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DEVELOPMENT OF ELECTROCHEMICAL SENSORS FOR HEAVY METAL IONS DETECTION IN ENVIRONMENTAL SAMPLES

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ABSTRACT

The work presented in this thesis was concerned with the development of single-use drop-on sensors incorporating a three-electrode configuration (graphite carbon-working electrode, carbon-counter electrode and silver/silver chloride - reference electrode) for on-site detection of toxic heavy metals in various environmental matrices.

The fabricated three-electrode configuration system was coupled with square-wave anodic stripping voltammetry (SWASV) or constant current stripping chronopotentiometry (CCSCP) in order to provide a means of a relatively inexpensive on-site detector for trace levels of lead (II), copper (II) and cadmium (II). Detections and determinations of these metals were carried out on bare screen-printed carbon electrodes (SPCEs), mercury film SPCE, bismuth film SPCE and SPCEs modified with Nafion, 2,5- Dimercapto-1, 3, 4- thiadiazole (DMTD), bismuth oxide (Bi_2O_3) and polyethyleneimine (PEI) using the optimised procedures developed for measurements. With the optimised working conditions, the results obtained indicate that the screen-printed electrochemical sensors are sensitive and reproducible enough for the CCSCP and SWASV determination of lead, copper and cadmium in the microgram per litre – milligram per litre range. Limits of detection below 20 μg l^{-1} were estimated for the trace metal detection of lead, copper and cadmium on both the bismuth and mercury film electrodes. For the bare SPCE, detection limits of 35, 45 and 59 μg l^{-1} were obtained for lead, cadmium and copper detection using CCSCP. The reproducibility of the measurements, which also contributed to the interest in developing the electrochemical sensing devices for metal ions, was below 15 % for the bare SPCE, bismuth film SPCE, and mercury film SPCE. Modifications of SPCEs with an ion-exchanger (Nafion) and a complexing agent (DMTD) provided means of increasing the sensitivity of stripping response obtained at the bare SPCE. Detection limits of 20 and 22 μg l^{-1} were estimated for lead (II) measurements at the Nafion modified SPCE and at the screen-printed DMTD modified electrode, respectively.

The application of the various electrodes to real samples is demonstrated and proved successful for both water and soil extracted samples including *in situ* measurements at a contaminated site.

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NOTATION

Symbols (units):

C	Concentration (Mol dm^{-3}, M)
D	Diffusion coefficient (cm^2 s^{-1})
e	Electron
E	Potential (volts, V)
F	Faraday's constant (96,485 C)
I	Current (Ampere, A)
j_o	Current density (A, cm^{-2})
k^0	Heterogeneous rate constant (cm s^{-1})
Q	Charge (Coulombs, C)
π	Archimedes number, 3.1416
α	Transfer coefficient
τ	Transition time (s)
r^2	Correlation coefficient
R	Resistance (Ω)
R	Universal gas constant (8.314 JK mol^{-1})
T	Absolute temperature (K)
t	Time (second, s)
μ	Micro
ψ	Kinetic parameter

Abbreviations:

AAS	Atomic Absorption Spectrometry
AdCSV	Adsorptive cathodic stripping voltammetry
AdSV	Adsorptive stripping voltammetry
Ag/AgCl	Silver/silver chloride
AgSPEs	Screen-printed silver electrodes
ASV	Anodic stripping voltammetry
BCR	Community Bureau of Reference
BFSPCE	Bismuth film screen-printed carbon electrode
Bi_2O_3	Bismuth (III) oxide

BSA	Bovine serum albumin
C.O.S.H.H	Control of substances hazardous to health
CadSV	Catalytic adsorptive stripping voltammetry
CCSCP	Constant current stripping chronopotentiometry
Cd	Cadmium
CE	Counter electrode
CME	Chemically modified electrode
CMSPE	Copper/mercury-plated screen-printed electrode
CPE	Carbon paste electrode
CRM	Certified reference material
CSV	Cathodic stripping voltammetry
Cu	Copper
CV	Cyclic voltammetry
ΔE_p	Peak separation (V)
DMG	Dimethylglyoxime
DMTD	2,5- Dimercapto-1, 3, 4- thiadiazole
DPASV	Differential pulse anodic stripping voltammetry
DPV	Differential pulse voltammetry
dt/dE	Inverse potential derivative with time
EC	European Community
E_{dep}	Deposition potential (V)
emf	Electromotive force
E_p	Peak potential (V)
EPA	Environmental Protection Agency
EU	European Union
FAAS	Flame atomic absorption spectrometry
GCE	Glassy carbon electrode
GFAAS	Graphite furnace atomic absorption spectrometry
HCl	Hydrochloric acid
Hg^{2+}	Mercury
HgO	mercury oxide
HMDE	Hanging mercury drop electrode
HNO_3	Nitric acid

