention would have clear end-user and market applications. Indeed, an analysis of the literature identifies numerous applications in which it is desirable to be able to measure trace levels of heavy metals in field locations, without recourse to centralised analytical facilities. This may be achieved through the use of low cost and hence single-use disposable electrodes. Given these demands, nowhere in the prior art is there evidence of low-cost electrode assemblies being used for trace metal determination without some form of electrode surface adulteration to facilitate deposition (preconcentration) of metal analyte species.

Disclosure of the invention

The invention comprises a method for determining trace metals that involves the provision of a plurality of flat printed electrodes, with at least one of the said flat printed electrodes serving as a site for deposition of target metal analyte species, without the requirement for subsequent electrode adulteration to facilitate metal analyte deposition. Samples are subsequently analysed for heavy metal content with said plurality of electrodes. At least one of the flat screen-printed electrodes serves to provide a reference potential, being for example an Ag/AgCl reference electrode. At least one of the electrodes is fabricated from a screen-printed or otherwise deposited film of a noble metal, such as gold.

The sample analysis procedure for the determination of heavy metal content may be voltammetric or potentiometric or any other electrochemical stripping method. The use of a deposition step may be used to preconcentrate target metal species upon one or more electrodes. Tests may be performed by applying appropriate volumes of test solution to the plurality of electrodes, which may be as little as microlitre volumes if desired. Alternatively, the electrode plurality may be submerged in a test solution.

Samples may be tested with or without stirring (or other method of agitation, such as shaking or sonication) as required and with or without deaeration as required.

The invention may enable the provision of disposable single-use electrodes for trace metal detection.

The invention may enable the provision of an apparatus or instrument, to which the electrodes can be connected, for applying, measuring and manipulating the electrical potentials and currents required for the analysis. Furthermore, the apparatus or instrument may be sufficiently compact to be easily portable.

The invention may enable the provision of a trace metal testing apparatus that is compatible with the usage of small (microlitre) volumes of sample. The approach is compatible with the determination of samples that have not been de-aerated, stirred or otherwise manipulated to facilitate the measurement process.

The invention may enable the use of low-cost electrodes for metal determination. The low cost and ease of mass manufacture of the devices may mean that the electrode assembly may be for single use or may be reused if desired. Disposability has the added advantage of removing the requirement to regenerate or clean fouled or otherwise altered electrode surfaces which is a further encumbrance when working with traditional electrode materials.

Use of the invention may lead to simplicity and rapidity of the assay procedure, which allows the apparatus to be readily used in decentralised or "field" locations without recourse to centralised facilities. However, The invention may be used within centralised locations if required.

Desirably the invention makes use of screen-printable inks that can be cured and stabilised onto a variety of backing substrates that can be subsequently used for the intended measurement purpose.

Desirably use is made of low temperature curing gold inks that do not require elevated temperatures to form stable structures for subsequent usage within liquidic sample solutions. Low temperature curing offers the advantage that the apparatus may employ backing substrates, e.g. plastic materials, that may otherwise become deformed or otherwise damaged when subjected to elevated temperature conditions.

The sample to be analysed may be extracted or pre-treated by appropriate physico-chemical means before it is brought into contact with the electrodes.

A benefit of using screen-printed noble metals, such as gold, over the materials used in the prior art is the fact that heavy metal species may be directly deposited out of solution onto the gold (noble metal) electrode surface without the need to adulterate or modify the screen-printed gold surface in any way. This differs from the prior art in which (a) mercuric ions must be added to the test solution and are then reduced onto the screen-printed electrode surface (usually carbon or similar) thus depositing metallic mercury on the electrode surface in order to allow depositioning of other (analyte) metals; (b) electrodes are microdosed with a layer of a mercuric salt and dried, thereby forming a mercury film during the depositioning process; or (c) electroplating of screen-printed electrodes (usually carbon) with gold or other nobel metal, thereby creating essentially gold electrode surfaces with underlying carbon contact pads.

A plurality of screen-printed electrode structures may be fabricated. Such an architecture may consist of one or more working (noble metal) electrodes, one or more screen-printed reference electrodes, most likely of the Ag/AgCl type, and one or more counter or auxiliary electrodes as required. In its simplest format, the electrode assembly would consist of one noble metal (gold) electrode and one reference electrode, both screen-printed.

The above assembly may be used for trace level determination of heavy metals in solution and provides both qualitative and quantitative information.

In one embodiment of the invention, the noble metal material, specifically gold material, may require high temperature curing to allow formation of a stable gold electrode preparation suitable for the intended end usage in liquidic solutions. In such situations, all other materials used to fabricate the electrode assembly, including the backing substrate material should be resistant to the applied heat. In such instances ceramic material or other suitable backing substrate may be considered.

In a more preferred embodiment of the invention, the noble metal material, specifically gold material, may not require high temperature curing to allow formation of a stable gold electrode preparation suitable for the intended end usage in liquidic solutions. In such situations, a lower requirement for thermal stability of all materials used in the electrode assembly preparation is evident. This allows electrode assemblies to be printed onto heat labile backing materials, e.g., plastic-based materials such as polyester or polyvinyl chloride, provided adequate material adhesion can be achieved.

The low cost and ease of mass manufacture of the screen-printed noble metal electrodes renders them disposable. Such an approach removes issues associated with extensive electrode preparation steps (electroplating of gold) or the use of mercuric salts in electrode preparation, undesirable from both environmental and toxicological viewpoints, allowing the usage of such devices in decentralised locations with a simplified measurement technology.

The test method may be voltammetric (to include anodic stripping voltammetry which may include linear voltammetric waveforms, normal pulse voltammetric waveforms or square wave voltammetric waveforms including cathodic stripping voltammetry or any other electrochemical stripping analysis procedure), or may consist of a potentiometric stripping analysis procedure.

The screen-printed noble metal provides a surface suitable for the accumulation of metals from the test solution during the sample deposition step. The inherent physico-chemical properties of the material allow heavy metals to be preconcentrated from solution onto the electrode surface throughout the deposition process. To a certain extent, prolongation of the deposition step influences the extent of heavy metal deposition at the electrode surface(s). The preconcentration step may also be in the form of an adsorptive accumulation step.

Samples may be applied as droplets of any suitable volume, most likely microlitre volume, directly onto the active electrode assembly such that all of the active areas of the exposed parts of the screen-printed electrode elements are in contact with said sample solution. The solution may be deaerated as required.

Alternatively, sample-electrode assembly contact may be achieved through suspending the electrode assembly in the sample solution to be measured. The solution may be stirred and/or deaerated as required.

The invention may be used to assess the heavy metal content of any liquidic solution. Specifically, the invention may be used to determine the heavy metal content of urine or any other body fluid, biomaterial or other material from which a liquid same can be prepared, such as hair samples, water, drinking water, waste water, process water and other waters; soil and sediment liquid extracts, liquid components of foods, such as heavy metals in fish products and liquid traps in which heavy metal species are collected from gaseous samples, including exhausts and emissions.

The screenprinted gold electrodes can be used to detect and quantify metals in biological fluids and materials such as urine, blood, saliva, hair, skin, nails and teeth. This may be particularly useful in the *medical diagnosis* of potentially harmful levels of any metal or metals in the human body or any part of the human body. In this respect, it may be especially useful for screening populations of individuals for their content of any metal or metals. It may also find application in the monitoring of populations deemed to be at some risk of exposure to a particular metal or metals, for example, populations of workers whose occupation may carry some risk of such exposure.

Use of screenprinted gold electrodes for detection or quantification of metals in biological materials need not be restricted to the field of medical diagnosis or the assessment of human health. There is also an important application in testing biological materials which are intended for human consumption as *foodstuffs*. The source of these materials may be animal (for example; cattle, sheep, pigs, poultry, fish and milk) or plant (for example; vegetables, fruit, grain). The metals present in, or contaminating the biological materials under test, may have accumulated during the lifetime of the relevant organism or may have been introduced after slaughter or harvest by way of cooking, storage, transport, processing, preservation or packaging.