ICP-AES	Inductively Coupled Plasma Atomic Emission Spectrometry
ICP-MS	Inductively Coupled Plasma- Mass Spectrometry
ISE	Ion-selective electrode
KCl	potassium chloride
LSASV	Linear sweep anodic stripping voltammetry
M	Moles per litre
MFSPCE	Mercury film screen-printed carbon electrode
$mg\ l^{-1}$	Milligram per litre
mM	Millimolar
ms	Millisecond
mV	Millivolt
NAA	Neutron Activation Analysis
nC	Nanocoulombs
$ng\ l^{-1}$	Nanogram per litre
PAN	1-(2-pyridylazo)-2-naphthol
Pb	Lead
PEI	Polyethylenimine
PESA	Poly(ester sulphonic acid)
PSA	Potentiometric stripping analysis
RE	Reference electrode
RSD	Relative standard deviation
S/N	Signal to noise ratio
SCE	Saturated calomel electrode
SDS	Sodium dodecyl sulphate
SMDE	Static mercury drop electrode
SPCE	Screen-printed carbon electrode
SPE	Screen-printed electrode
SWASV	Square-wave square-wave anodic stripping voltammetry
SWV	Square-wave voltammetry
TMF	Thin mercury film
Tx-100	Triton X-100
$\mu g\ l^{-1}$	Microgram per litre
UK	United Kingdom

UV	Ultraviolet
w/v	Weight per volume
w/w	Weight per weight
WE	Working electrode
WHO	World Health Organisation
XRF	X-Ray Fluorescence

CHAPTER 1

• 1 INTRODUCTION

1.1 OUTLINE

In this Chapter, the problem of heavy metals in the environment and their toxicity is presented as well as the legislation governing them. Moreover, a brief account of analytical methods used for metal ions detection and determination is outlined; particular attention is given to the principle and theory underpinning electrochemical measurements. The aims and objectives of the project are also stated.

1.2 BACKGROUND

A long history of environmental pollution is stored in soils and sediments (Concerted Actions CLARINET, NICOLE, ETCA and SENSPOL, 2000) because they act as massive reservoir for many pollutants. Most soil contamination occurs from the disposal of mining and industrial wastes, or from land disposal of domestic and municipal wastes. The mining and industrial wastes can contain high concentrations of specific contaminants, which are very toxic, while domestic waste usually is very heterogeneous and contains fewer toxic contaminants (Kebbekus and Mitra, 1998). These contaminants at very unacceptable levels can be leached into the groundwater, surface water and finally drinking water.

There have been several well- publicised instances of aquatic pollution by toxic heavy metal due to mining accidents in the last few years (Kroll *et al.*, 2002). These include the mine tailings dam failures of the Los Frailes lead-zinc mine at Aznalcollar near Seville, Spain in 1998 and the one at Baia Mare, Romania in the year 2000. The Aznalcollar accident was caused by a large spill of mine water and sludge, heavily contaminated with toxic metal ions into both soil and water in the surroundings of Doñana Natural Park (Muraviev and Valiente, 1998; Kroll *et al.*, 2002).

Due to these accidents and others, proliferation of heavy metal contaminants has received a lot of attention. Hence, there is an increasing demand for simple screening methods for heavy metal analysis in industrial wastes, leachates, landfills, groundwater and surface waters. Heavy metals, recognised as highly toxic and dangerous pollutants, are placed second only to pesticides in environmental importance (Wang, 1985). Heavy metals are metals with atomic density over 5 g cm^{-3} (e.g., copper - 8.9 g cm^{-3}, cadmium - 8.7 g cm^{-3}, lead - 11.3 g cm^{-3}, mercury - 13.5 g cm^{-3}, etc) (Alloway, 1990; Palchetti, 1998 and Baird, 1999). These metals are natural constituents of rocks, soils and sediments. Perhaps the most important feature distinguishing heavy metals from other toxic pollutants is that metals are not biodegradable (Baird, 1999; Palchetti *et al.*, 2000). Hence they are retained in the environment and in the food chains. Some of the metals, which are of primary concern include, cadmium, copper, and lead (Alloway, 1990; Palchetti, 1998; Baird, 1999; Manahan, 2001). These metals are further explored here since they were investigated in this research.