Screenprinted gold electrodes may also be used for detection/quantification of metals in *animal feeds* (such as grain products, fish products, offal and other abattoir products, human food waste) and also plant fertilizers or other soil fertility enhancers such as sewage or silage or organic or inorganic wet or dry composite preparations or treatments. One application of such use might be the prevention of metals accumulation in humans by way of investigation or monitoring of potential or actual pathways of human foodborne metal contamination.

Another application of this technology in relation to metals detection in biological materials could concern testing non-food materials of a *biological origin or else containing some component of a biological origin.* Such materials frequently have direct or indirect human contact or useage and in some circumstances it may be deemed necessary to determine their metals content, possibly in order to assess their potential toxicity or risk to human health. Examples are cosmetics (for example, shampoos, soaps, cremes and perfumes), medicines or medical preparations (for example linctuses, pills, lotions, ointments, tonics), paper or cardboard packaging for food or other materials, clothing, bedding, soft furnishings, furniture, flooring, carpeting, wallpaper, paint, varnish, panelling or tiling.

The invention allows both quantitative and qualitative information to be obtained regarding the heavy metals composition of a sample.

The quantitative and qualitative information obtained by the method allows the simultaneous detection of two or more metals in the same test solution. The invention has use as a method for providing an indication of the total heavy metals content of a given sample.

The device may be used in combination with other electrode materials, such as carbon inks, to allow more complex measurements to be performed.

Further, the method can be combined with appropriate data treatment procedures, such as chemometrics and machine learning, to allow complex voltammetric or potentiometric profiles to be interrogated with respect to the qualitative and/or quantitative composition of the solution under test.

The measurement procedure utilises the unadulterated surface of the screen-printed electrode element as a means of direct deposition and pre-concentration of metals from solution. The method is therefore not limited with respect to the potential window otherwise dictated by the use of additional deposition films, such as mercury. The method is therefore amenable to measuring those metals undergoing oxidation at more electropositive potentials and is therefore more useful as a means of semi-quantitatively determining the total metal content of a sample.

The measurement procedure and electrode assembly can be under the control of a measurement apparatus that is both portable and simple to use, if necessary containing on-board software to allow requisite measurements to be performed, analysed and stored as appropriate. The device may be capable of performing both stripping voltammetric and potentiometric stripping analysis as required.

Brief Description of drawings

Fig 1(a) is an anodic stripping voltammogram ("ASV") showing detection of mercury ("Hg") and a blank ("B") at a screen-printed gold electrode.

Fig 1(b) is an ASV corresponding to Fig 1(a) determined with a solid gold disc electrode.

Fig 2(a) shows a set of ASVs for different mercury concentrations (i), and a corresponding graph (ii) of peak area vs concentration.

Fig 2(b)(i) and (ii) correspond to Fig 2(a)(i) and (ii) for a smaller range of concentrations (10-400μg/l).

Fig 3(i) and (ii) correspond to Fig 2(a)(i) and (ii) for experiments with longer deposition times.

Fig 4(i) and (ii) correspond to Fig 2(a)(i) and (ii) but relate to the determination of arsenite.

Fig 5 shows a set of ASV, for mixtures of arsenite and mercury.

Fig 6(i) and (ii) correspond to Fig 2(a)(i) and (ii) and were determined for solutions of reduced arsenate.

Figs 7, 8 and 9 show ASVs for Cd, Pb and Cu respectively.

Fig 10 is a schematic view of an apparatus embodying this invention.

Modes for carrying out the Invention

The preferred device consists of a plurality of planar electrodes screen-printed, or deposited by an analogous method such as stencil depositing, onto a suitable supporting material. Trace metal analysis is achieved through the application of suitable voltammetric or potentiometric stripping procedures. Electrochemical measurements were performed using either a computer controlled Autolab PGSTAT-10 (Eco Chemie, Utrecht, Netherlands) with a general-purpose electrochemical software operating system (GPES3) or a hand-held PalmSens electrochemical analyser (Palm Instruments BV, Utrecht, Netherlands).

Many voltammetric and potentiometric stripping analyses have been performed either by applying discrete volumes of test solution directly to the planar electrode assembly or by suspension of the assembly in the test solution, typically of 2-20 ml capacity. The preferred measurement protocol involves the application of microlitre or multiple microlitre volumes of test solution directly to the plurality of printed planar electrodes. Alternatively the electrode assembly may be suspended in test solutions (held within test cells if so required). However the first approach, not requiring immersion, is well suited to simple, rapid decentralised testing operations. Stripping analyses (voltammetric and potentiometric) were performed using the PGSTAT-10 device. A number of voltammetric experiments were performed using the PalmSens electrochemical analyser.

The electrode assembly may be fabricated by any suitable printing process that involves the deposition of conducting and insulating preparations, as appropriate, onto suitable supporting substrates. The preferred embodiment of the device involves the use of screen-printing techniques to sequentially deposit the requisite electrode assembly preparation materials onto selected substrates.

The screen-printed electrodes used to illustrate the applicability of the invention consisted of a planar 3-electrode assembly 8 (see Fig 10). The electrode strips comprised 3 electrodes – a working electrode 10 fabricated from a proprietary printable gold-containing preparation, carbon counter electrode 12 and Ag/AgCl reference electrode 14. Underlying conducting tracks 16 were fabricated from carbon-based material. The backing substrate 18 was based on a plastic (polyester) material. The uppermost printed layer 20 consisted of an insulation shroud with a circular aperture (target area) 22 to allow exposure of the working, reference and counter electrodes to the test solution whilst protecting the underlying conducting tracks from the test solution. The hydrophobic nature of the insulation shroud allowed application of appropriate volumes (typically 50-100 microlitre) of test solution to the target area such that the solution was contained within the circular aperture and directly over the exposed portions of the printed electrode elements (Fig 10 actually shows the assembly 8 immersed in a test solution 24 in a vessel 26). An optional stirring means (stirrer bar 28 and magnetic stirrer unit 30) and a deaerating means (tube 32 for bubbling nitrogen) are shown in phantom. The electrodes 10, 12, 14 are connected to an electrochemical analysis operating system 34.)

Most experiments were performed using the 3 electrode elements. A number of experiments were performed using only a 2 electrode system in which the counter electrode was excluded from the circuit and its function was performed instead by the Ag/AgCl reference electrode, which was in this case working as both counter and reference electrode. The working electrode was always placed in direct proximity to the reference electrode.

Conventional electrodes were used for comparative analysis and included a platinum (Pt) wire counter electrode and Ag/AgCl solid reference electrode.

All solutions were prepared using HPLC grade water. The metal-containing test solutions were atomic absorption standard solutions purchased from commercial organisations. High purity standard/stock solutions of 1000 mg l^{-1} were used to prepare 50 mg l^{-1} working standards for each analyte of interest. Standard mixtures were prepared at the required concentration, in 0.1 M potassium chloride (KCl) and 2-4M nitric acid (HNO_3) for subsequent ASV analysis. Potassium chloride served a dual purpose as a supporting electrolyte, and to stabilise the reference potential of the Ag/AgCl electrode. Nitric acid was used to obtain a low and relatively constant pH and ensure the solubilisation of the analytes being determined. Urine samples were collected from a healthy volunteer.

The preferred anodic stripping voltammetry (ASV) and potentiometric stripping analysis (PSA) procedures will now be described. Both types of procedural method involved a common deposition stage followed by the relevant stripping steps. In a typical procedure one hundred microlitre volumes of test solution containing nitric acid and supporting KCl electrolyte were applied to the 3 electrode assembly, forming a solution bead bounded by the edge of the electrode target area. The exact test solution volume may be varied according to experimental requirements. Metals were deposited from solution onto the gold working electrode by application of a suitable deposition potential (varied according to the analyte of interest as illustrated in the examples to follow) for a fixed period of time (again varied according to the analyte of interest and preferred detection limit as illustrated in the examples to follow). Experiments were usually performed on non-deaerated and non-agitated samples. In another type of embodiment of the procedure, the electrode assembly may be suspended in a suitable volume of test solution, containing nitric acid and supporting KCl electrolyte, within an electrochemical cell. The sample may be deaerated and agitated if so desired. (See Fig 10.)