1.2.1 Cadmium (Cd)

Cadmium (Cd) belongs to group 11B of the periodic table, and is relatively rare metal, being 67th in order of elemental abundance. Unlike lead, copper, and mercury, which have been utilised for centuries, Cd has only been widely used recently. More than half of the Cd ever used in industry was produced in the last 20 years (Alloway, 1990). It is obtained as a by-product of the smelting of zinc (Zn) and other base metals, and no ores are used primarily as source of Cd. Chemically, Cd is very similar to Zn, and these two metals frequently undergo geochemical processes together (Palchetti, 1998; Manahan, 2001). Both metals are found in water in the +2 oxidation state. Other sources of soil contamination by Cd are the atmospheric pollution from metallurgical industries, the disposal of wastes containing Cd such as incineration of plastic containers and batteries, sewage sludge application to land; and the burning of fossil fuels.

Cadmium has no essential biological function, and is highly toxic to plants and animals (Alloway, 1990). It is a priority pollutant and in view of the dangers of the chronic accumulation of cadmium in the human body, the factors influencing its concentration in the components of diet are of great importance. With the estimated half-life for Cd in soils varying between 15 and 1100 years, this is obviously a long-term problem and pollution needs to be prevented or minimised wherever possible.

Several countries have restricted the use of Cd, or are planning to, but nearly all have a legacy of pollution from its many sources. The effects of acute Cd poisoning in humans are very serious. Among them are high blood pressure, kidney damage, destruction of testicular tissue, and destruction of red blood cells. It is believed that much of the physiological action of Cd arises from its chemical similarity to Zn. Specifically, Cd may replace Zn in some enzymes, thereby altering the stereostructure of the enzyme and impairing its catalytic activity. Disease symptoms ultimately result (Alloway, 1990; Palchetti, 1998).

Cadmium is a priority pollutant and its anthropogenic discharges into the aquatic and terrestial environment need to be curtailed.

1.2.2 Copper (Cu)

The abundance of copper (Cu) in the Earth's crust is reported as ranging from 24 – 55 mg/kg (Alloway, 1990; Stringer *et al.*, 2001). Copper is a relatively abundant "trace" metal, found at varying concentrations in nearly all uncontaminated aquatic and terrestrial ecosystems. However, as anthropogenic emissions of Cu exceed those from natural sources, elevations above these natural, background concentrations are often found. Copper may exist in natural waters either in the dissolved form as the cupric (+2) ion or complexed with inorganic anions or organic ligands (e.g. carbonates, chlorides, humic and fulvic acids). It may also be present as an insoluble precipitate (e.g. a hydroxide, phosphate, or sulphide) or adsorbed onto particulate matter. Alternatively it can be adsorbed to bottom sediments or exist as settled particulates. The relative concentrations of each of these forms is dependent upon a number of chemical parameters, including pH, salinity, alkalinity and the presence of organic ligands, inorganic anions and other metal ions. However studies have frequently shown that the free +2 ion concentration is low, compared to the levels of copper associated with suspended and bottom sediments (Stringer *et al.*, 2001).

In soils, Cu has a high affinity for sorption by organic and inorganic ligands (e.g. humic and fulvic acids, hydroxides of iron, aluminium and manganese). However it can also exist as soluble ions and complexes. Copper in a soluble form is far more bioavailable and far more likely to migrate through the environment, than if it is bound to organic matter or present as an insoluble precipitate. Therefore, copper sulphate, or chloride, present in incinerator ash or mine tailings, is far more bioavailable and migratory than the organically bound copper found in sewage sludge (Alloway, 1990; Stringer *et al.*, 2001). Higher concentrations of Cu in soils, is an indication of additions from smelters, fertilisers, sewage sludges, fungicides and bactericides. Copper ions are very toxic while copper bound to natural organic matter is less harmful (Sterner, 1999).

In spite of the fact that Cu is essential, it is a very toxic metal of which any organism in principle would only be able to tolerate within a narrow concentration range. Human deficiency is characterised by anaemia, resulting from defective haemoglobin synthesis. However at the other extreme, vomiting, hypotension, jaundice, coma and even death, can result from acute poisoning (Stringer *et al.*, 2001). In mammals, evolution has solved this dilemma in an elegant way, by for example making sure that excess Cu is eliminated rapidly and that the Cu that the cells make use of is tightly bound to specific proteins and not available for other reactions. As a result of this, we are less sensitive to intoxication by Cu than one might fear. However, excess Cu is highly toxic to microorganisms (Sterner, 1999). Copper is not a priority pollutant but is included on the majority of subsidiary and secondary pollutant lists.

1.2.3 Lead (Pb)

Lead (Pb) is one of the most ubiquitous toxic metals. It is a member of Group IVB of the Periodic Table of elements. Two oxidation states of lead (Pb (+2) and Pb (+4)) are stable, but the environmental chemistry of the element is dominated by the plumbous ion, Pb^{2+}, its compounds and complexes. When Pb is released into the environment it has a long residence time compared with most other pollutants. As a result, Pb and its compounds tend to accumulate in soils and sediments where, due to their low solubility and relative freedom from microbial degradation, they will remain accessible to the food chain and to human metabolism far into the future (Alloway, 1990). In general, the highest Pb concentrations are found in aquatic and terrestrial organisms that live near to Pb mining, smelting and refining facilities, storage battery recycling plants, areas affected by high automobile and truck traffic, sewage sludge and spoil disposal areas, sites where dredging has occurred, areas of heavy hunting (spent lead shot), and in urban and industrialised areas (Stringer *et al.*, 2001). Lead from leaded gasoline used to be a major source of atmospheric and terrestial Pb, much of which eventually entered natural water systems (Manahan, 2001). Due to this, the use of leaded gasoline was phased out in the early 1990s and also because research indicated that 60 % of lead emission came from automobile exhaust (Palchetti, 1998).

Lead has no known nutrition, biochemical or physiological function (Stringer *et al.*, 2001). Acute Pb poisoning in humans may cause severe dysfunction of the kidney, reproductive system, liver, brain, and central nervous system leading to sickness or death (Manahan, 2001). Lead poisoning from environmental exposure is thought to have caused mental retardation in many children. Mild Pb poisoning causes anaemia. The victim may have headaches and sore muscles, and may feel generally fatigued and irritable. Except in isolated cases, Pb is probably not a major problem in drinking water, although the potential exists in cases where old Pb pipe is still in use (Alloway, 1990; Baird, 1999; Manahan, 2001).

Lead is not generally treated as a priority pollutant. However, anthropogenic discharges of Pb into the aquatic environment still need to cease and therefore Pb is included on the majority of subsidiary and secondary lists.

1.3 LEGISLATION CONCERNING HEAVY METALS

Since the mid 1970s, the European Community (EC) has adopted a series of legislation, which addresses the issues of environmental protection in response to well- publicised incidents of pollution resulting from the escape of materials from industrial premises and agricultural sites. These incidents have translated into pressures on industry to comply with the legislation in force, on the Government to bring in more stringent legislation and on the statutory authorities to increase and improve their policing of the legislation and to act strongly in enforcing it through court action. There is a great deal of UK and EC legislation, which is aimed at controlling incident risks and pollutant discharges, and for the establishment of water quality objectives and standards. The legislation is essentially aimed at controlling known, or planned, point discharges of trade and sewage effluents to the environment (Her Majesty Industrial Pollution Inspectorate, 1999).

The principle legislative driving force for nations in the European Union (EU), on the environment is the series of Directives issued by the EC. These are intended to provide a basis for specific legislation enacted by each member state (Gopel *et al.*, 1993). The key EC directives affecting pollution control in water and on land are as follows:

- Urban Wastewater Directive (91/271): Reduction of pollution in freshwater, estuarial and coastal waters due to domestic sewage, industrial effluent and agricultural run-off.
- Sewage Sludge in Agriculture Directive (86/278): Correct use of sewage sludge on land.
- Drinking Water Directive (80/778): Quality standards for water for drinking and food processing.
- Groundwater Directive (80/68): Regulates direct and indirect discharges of waste into groundwaters.
- Dangerous Substances in Water Framework Directive (76/464): Establishes a system for prescribing substances as needing control or limits. Substances for which a discharge reduction programme must be developed are entered on the 'grey list' (List II) with more toxic substances requiring strict limitation going on the 'black list' (List I).
- Surface Water Directive (75/440): Quality of surface waters intended for abstraction for drinking water.

Cadmium and mercury are the only two heavy metals on the 'Black list' (List I). They are considered to be significantly toxic, persistent and liable to bioaccumulation in the aquatic environments. Arsenic, copper, chromium, lead, nickel and zinc are included on the 'Grey List' (List II) and are considered to exert deleterious impacts on the aquatic environments on a local level (not affecting ecosystems on regional or global scales but confined to specific areas) (Phillips and Rainbow, 1993; Chave, 2001). All the listed Directives are implemented as a variety of legislative instruments in member countries of the EU, and are reflected also in the legislation of many non-member countries. Together with its equivalent in the USA, the framework provided by the Directives is regarded as a model for environmental legislation in the rest of the world (Gopel *et al.*, 1993).

The maximum allowable concentration of metals in water and soil permitted by environmental bodies in various countries, continental and world organisations are presented in Table 1.1 and Table 1.2.

Table 1.1: Maximum concentration values of metals as specified in different regulations (Palchetti, 1998; Baird, 1999; Her Majesty Industrial Pollution Inspectorate, 1999).