All stripping steps were performed on quiescent solutions. In the voltammetric procedure, the potential was scanned, usually (but not necessarily) with a differential pulse waveform, from the depositioning potential to a more positive potential, at a fixed scan rate. The scan was stopped at a potential dependant upon the analytes of interest, as illustrated in the examples to follow. Potentiometric stripping was performed by application of a constant oxidation current, typically in the region of 1 microAmp, but this may be varied according to application.

In a typical procedure, each printed electrode assembly was used once before disposal. Such an approach is possible given the ease and low-cost of mass manufacture of the screen-printed electrode assemblies.

Example 1

The electrochemical detection strip (approx. 1cm x 3.5cm) was screen-printed on polyester (125μm) and comprised 3 electrodes; gold (working electrode); carbon (counter electrode); Ag/AgCl (reference electrode). An example of the utility of this invention is indicated by the following case of metal detection; in this case, mercury in aqueous solution. Figure 1 compares stripping voltammograms for an unstirred and non-deaerated solution containing 100μg/l mercury and a reagent blank at a screen-printed gold electrode (Figure 1(a)) and a solid gold disk electrode (Figure 1(b)). Further (a) used a screen-printed Ag/AgCl reference electrode, while a conventional Ag/AgCl electrode was employed in (b). Metal deposition was carried out at +0.2V for 30 seconds before the potential was swept to +0.7V. A differential pulse waveform of 20 mV/s scan rate and amplitude of 50 mV was applied. The gold screen-printed electrode of the invention exhibits a well-defined stripping response for mercury and low background current. A short (30 seconds) preconcentration time allows quantification in the micrograms per litre range. Comparison to the more traditional solid gold disc electrode indicates that the gold screen-printed electrodes of this invention are less affected by the use of unstirred and non-deaerated solutions, exhibiting lower background currents.

Example 2

A screen-printed detection strip as used in Example 1 was connected (in a flat, horizontal position) to an electrochemical analyser and a drop (100µl) of suitably pre-treated sample (containing mercury) placed across the three electrodes. Figure 2(a) illustrates differential pulse anodic voltammetric stripping responses for gold screen-printed electrodes for unstirred and non-deaerated solution of increasing mercury concentrations, from 10-2000 µg/l. Metal deposition was carried out at +0.2V for 30 seconds before the potential was swept to +0.7V, stripping out the mercury at around 0.4V. Stripping peak areas were calculated and plotted against the mercury concentration in µg/l (parts per billion). In this analysis, each electrode was used once and discarded. Fourteen concentration increments from 0-2000 µg/l resulted in a linear calibration plot with a correlation coefficient of 0.9971.

Linearity was maintained in the concentration range of 0-400 µg/l yielding a correlation coefficient value of 0.9989 (Figure 2(b)). An LOD, calculated according to the IUPAC convention ($S/N = 3$) using a 20µg/l mercury solution was calculated as 2.7 µg/l following 30 s depositioning. The reproducibility was estimated from a series of 10 successive measurements of a 10 and 100 µg/l mercury solution. Mean I_p values of 0.05µA and 0.0044µA with RSD of 8.4% and 9.1% for 100 and 10 µg/l mercury respectively, were achieved using a deposition time of 30 seconds.

Example 3

To achieve a lower limit of detection, the mercury deposition time was increased to 120 s. All other parameters were identical to those in Example 2. Figure 3 shows linearity was maintained in the range 0-10µg/1, yielding a correlation coefficient value of 0.9989. A limit of detection, calculated according to the IUPAC convention

(*S/N* = 3) using a 10µg/l mercury test solution was calculated as 0.15 µg/l. The use of gold screen-printed electrodes in this invention has provided a low cost technique for the detection of Hg. The experimental protocol is simple to perform, allowing semi-skilled personnel the ability to rapidly measure Hg in field conditions.

Example 4

Figure 4 illustrates the applicability of the gold screen-printed electrodes to the analysis of Arsenite (As^{3+}). The gold screen-printed electrode was connected to the analyser and a 100µl sample droplet placed onto the gold screen-printed electrode such that is completely covered the 3-electrode assembly. A differential pulse waveform of 20 mV/s scan rate and amplitude of 50 mV was applied. Figure 4 illustrates the voltammetric stripping responses for gold screen-printed electrodes for unstirred and non-deaerated solution of increasing arsenite concentrations, from 0-800 µg/l in 4 M HCl supporting electrolyte. Metal deposition was carried out at 0V for 30 seconds before the potential was swept to +0.5V, stripping out the arsenite at around 0.15V. Stripping peak areas were calculated and plotted against the arsenite concentration in µg/l (parts per billion). In this analysis, each electrode was used once and discarded. The calibration plot was linear with a correlation coefficient value of 0.9939.. A relative standard deviation of <10% was obtained (n=6) at each arsenite concentration. The limit of detection, calculated according to the IUPAC convention (*S/N* = 3) using a 50µg/l arsenite test solution was calculated as 1 µg/l. The gold screen-printed electrode of the invention exhibits a well-defined stripping response for arsenite and despite the short (30 seconds) deposition time allows quantification in the micrograms per litre range.

Example 5
Figure 5 illustrates stripping voltammograms of an unstirred and non-deaerated solution containing equal concentrations of arsenite and mercury in the range of 0-800 µg/l in 4 M HCl supporting electrolyte. Metal deposition was carried out at 0V for 30 seconds before the potential was swept to +0.7V, stripping out the arsenite at around +0.15V and mercury at +0.4V. The calibration plot was linear with a correlation coefficient value of 0.9824 and 0.9885 for arsenite and mercury respectively. The gold screen-printed electrode of the invention exhibits a well-defined stripping responses and good resolution between the stripping peaks of arsenite and mercury at these concentrations.

Example 6
The measurement of both arsenite (As^{3+}) and arsenate (As^{5+}) is important for environmental toxicity studies. For the speciation measurement of As^{5+}, a pre-reduction step, to reduce As^{5+} to As^{3+}, henceforth referred to as As^{5+}_{RED}, is necessary. This was performed by adding 0.1ml 20% v/v cysteine to test solutions containing As^{5+}, and heating at 75°C for 10 minutes. The reduction procedure was simple and rapid (10 min) to perform and by using a commercially available portable heating system, could easily be carried out under field conditions. Following reduction of As^{5+} □□As^{5+}_{RED} a calibration plot (Figure 6) of the As^{5+}_{RED} stripping response versus its concentration in the range of 0-800 µg l^{-1}, in 1 M HCl supporting electrolyte, yielded a correlation coefficient value of 0.9852. Metal deposition was carried out at -0.3V for 30 seconds before the potential was swept to +0.5V, stripping out the As^{5+}_{RED} at around 0.25V. A RSD of □15% was obtained (n=6) at each concentration increment. The limit of detection, calculated according to the IUPAC convention (*S/N*

= 3) using a 100μg/l $As^{5+}{}_{RED}$ test solution was calculated as 42 μg/l. The voltammograms illustrated in Figure 6 show a different shape of signal was obtained when analysing the pre-reduced $As^{5+}{}_{RED}$ than the original trivalent As^{3+}. The stripping peaks obtained were broader and shifted to more positive potentials. These preliminary findings have demonstrated the applicability of the sensor to determining the less toxic As^{5+} when speciation studies are necessary.