	EPA Drinking Water	**EU Drinking Water**	**Germany Soil to Groundwater**	**WHO Drinking Water**	**Dutch Groundwater**
METAL	mg l^{-1}	mg l^{-1}	mg l^{-1}	mg l^{-1}	mg l^{-1}
Arsenic	0.05	0.05	0.01	0.01	0.06
Cadmium	0.005	0.005	0.005	0.003	0.006
Chromium	0.1	0.05	-	-	0.03
Copper	1.3	0.5	0.05	-	0.075
Lead	0.015	0.05	0.025	0.01	0.075
Mercury	0.002	0.001	0.001	0.001	0.003
Nickel	0.1	0.05	0.05	-	0.075
Zinc	-	1	0.5	-	0.8

EPA: Environmental Protection Agency, **EU:** European Union, **WHO:** World Health Organisation

Table 1.2: Maximum allowable concentration values for heavy metals in soil as specified in different regulations (Wild, 1993; Kabata-Pendias, 1995).

METAL	AUSTRIA mg kg^{-1}	CANADA mg kg^{-1}	POLAND mg kg^{-1}	JAPAN mg kg^{-1}	UK mg kg^{-1}	GERMANY mg kg^{-1}	EU mg kg^{-1}
Cadmium	5	8	3	-	3	2	3
Chromium	100	75	100	-	50	200	-
Copper	100	100	100	125	100	50	140
Nickel	100	100	100	100	50	100	75
Lead	100	200	100	400	100	300	300
Zinc	300	400	300	250	300	300	300

EU: European Union, **UK:** United Kingdom

With Environmental monitoring driven primarily by legislation drafted as a result of continuing health concerns, the need for suitable analytical methods for quantitation is important. Suitable analytical methods are needed for the rapid assessment and remediation of contaminated sites and also for the enforcement of the regulations on heavy metal discharge from many industrial, agricultural and domestic sources. Also the quality of drinking water needs to be monitored so that the set limits are not exceeded. Since the work carried out in this research is about metal ions analysis, Section 1.4 details some of the analytical methods used for heavy metal analysis.

1.4 ANALYTICAL METHODS FOR HEAVY METAL ANALYSIS

The increasing need for trace metal analysis is associated with many practical applications. Among these are the establishment of meaningful tolerance limits for trace metal pollution and elucidation of the roles of trace metals in biological function. A thorough understanding of the effects of trace metals depends largely on the availability of sensitive and reliable analytical techniques (Wang, 1985).

There are numerous analytical methods and devices that determine heavy metal concentrations in water and soil samples with accuracy down to the milligram per litre (mg l^{-1}) level or below. Examples of these are:

- Atomic Spectrometry
- Visible Spectrophotometry
- X-Ray Fluorescence (XRF)
- Instrumental Neutron Activation Analysis (INAA)
- Immunoassay
- Biosensors (Electrochemical, Optical, Piezoelectrical, Thermal)
- Electrochemical techniques (e.g., Potentiometry, Electrochemical Stripping analysis, etc.)

In various matrices the normal concentration of various important metals is often near the detection limits of some of these techniques. Many factors, such as detection limits, instrumentation cost, analysis time, and sample preparation or selectivity, should be considered in judging the suitability of a specific technique for practical problem of trace analysis. Tradeoffs are inevitable and the attempt to balance one factor against another leads to many critical decisions (Wang, 1985).

1.4.1 Atomic Spectrometry

The area of heavy metal ions analysis is currently dominated by techniques, which can be grouped together under the general title of atomic spectrometry (Reeve, 1994). The individual techniques are:

- Flame atomic absorption spectrometry (FAAS);
- Flameless or Graphite furnace atomic absorption spectrometry (GFAAS);
- Inductively coupled plasma atomic emission spectrometry (ICP-AES);
- Inductively coupled plasma mass spectrometry (ICP-MS).

FAAS is one of the most widely used techniques for the determination of heavy metals and also the choice of smaller laboratories (Bersier *et al.*, 1994; Reeve, 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 (the sample is aspirated into a chamber in which the fuel, oxidant, and the analyte solution droplets are mixed). Light of a characteristic wavelength of the element of interest passes through the flame. The amount of light absorbed is directly proportional to the concentration of the analyte in the flame and is derived from the Beer's Law: $A = \varepsilon bC$, where A is absorbance; ε is the molar absorptivity (a wavelength dependent parameter characteristic of absorbing substance); b is the path length of the light through the absorbing solution and C is the concentration of absorbing substance. The linear relationship between the measured absorbance and the concentration of analyte atoms in the flame, which is proportional to the concentration of atoms in sample solution, holds in practice, over some two orders of magnitude. The precise relationship between absorbance and solution concentration of the analyte is established by analysing a series of standard solutions with known analyte concentrations covering the range required or, in some cases, by the method of standard additions. Detection limits are in the range 0.05 - 1 mg l^{-1} for most metals with a precision of 2 - 5 % RSD (relative standard deviation) (Alloway, 1990). AAS is a rapid technique, and sample-handling, measurement, computation and printout are available in automatic form from all commercial manufacturers. The interferences are few and well documented. The technique is rather slow when more than two or three analytes must be determined from each sample (Bersier *et al.*, 1994; Reeve, 1994). Sample requirements in the 2 - 10 ml range are usual, and smaller samples of about 50 - 100 µl can be accommodated by pulse nebulisation techniques. The limitations of FAAS in sensitivity resulting from the inefficiency of sample introduction by pneumatic nebulisation (< 10 % efficient) and the large dilution introduced by the expanding flame gases have been overcome by the use of the electrothermal atomiser (Alloway, 1990). This generally takes the form of a cylinder of graphite (graphite furnace atomic absorption spectrometry, GFAAS), which can be heated by the passage of an electric current through it. Samples (5 - 100 µl) are introduced into the furnace through a small, central hole. The furnace is protected from oxidation by a surrounding atmosphere of a protective gas, usually argon or nitrogen. After injection of the sample solution, the atomiser is programmed through a drying stage, a pyrolysis stage, and an atomisation stage by passing progressively higher currents, for selected times, through the atomiser. At the atomisation stage, an atomic absorption measurement is made through the tube. In terms of concentration, the detection limits obtained with GFAAS are 2 – 3 orders of magnitude better than those of AAS and ICP-AES (Bersier *et al.*, 1994). 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. GFAAS determines one element at a time.

ICP because of its widely acknowledged attributes, including low detection limits, a wide linear dynamic range and high precision, is becoming the dominant technique for rapid spectroscopic multi-elemental analysis (Bersier *et al.*, 1994). Inductively coupled plasma-atomic emission spectrometry (ICP-AES) uses, in principle, the emission from the flame-like plasma formed on a quartz touch by coupling a radio-frequency electromagnetic field to the electrons in ionised argon 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. This, in conjunction with the torch parameters, shapes the plasma into a toroidal form. Sample aerosol is directed into the central hole of the plasma toroid (Alloway, 1990). Temperatures in the plasma are in the range 6000 - 10000 K and are typically of the order of 6500 K in the analytical measuring zone. At these high temperatures atomisation is virtually complete for most elements, and strong atomic and ionic line emission can occur. Samples are usually introduced in the form of aerosols produced by pneumatic nebulisers, which are often fed from a peristaltic pump. Simultaneous ICP-AES can determine 60 or more elements at once by monitoring at preset wavelengths (Reeve, 1994). Sequential spectrometers, which are more common for water analysis, are restricted to a smaller number of elements, determined by the requirements of the analysis, measured in succession by rapid changes in the detection wavelength. The advantages of remarkable simplicity and freedom from interferences are offset by the large number of spectral emission lines associated with each element, such as Fe, Ni, U, and rare earths (Bersier *et al.*, 1994).

A more recent development is to use the ICP as an ion source for a mass spectrometer. The charged ions are produced by the plasma flame and are separated according to their mass/charge ratios. Although the technique is not strictly simultaneous, the ions being determined sequentially, determination of 20 elements is possible within 4 s (Reeve, 1994). ICP-MS possesses the multi-element capabilities of ICP-AES and the excellent detection limits of GFAAS. An isotope dilution (ID) procedure when coupled to ICP-MS (ID-ICP-MS) is listed as the 'definitive' method, because of its inherently greater accuracy than other techniques. The isotope of the analyte of interest is added to the sample preparation. The amount of the analyte in the sample can be calculated when comparing the known original isotope ratios and the measured altered isotope ratio. ICP-MS is free of spectral interferences but has interferences of its own (due to presence of chlorides and sulphates), in addition to the overlap of isotopes of different elements with nearly equal mass. Restrictions on the sample's salt content are more severe than those for ICP-AES. Interferences from the matrix, especially from chlorides and sulphates, are well known. The carrier gas, usually argon, forms an incredible array of vapour-phase combinations with materials from the matrix, including oxygen, hydrogen, chlorine, nitrogen, carbon, phosphorus, etc (Bersier *et al.*, 1994)

Each development in atomic spectrometry has brought with it a significant increase in instrument capital cost. The cost of instrumentation is generally FAAS< GFAAS< ICP-AES< ICP-MS (Reeve, 1994).

1.4.2 Visible Spectrophotometry

Until the widespread use of atomic spectrometric techniques, visible spectrophotometry was the most commonly used technique for heavy metal ion analysis (Reeve, 1994). Selectivity in the analysis is achieved in two different ways:

- solvent extraction with precise control of pH and the use of masking agents.
- colour-forming complexing agent, which is sufficiently sensitive and selective for use in the aqueous sample without extraction being necessary.