Example 7

Metals such as cadmium, lead, and copper are generally measured by amalgamation in a mercury film electrode. In this instance, the metals are deposited directly onto the bare gold screen-printed working electrode, providing a mercury free procedure. The gold screen-printed electrode was connected to the analyser and a 100μl sample droplet was placed onto the gold screen-printed electrode such that completely covered the 3-electrode assembly. A differential pulse waveform of 20 mV/s scan rate and amplitude of 50 mV was applied. Figure 7 illustrates the voltammetric stripping response for 200 μg/l lead, while Figure 8 shows the response of 1mg/l cadmium solution; both in 0.1M KCl and 1%v/v nitric acid supporting electrolyte, at the gold screen-printed electrodes. Reagent blanks are also shown. Metal deposition was carried out at -1.1V for 165 seconds before the potential was swept to 0V, stripping out lead at around -0.2V and cadmium at around -0.3V. Figure 9 illustrates stripping voltammograms for copper (0.2-1mg/l) following a 30 second deposition at 0V. The gold screen-printed electrode of the invention exhibits a wide potential window, with well-defined stripping responses for these metals at both negative (lead and cadmium) and positive (copper) potentials. These preliminary findings have demonstrated the applicability of the gold screen printed electrodes to determining a range of metals environmentally important metals.

Example 8

A sample of fresh urine was spiked with a solution of mercuric nitrate such that it contained 100 ppb mercury. The concomitant urine dilution was 1 %. This spiked urine was then diluted tenfold with a solution of 6% (v/v) nitric acid containing 0.1M KCl. An unspiked urine sample was also diluted in the same way. Aliquots (5 ml) of both spiked and unspiked acidified urine were then sealed in 20 ml screw-top glass Universal containers and autoclaved for 15 min at 120°. After cooling, samples were tested for mercury content by placing 100µl on the target area of a gold screenprinted electrode which was held horizontally and connected to an Autolab electrochemical workstation programmed to perform the ASV analysis. The three mercury-containing urine samples all showed a pronounced stripping peak at approximately 0.375 V, the two urine samples lacking mercury showed no stripping peaks. ASV parameters were as follows: deposition potential, 0.2V; deposition time, 30s; modulation time, 0.05s; interval time, 0.5s; final potential 0.7V; step potential, 0.00495V; modulation potential 0.04995V.

Claims

1) Apparatus for use in electrochemical stripping analysis, said apparatus comprising an electrode assembly including at least a working electrode and a reference electrode constituted by films deposited on an insulating substrate, wherein the working electrode is a noble metal electrode.

2) Apparatus according to claim 1 wherein said noble metal electrode contains as the noble metal gold, silver, platinum, palladium, iridium, rhenium, ruthenium or osmium.

3) Apparatus according to claim 2 wherein said noble metal is gold.

4) Apparatus according to any preceding claim wherein at least the noble metal electrode was deposited by screen printing or stencil depositing.

5) Apparatus according to claim 4 wherein at least the noble metal electrode was deposited by screen printing.

6) Apparatus according to any preceding claim wherein the electrode assembly provides said working and reference electrodes and a counter electrode.

7) Apparatus according to any preceding claim wherein the insulating substrate bears the electrodes and conductive tracks for conveying currents between respective electrodes and edge regions of the substrate, and there is an insulating layer overlying

the tracks and having an opening through which at least part of each electrode is exposed.

8) Apparatus according to any preceding claim including an electrochemical analysis operating system coupled to the electrodes.

9) A method of analysis of at least one metallic species in a solution comprising apparatus according to any preceding claim; and carrying out electrochemical stripping analysis to determine the species quantitatively and/or qualitatively.

10) A method according to claim 9 wherein said analysis includes a preliminary step of effecting deposition of the or each species to be analysed onto the working electrode.

11) A method according to claim 10 wherein said deposition is electrochemical.

12) A method according to any of claims 9-11 wherein said analysis is anodic stripping voltammetry.

13) A method according to any of claims 9-11 wherein said analysis is potentiometric stripping analysis

Abstract

An electrode assembly (8) for determining metals by electrochemical stripping analysis consists of film electrodes (10, 12, 14) deposited, e.g. by screen printing, on an insulating substrate (18). The working electrode (10) is of noble metal, preferably gold, applied by the screen printing or analogous process. In analysing a solution of a metal analyte, the metal may be electrolytically deposited on the working electrode, and then stripped off, with monitoring of the stripping current and/or potential.

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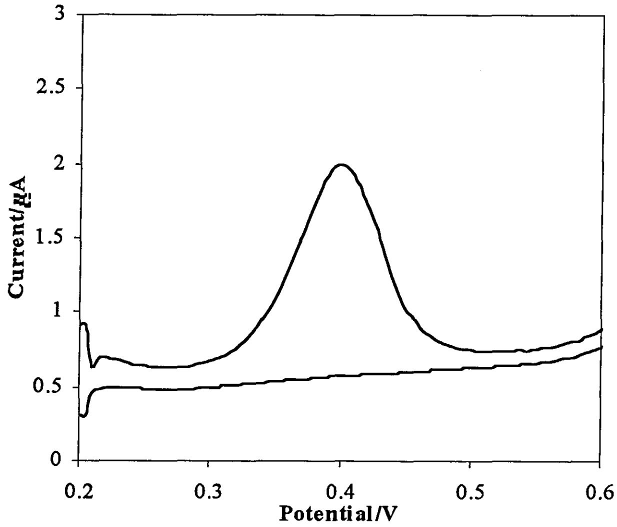


FIGURE 1

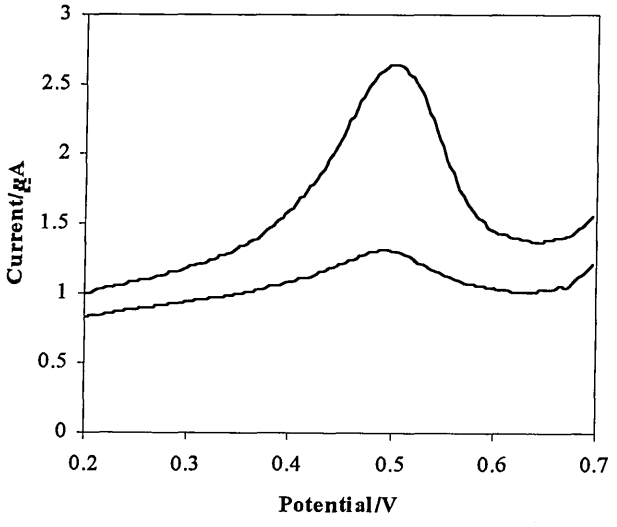


FIGURE 1(b)

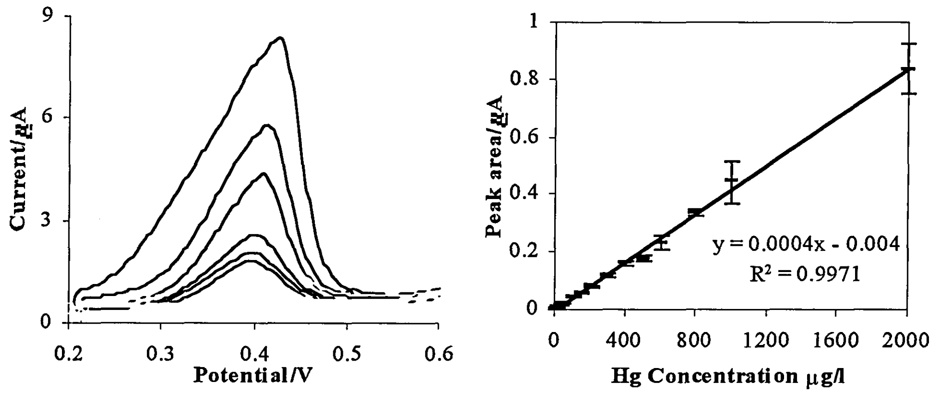


FIGURE 2(a)

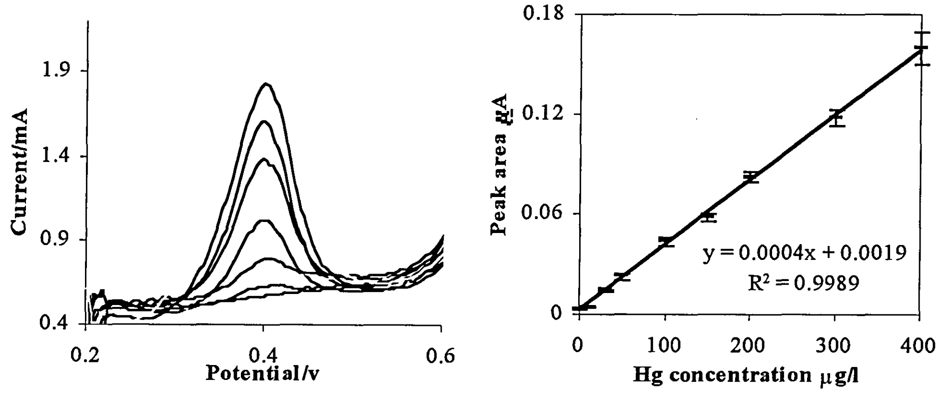


FIGURE 2(b)