The selectivity is provided by the colorimetric reaction, with the absorbance in the visible or ultra-violet region used for quantitation. The main advantage of spectrophotometry is that the analysis can be done using a simple, inexpensive spectrophotometer. The availability of battery powered spectrophotometers (Kebbekus and Mitra, 1998) makes spectrophotometry easily adaptable to field use. Commercially available spectrophotometric test kits for heavy metals based on colorimetric reagent can be obtained from the Hach Company (USA), Aquatic Life Ltd (Canada) and LaMotte Company (USA). These spectrophotometric methods are not as sensitive as other analytical methods for detecting heavy metals at very low concentration. Also they are often affected by interference from other elements (Reeve, 1994; Kebbekus and Mitra, 1998).

1.4.3 X-ray Fluorescence Spectrometry (XRF)

X-ray fluorescence spectrometry is a 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.

There are already portable X-ray fluorescence (XRF) spectrometric analyzers for detecting heavy metals (Driscoll *et al.*, 1991; Bernick *et al.*, 1995; Argyraki *et al.*, 1997). Development of these units has offered a potentially fast and inexpensive method of determining levels of heavy-metal contamination in soils. Being able to take a lot of readings a day and having the data available immediately are the major benefits of portable XRF units. Ordinarily, one would have to wait weeks for a laboratory to report analytical results. With a small portable unit, a user can quickly determine the scope of metal contamination and then use laboratory results to verify field measurements. Compared to AAS, ICP-AES and ICP-MS, XRF spectrometry does not require the digestion of samples since it measures the metals in whatever forms present (dissolved, complexed, or solid). The major barrier to the use of XRF for water analysis is that detection limit of the technique is in the low milligram per litre (mg l^{-1}) range (Rogers and Mascini, 1998) while the concentrations of metal ions found in surface or ground waters are mostly at microgram per litre ($\mu g\ l^{-1}$) levels. Also the XRF instrument contains a radioactive source and may need to be cleared by a radiological control officer before it is used at some contaminated sites. Along with atomic spectrometric techniques, XRF only determine total metal concentration, and cannot directly provide speciation information.

1.4.4 Instrumental Neutron Activation Analysis (INAA)

Instrumental neutron activation analysis is a multi-element, solid sample technique for the analysis of soils. 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.

Sensitivity multi-element analysis requires a combination of the appropriate exposure (irradiation) and radiation decay times before analysis of the γ-spectrum, and these are dependent on the analyte and on the nature of the matrix components and their interfering radiation. Sophisticated instrumentation and computer facilities as well as experienced staff are thus essential for this technique. Sample requirements are generally in the range 50 – 2000 mg of finely ground (< 200 μm) soil. The detection limits for heavy metals using this technique, are in the range 1 mg kg^{-1} to 0.01 mg kg^{-1} (Alloway, 1990). Using the technique is costly in terms of financial and work expenditure (Bersier *et al.*, 1994).

1.4.5 Immunoassay

Immunoassay technology, which is well established in the field of organic environmental analysis, has now been extended to heavy metal analysis (Blake *et al.*, 1998; Khosraviani *et al.*, 1998; Blake *et al.*, 2001; Darwish and Blake, 2001). Immunoassays are appropriate for characterisation, field screening and compliance monitoring. The method is inexpensive, simple to perform, and reasonably portable. Immunoassay technology relies on an antibody that is developed to have a high degree of sensitivity and specificity to the target metal. The antibody's high specificity is coupled within a sensitive colorimetric reaction that provides a visual result.

1.4.6 Biosensors

A biosensor is an analytical device incorporating a biological or biologically derived sensing element either intimately connected to or integrated within a transducer (Pravda, 1998). Typically, a biospecific-sensing element can be an enzyme, antibody, biomembrane component, or microorganism. Specific interactions between the target analyte and complementary biorecognition layer, produces a physico-chemical change, which is detected and may be measured by the transducer. The signal generated by the transducer requires amplification and processing; hence a signal processor is employed to convert this signal into a processable form.

Biosensors using different methods of detection (electrochemical, optic, mass (piezoelectric), fluorescence, luminescence) have been reported for heavy metal detection. These biosensors were based on the use of enzymes (Volotovsky *et al.*, 1997; Fennouch *et al.*, 1998; Kukla *et al.*, 1999; Compagnone *et al.*, 2001), proteins (Bontidean *et al.*, 1998), whole cells (e.g. bacteria, fungi, yeast, lichen etc.) (Rogers and Mascini, 1999; Leth *et al.*, 2002; Shetty *et al.*, 2003) and apoenzymes (Satoh *et al.*, 1990; Satoh, 1993) as recognition elements. The major problem with biosensors is their lack of robustness and the instability of the biological recognition element (Prichard *et al.*, 1996).