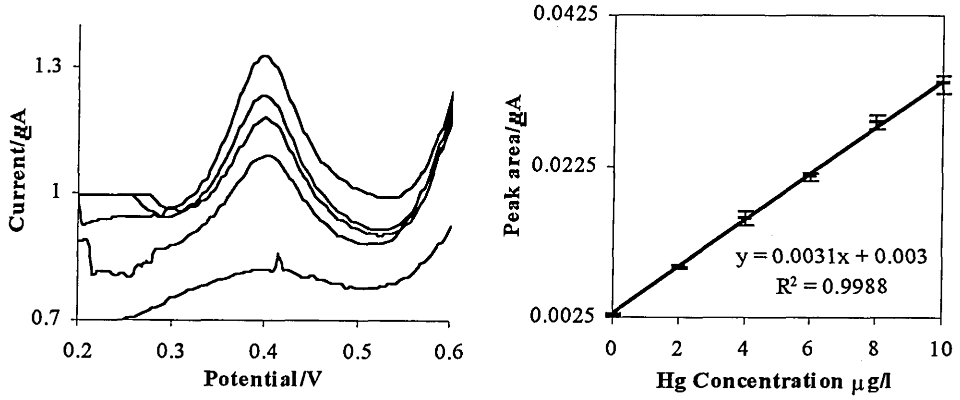


FIGURE 3

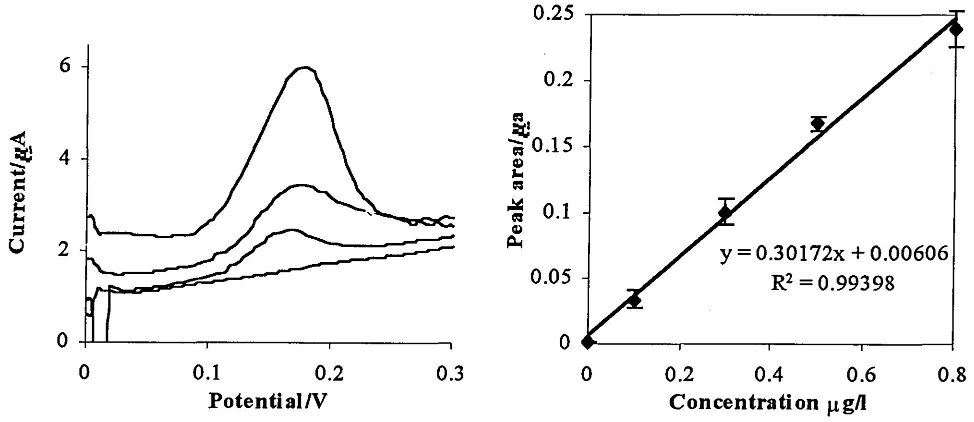


FIGURE 4

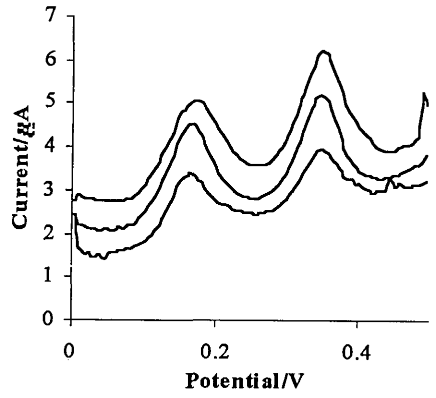


FIGURE 5

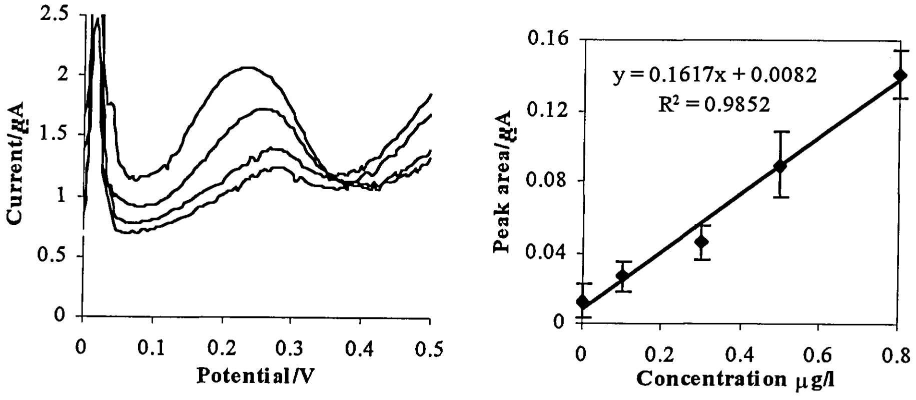


FIGURE 6

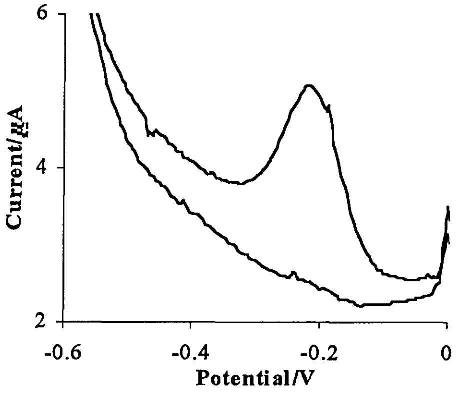


FIGURE 7

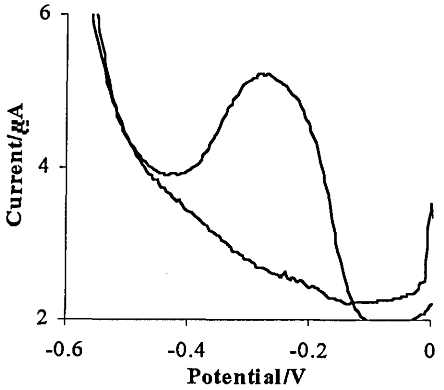


FIGURE 8

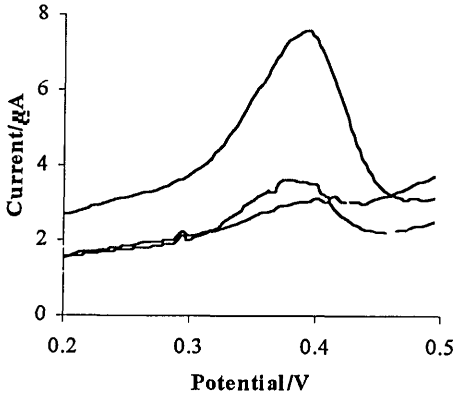
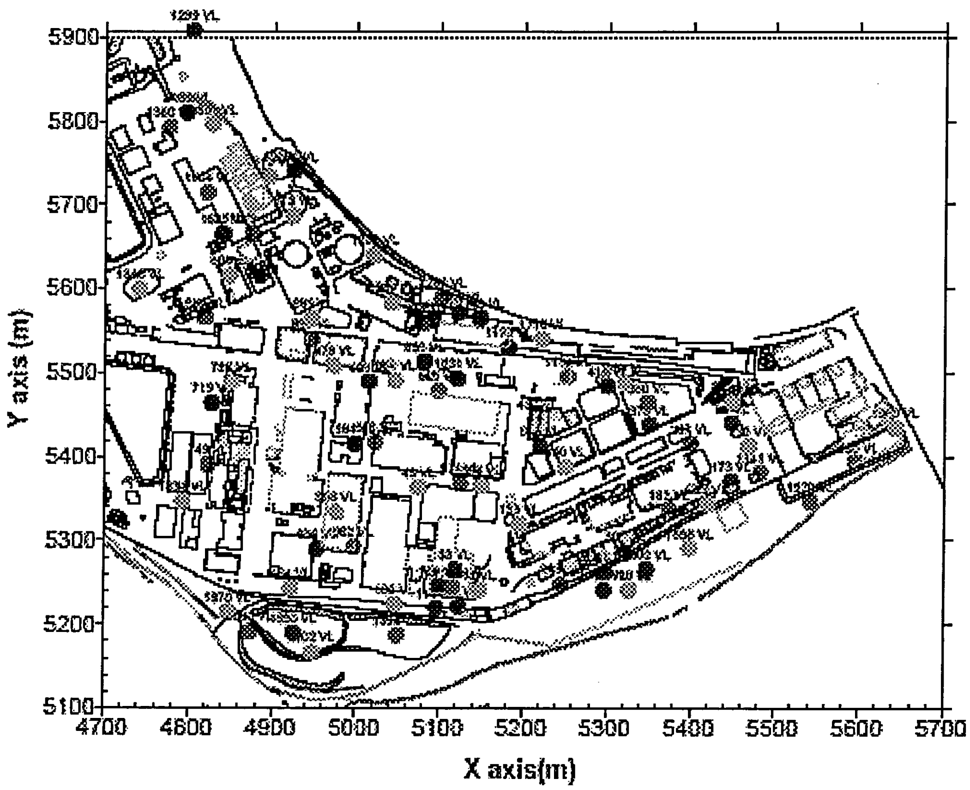


FIGURE 9

APPENDIX A2

SITE MAP AND STANDARD LABORATORY ANALYSIS FOR PURE SOIL SAMPLES

Site map showing location of boreholes where soil samples of the first set (25, red circles), and the second set (30, green circles, 27, blue circles) were collected.



ICP-MS standard laboratory analysis values for first set (25) of soil samples supplied by PURE project partners, mg kg^{-1} dried soil

x_map	y_map	Boring	Sample_ID	As	Cd	Hg	Pb	Cu	Zn
5116.512	5244.68	A1.S.11.9	116 VL	21	0.4	0.3	39	66	115
5322.06	5287.016	A2.S.17.9	305 VL	44	0	0.2	141	25	46
5374.121	5337.106	A2.S.35.9	183 VL	33	0.5	0.1	88	31	56
5245.328	5248.046	A2.S.5.9	285 VL	17	0.4	0.5	32	27	68
5482.408	5381.967	A2.S.57.9	1111 VL	27	0.1	0.2	29	29	134
5398.851	5416.423	A2.S.62.9	237 VL	24	0.2	0.1	29	18	73
5216.331	5445.51	A3.S.12.9	430 VL	31	0.3	0.4	51	12	41
5347.977	5465.138	A3.S.25.9	380 VL	29	0	0.1	118	22	34
4996.706	5291.506	A4.S.17.9	962 VL	49	0	0.1	29	18	257
5124.354	5368.525	A4.S.35.9	524 VL	11	0	0.8	15	11	44
5022.414	5417.713	A4.S.54.9	616 VL	19	0.3	0.9	46	26	83
5098.07	5478.995	A4.S.93.9	625 VL	11	0.2	0.2	122	49	80
4818.692	5566.033	A4B.S.39.9	669 VL	4	0	0	7	22	47
4822.779	5390.588	A4B.S.9.9	749 VL	33	0.1	0.2	26	54	211
5595.03	5394.106	A5.S.103.9	1930 VL	16	0.1	0.1	17	47	42
4874.137	5191.103	A5.S.28.9	2060 VL	18	0.1	0.8	29	15	26
5049.5	5187.587	A5.S.35.9	1990 VL	34	0.4	0.3	54	25	53
5542.449	5346.718	A5.S.96.9	1970 VL	29	0.3	0	24	28	55
4945.655	5539.986	A6.S.23.9	899 VL	18	0.3	0.1	16	21	199
5080.064	5558.913	A6.S.42.9	933 VL	23	0	3.6	36	16	65
5101.793	5590.167	A6.S.56.9	1289 VL	21	0.1	0.5	21	16	93
5252.598	5496.048	A6.S.6.9	1153 VL	102	0.1	5.8	484	25	87
4874.341	5664.911	A6.S.74.9	1279 VL	12	0.2	0.2	17	34	88
4820.86	5714.155	A6B.S.45.9	1604 VL	29	2.2	16.2	17	35	243
4775.587	5793.093	A6B.S.65.9	1360 VL	25	0.1	0.3	19	33	85

ICP-MS standard laboratory analysis values for second set (57) of soil samples supplied by PURE project partners, mg kg^{-1} dried soil

x_map	y_map	ID_Boring	Sample_ID	As	Cd	Hg	Pb	Cu	Zn
5047.004	5224.536	A1.S.1.9	106 VL	32	0.2	0.4	142	82	124
5099.851	5246.836	A1.S.10.9	109 VL	17	0.9	0	18	26	53
5147.867	5241.707	A1.S.12.9	11 VL	19	1.8	0.2	38	32	108
5118.772	5265.114	A1.S.17.9	33 VL	22	0.7	0.1	21	27	84
5096.551	5219.35	A1.S.3.9	114 VL	22	0.1	0	28	27	76
5074.415	5363.763	A1.S.39.9	46 VL	34	0.5	0.1	37	24	87
5123.372	5220.546	A1.S.4.9	281 VL	31	0.6	0.5	125	33	114
5195.093	5319.576	A2.S.19.9	153 VL	18	0	0	19	17	60
5420.057	5343.326	A2.S.37.9	229 VL	20	0.1	0.2	18	26	74
5447.737	5370.938	A2.S.47.9	173 VL	24	0.2	1.6	30	32	123
5468.942	5412.855	A2.S.65.9	130 VL	21	0.1	0	34	32	107
5448.4	5440.612	A2.S.68.9	93 VL	25	2.5	0.3	24	35	132
5448.939	5465.674	A2.S.70.9	315 VL	15	0	0.1	28	29	66
5349.783	5438.52	A3.S.17.9	370 VL	31	0	0.6	57	33	60
5300.064	5484.98	A3.S.30.9	410 VL	29	0.4	0.2	40	54	88
5320.522	5488.843	A3.S.31.9	360 VL	21	0	0.2	51	9	74
5250.009	5387.367	A3.S.5.9	450 VL	39	2.5	0.7	21	35	74
5219.825	5411.713	A3.P.7.9	982 VL	39	2.2	0.4	118	33	210
4953.023	5289.612	A4.S.15.9	494 VL	14	0.7	0.2	29	44	904
4976.159	5333.729	A4.S.22.9	996 VL	21	0.3	0.4	20	18	123
4922.77	5244.007	A4.S.3.9	464 VL	16	0.2	0.2	23	11	51
5149.881	5365.434	A4.S.36.9	596 VL	30	0.1	0.2	112	13	41
4998.361	5415.655	A4.S.53.9	584 VL	25	2.9	0.5	94	17	54
5014.882	5489.639	A4.S.90.9	659 VL	21	0.2	0.1	22	16	62
5045.858	5491.716	A4.S.91.9	564 VL	21	0.3	0.1	25	14	64
4827.286	5463.912	A4B.S.21.9	719 VL	25	0.2	0.2	36	17	68
4850.006	5488.258	A4B.S.26.9	786 VL	24	0.3	0.1	24	68	166
4792.739	5346.935	A4B.S.4.9	1634 VL	44	0.7	0.2	233	56	71
4848.668	5616.678	A4B.S.49.9	801 VL	4	0.5	0.1	19	440	100
4840.323	5665.69	A4B.S.53.9	1625 VL	25	0.2	1.1	30	29	166
5642.352	5434.343	A5.S.107.9	1960 VL	18	0.4	0	36	10	38
4948.169	5166.516	A5.S.18.9	2102 VL	33	0.3	0.3	41	15	40
4926.02	5190.242	A5.S.30.9	1850 VL	37	8.6	0.3	33	55	52
4847.614	5215.355	A5.S.46.9	1870 VL	31	0	0.2	76	67	67
5296.8	5240.836	A5.S.64.9	1773 VL	20	0.8	0.2	28	26	99
5326.597	5241.391	A5.S.65.9	1730 VL	27	0.2	0	27	23	101
5298.668	5262.247	A5.S.71.9	1750 VL	23	0.2	0.2	174	28	105
5347.476	5265.781	A5.S.73.9	2000 VL	24	0.1	0	28	12	52
5398.581	5291.251	A5.S.82.9	1558 VL	22	0.3	0.4	101	27	111
5120.977	5492.975	A6.S.1.9	1031 VL	33	0.1	0.2	31	20	396
5081.145	5514.014	A6.S.13.9	953 VL	45	0.1	0.8	19	37	416

5181.466	5529.775	A6.S.32.9	1173 VL	30	0.4	0.6	19	24	171
5221.642	5540.702	A6.S.34.9	1710 VL	20	0.1	0	19	28	78
4946.847	5565.689	A6.S.37.9	889 VL	8	0.2	0.1	9	29	286
5089.817	5563.463	A6.S.43.9	1041 VL	8	0	3.3	28	19	327
5121.754	5571.394	A6.S.44.9	1232 VL	18	0.2	0.9	10	50	309
5147.114	5565.266	A6.S.45.9	1143 VL	8	0.2	0.2	10	15	88
5041.066	5583.447	A6.S.54.9	1269 VL	8	0.1	1.2	12	20	94
5020.556	5640.389	A6.S.72.9	1206 VL	27	5.8	0.2	28	41	109
4923.013	5686.28	A6.S.83.9	813 VL	12	0.2	0	16	15	91
4972.48	5508.611	A6.S.9.9	879 VL	22	0	0.1	25	20	202
4893.699	5732.998	A6.S.94.9	1310 VL	11	1.1	0.1	7	17	120
4923.084	5740.032	A6.S.95.9	2091 VL	8	0.1	0	11	13	44
4740.831	5598.99	A6B.S.14.9	1340 VL	37	0.3	1.7	197	118	161
4826.622	5796.444	A6B.S.67.9	1501 VL	8	0.1	0	14	14	75
4795.833	5810.489	A6B.S.73.9	1469 VL	36	0.3	0.2	21	50	82
4803.073	5907.559	A6B.S.88.9	1299 VL	102	5.7	0.3	19	71	109

APPENDIX A3

STANDARD LABORATORY ANALYSIS FOR SOIL SAMPLES SUPPLIED DURING THE TECHNICAL WORKSHOP, SEVILLE

ICP-OES laboratory analysis values in mg kg^{-1} dried soil.

	acetic acid extracts				aqua regia extracts			
sample	**Cd**	**Pb**	**Cu**		**Cd**	**Pb**	**Cu**	
GTS00683	**2.4**	**119.4**	**46.9**		**4.0**	**872.0**	**666.0**	
SD	0.0	1.0	1.4		1.3	7.5	1.3	
CV	3.0	1.0	3.0		32.0	1.0	0.2	
GTS00684	**0.6**	**58.5**	**10.0**		**<2**	**977.0**	**348.7**	
SD	0.1	1.0	1.6			8.4	0.9	
CV	12.0	2.0	16.0			1	0	
GTS00685	**1.0**	**5.7**	**38.8**		**<2**	**292.0**	**254.0**	
SD	0.1	1.0	1.4			4.7	1.0	
CV	8.0	22.0	4.0			2.0	0.4	
GTS00686	**< 0,2**	**1.5**	**1.5**		**<2**	**47.0**	**34.0**	
SD		1.0	1.7			5.0	1.1	
CV		83.0	116.0			11	3	
GTS00687	**< 0,3**	**1.2**	**1.8**		**<2**	**140**	**66**	
SD		1.0	1.7			4.3	1.0	
CV		110.0	93.0			3.0	1.0	
GTS00688	**< 0,4**	**6.9**	**5.3**		**<2**	**179.0**	**72.0**	
SD		0.3	1.7			5	1.0	
CV		0.2	1.0			3	1.00	
GTS00689	**< 0,5**	**< 0,4**	**< 0,4**		**<2**	**13.0**	**12.0**	
SD						5.5	1.2	
CV						41.0	9.0	
GTS00690	**< 0,6**	**< 0,4**	**< 0,4**		**<2**	**40.0**	**20.0**	
SD						5	1.2	
CV						14	6.0	
GTS00691	**7.5**	**109.3**	**72.1**		**20.0**	**1116.0**	**518.0**	
SD	0.1	1.0	1.4		13.8	17.5	16.8	
CV	0.0	1.0	2.0		70.0	2.0	3.0	
GTS00692	**4.1**	**13.7**	**276.0**		**19.0**	**1111.00**	**508.0**	
SD	1.0	0.3	4.8		12.2	17.5	15.5	
CV	0.9	0.1	0.1		64.0	2.0	3.0	
GTS00721	**3.8**	**2.3**	**8.9**		**16.0**	**245.0**	**199.0**	
SD	1.0	0.3	2.0		6.0	9.0	15.1	
CV	1.0	0.5	1.0		14.0	1.4	3.0	
GTS00722					**12.0**	**1853.0**	**419.0**	
SD					4.3	30.6	14.0	
CV					12.0	0.6	1.0	

APPENDIX A4

COST CALCULATIONS FOR REAGENTS, CONSUMABLES AND SPES FOR ANALYSIS USING THE *IN SITU* ELECTROCHEMICAL SCREENING SENSOR

These calculations are based upon one operator performing 4680 *in situ* soil tests per annum.

	Dilution	per test	diluted volume used	contingency	actual volume	volume/mass per annum (ml/g)	Volume mass per kg	cost (per liter or kg) £	**cost per annum**
Sample analysis									
Aqua regia	1M	5							
Nitric (1)	0.1	1.25	0.125	20%	0.15	702	0.702	16.8	**11.79**
HCl	0.1	3.75	0.375	20%	0.45	2106	2.106	14.8	**31.17**
Water	1	5	5	20%	6	28080	28.08	4.2	**117.94**
KCl							0.21	26	**5.40**
Standards									
Cu								25	**25.00**
Cd								25	**25.00**
Pb								25	**25.00**
Hg								25	**25.00**
Filtration		£0.6						2808	**2808.00**
Other									
Pipette tips (100 µg/l)	£20 per 2000	£0.01							**46.80**
Pipette tips (5 ml)	£30 per 500	£0.06							**280.80**
Universals	£75 per 400	£0.1875							**877.50**
Glassware									**100.00**

Based on previous internal studies the cost per sheet (100 electrodes) when producing 100,000 electrodes at Cranfield University, including personnel, was £0.40 (£0.004 per single electrode).

APPENDIX A5

ENVIRONMENT AGENCY NATIONAL LABORATORY SERVICE SAMPLE ANALYSIS QUOTATION

NATIONAL LABORATORY SERVICE

ENVIRONMENT AGENCY

QUOTATION FOR ANALYSIS : LL03-00579

Joanne Cooper

10 November 2003

Dear Joanne,

Thank you for your query regarding analysis

The attached quotation lists the price per sample for the analysis you requested.
Please specify the quote and group number when sending in samples and complete the attached Hazard Form .

If I can be of further assistance, please contact me.

Rhian Roberts - Team Leader
Tel: 01554 757031ext 3346
Fax: 01554 771256

The Environment Agency carries out analytical work to high standards and within the scope of it's UKAS accreditation but has no knowledge of the circumstances or the validity of the procedures used to obtain the samples provided to the laboratory were representative of the need for which the information was required.
The Environment Agency and/or it's staff does not therefore accept any liability for the consequences of any act or omissions made on the basis of the analysis or advice or interpretation provided.

Llanelli Laboratory, Penyfai House, 19 Penyfai Lane, Furnace, Llanelli, SA15 4EL
Phone: 01554 757031ext 3346, Fax: 01554 771256

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NATIONAL LABORATORY SERVICE

ENVIRONMENT
AGENCY

QUOTATION FOR ANALYSIS : LL03-00579

Joanne Cooper 10 November 2003

GROUP 1 Soil analysis

Analyte	Units:	MRV	Accreditation	Bottles Required
CADMIUM (DRY WEIGHT) - AS CD	mg/kg	0.01	UKAS	SHELM:Re-sealable polythene bag
COPPER (DRY WEIGHT) - AS CU	mg/kg	0.1	UKAS	
LEAD (DRY WEIGHT) - AS PB	mg/kg	0.2	UKAS	

GROUP 1 Charge per Sample: £43.77

Please Note: There is a minimum Invoice charge of £50 or minimum batch charge of £25, whichever is the higher. Supply, but not delivery, of sample bottles is included. The above price does not include VAT.

Minimum Reporting Value limits quoted are dependent upon nature of sample received.

Key to Accreditation Status: UKAS = Method accredited to ISO/IEC 17025, NONE = Method not

Key to "Analysing Lab"
Llanelli = Analysed at NLS Laboratory at Llanelli, SA15 4ELLlanelli Laboratory, Penyfai House, 19 Penyfai Lane, Furnace, Llanelli, SA15 4EL

Phone: 01554 757031 ext 3346, Fax: 01554 771256

APPENDIX A6

MATLAB PARTIAL LEAST SCRIPT

```
% loads three component, three level calibration data
% into data matrix X
%
% matrix Y contains concentration data

clear all

code = 'abc';

row = 1;
for i = 1:3
   for j = 1:3
       for k = 1:3
          filename = [code(i) code(j) code(k)];
          disp(filename)
          A = dlmread([filename '.oea'],',',1,0);
          X(row,:) = A(:,2)';
          Y(row,:) = ([i j k] - 1) * 400;
          row = row + 1;
       end
   end
end

% load central repetitions
for r = 2:5
   filename = ['bbb' int2str(r) '.oea'];
   A = dlmread(filename,',',1,0);
   disp(filename)
   X(row,:) = A(:,2)';
   Y(row,:) = [400 400 400];
   row = row + 1;
end

plot(X');

% generate PLS model
LVs = 5;   % number of LVs
[b,ssq,p,q,w,t,u,bin] = pls(X(1:27,:),Y(1:27,:),LVs);

disp(['as/cu/hg']);

% prediction for bbb:
as = X(28,:)*b(3*LVs-2,:)';
cu = X(28,:)*b(3*LVs-1,:)';
hg = X(28,:)*b(3*LVs,:)';
disp([as cu hg]);

% prediction for bbb:
as = X(29,:)*b(3*LVs-2,:)';
cu = X(29,:)*b(3*LVs-1,:)';
hg = X(29,:)*b(3*LVs,:)';
disp([as cu hg]);
```

```
% prediction for bbb:
as = X(30,:)*b(3*LVs-2,:)';
cu = X(30,:)*b(3*LVs-1,:)';
hg = X(30,:)*b(3*LVs,:)';
disp([as cu hg]);

% prediction for bbb:
as = X(31,:)*b(3*LVs-2,:)';
cu = X(31,:)*b(3*LVs-1,:)';
hg = X(31,:)*b(3*LVs,:)';
disp([as cu hg]);

plot(X');

% Maximum number of LVs is 15
LVas = 5;   % number of LVs to use for arsenic
LVcu = 5;   % number of LVs to use for copper
LVhg = 5;   % number of LVs to use for mercury

% load test data
filelist = dir('*.oea');
for filenumber = 1:length(filelist)
    filename = filelist(filenumber).name;
    A = dlmread(filename,',',1,0);
    x = A(:,2)';
    disp(filename);
    as = x*b(3*LVas-2,:)';
    cu = x*b(3*LVcu-1,:)';
    hg = x*b(3*LVhg,:)';
    disp([as cu hg]);
end
```

APPENDIX A7

LIST OF PUBLICATIONS

Publications:

S.J.Setford, J. Cooper, P.Rigou, J.A.Bolbot, S Saini. Field-based analytical methods for monitoring at industrially contaminated sites. Proceedings, 1st SENSPOL workshop, University of Alcala, Alcala de Henares, Spain, 9-11 May 2001, pp76-87.

J.Cooper, S.J. Setford, J.A. Bolbot and S. Saini. Electrochemical characterisation of screen-printed carbon electrodes. Workshop on the Protection of European Water Resources, Concerted Action on Environmental Technologies (ETCA), Harrogate, UK, 21-23 May 2001, p257-258.

J.Cooper, S.J. Setford, J.A. Bolbot and S. Saini. Electrochemical method for the rapid *in situ* screening of Cd, Pb and Cu in soil and water samples. Environmental Science and Technology, Web submission 6 January 2004.

J.Cooper, S.J. Setford, J.A. Bolbot and S. Saini. An electrochemical method using disposable gold screen-printed electrodes for rapid *in situ* deteremination of mercury and arsenic. The Analyst, Web submission 23 March 2004.

Conferences attended:

Electrochemical characterisation of screen-printed carbon electrodes for on-site heavy metal monitoring applications. Cooper, J., Bolbot J.A., Setford S.J., and Saini S. *Poster, New Directions in Electroanalysis. Royal Society of Chemistry Electroanalytical Group, University of Salford, UK. 22-25 April 2001.*

Electrochemical characterisation of screen-printed carbon electrodes for on-site heavy metal monitoring applications. Cooper, J., Bolbot J.A., Setford S.J., and Saini S. *Poster, ETCA Workshop – On the protection of European Water Resources. ETCA Science Panel, Harrogate, UK. 21-23 May 2001.*

Electrochemical method for the rapid on-site screening of heavy metals in soil, water and plant samples. J Cooper, S Setford, J Bolbot, S Saini. *Poster, Fifth Workshop on Biosensors and Biological Techniques in Environmental Analysis. Cornell University, Ithaca, NY. May 31-June 4 2002.*

Electrochemical method for the rapid on-site screening of heavy metals in soil, water and plant samples. J Cooper, S Setford, J Bolbot, S Saini. *Poster, SENSPOL, Response to New Pollution Challenges Workshop, Kings College, London. 4-7 June 2002.*

Electrochemical method for the rapid on-site screening of heavy metals at industrially contaminated sites. J Cooper, S Setford, J Bolbot, S Saini. *Oral, Senspol Technical*

Meeting on Sensors for Characterisation and Monitoring of Contaminated Sites, Seville. 6-9 November 2002.

Electrochemical method for the rapid on-site screening of heavy metals at industrially contaminated sites. J Cooper, S Setford, J Bolbot, S Saini. *Abstract submitted, Consoil 2003.*

Monitoring Industrially Contaminated Sites. Steven Setford, Hans van Duijne' Peggy Rigou, Joanne Cooper, John Bolbot, Selly Saini, Ken Killham, David Mardlin, Rolf Henckler Alfredo Battistelli, Linet Ozdamar, Melek Demirhan. Response to New Pollution Challenges, 2nd SENSPOL workshop, King's College London, 4-7 June 2002.

Organism & whole cell-based biosensors: Comparison of electrochemical and biosensor methods for the rapid on-site screening of heavy metals in soil, water and plant samples. J Cooper, S Setford, J Bolbot, S Saini *Poster Biosensors 2002, 7th World Congress on Biosensors, Kyoto, Japan, 15-17 May 2002.*

Protection of groundwater resources at industrially contaminated sites. J Cooper, S Setford, J Bolbot, S Saini. *Oral, Silsoe Conference, Cranfield University, Silsoe, Bedfordshire. 15 June 2002.*