1.4.7 Electrochemical techniques

1.4.7.1 Potentiometry - Ion-selective electrode

The analytical applications utilising potentiometry would be quite limited were it not for the development of ion-selective electrodes (ISEs) (Kennedy, 1990). These electrodes as the name implies, are capable of selectively measuring the activity (concentration) of a particular ionic species. The functioning of an ISE is based on the selective passage of charged species from one phase to another leading to the creation of a potential difference. The use of an ISE system involves dipping the electrodes in the test solution and reading a signal from the voltmeter. ISEs are reasonably sensitive (Prichard *et al.*, 1996). Some commercially available ISEs for metal ions (copper, lead, cadmium, silver, etc.) operate in the range 10^0 to 10^{-7} M range (Kennedy, 1990; Ahmad *et al.*, 2001). All the analytes to which ISE are applicable must be either ions or molecules that ionise. Like most analytical techniques, ISE work better in 'clean' solutions without interferences. They are ideally suited to analyses in flowing streams such as rivers or industrial processes, as they can be adapted to almost any size, shape, or material in order to fit in with their location (Prichard *et al.*, 1996). The main disadvantage in the use of ISEs is that the limit of detection in some kinds of environmental samples can be high and the selectivity, poor.

1.4.7.2 Stripping Analysis

Electrochemical methods like stripping analysis are widely used for the detection of heavy metals in various environmental matrices. Interest in stripping analysis (stripping voltammetry and stripping potentiometry), is sparked by its ability to measure, simultaneously four to six trace metals at concentration levels down to fractional microgram per litre (sub-μg l^{-1}), utilising inexpensive instrumentation (Wang, 1985). Amongst stripping analysis spectroscopic competitors, only flameless atomic absorption has nearly the same sensitivity, but at much higher costs. At even higher instrumentation cost, ICP-AES and ICP-MS offers detection limits at the μg l^{-1} concentration level coupled with simultaneous determination capability (Wang, 1985; Palchetti, 1998). Stripping analysis offers the advantage of species characterisation; i.e., through judicious experiments it is possible to determine the exact chemical nature of the trace constituent (Wang, 1985). Another advantage of stripping analysis is its suitability for automatic on-line monitoring and for *in situ* measurements (direct measurements in the field based on the nature of electrochemical processes and portability of equipment) (Wang, 1994; Palchetti, 1998; Wang *et al.*, 1999). Like all analytical methods, the use of stripping analysis has limitations and certain difficulties. There may be problems with the stability of the electrodes as well as fouling and interferences when electrodes are in physical contact with sample solutions (Prichard *et al.*, 1996). However, continuing developments in the field of microelectronics and microsensor/microelectrode technology are addressing these problems. Examples of environmental applications of stripping analysis are shown in Table 1.3 for heavy metal analysis.

Table 1.3: Examples of Environmental Applications of Stripping Analysis.

METAL	MATRIX	ELECTRODE	METHOD	DETECTION LIMIT	REFERENCE
Mercury Selenium Copper Lead	River water and seawater	Gold film on GCE	PSA	Mercury- 15 ng l^{-1} Selenium- 0.1 μg l^{-1}	Gil and Ostapczuk, 1994
Cadmium Chromium Copper Lead Zinc	Soil and sediment samples	SMDE Mercury plated GCE	ASV PSA AdSV	1 μg l^{-1}	Olsen *et al.*, 1994.
Arsenic	Stream sediment and water samples	HMDE	CSV	0.6 nM (4.4 μg l^{-1})	Greulach and Henze, 1995.
Zinc	Natural water, domestic wastewater, sewage sludge and sediment samples	Mercury plated GCE	ASV	-	Lau and Cheng, 1998.
Mercury	Seawater	Gold wire electrode	CCSCP	0.3 μg l^{-1}	Wang, *et al.*, 1998a.
Nickel Cobalt	Tap water, swamp water and sediment samples	In situ plated mercury GCE	AdCSV	0.1 μg l^{-1}- Nickel 0.03 μg l^{-1}- Cobalt	Adeloju and Hadjichari, 1999.
Copper	Drinking water and seawater	Rotating disc gold electrode	ASV	0.2 nM (13 ng l^{-1})	Bonfil *et al.*, 1999.
Lead Cadmium	Lake waters and certified seawater	Mercury film plated in situ on GCE	ASV	8 pM- Lead 5 pM- Cadmium	Fisher and van den Berg, 1999.
Lead	River water and drinking water	Silver electrode	ASV	0.5 nM (100 ng l^{-1})	Kirowa-Eisner *et al.*, 1999.
Arsenic - Selenium Copper Lead Zinc Cadmium	Estuarine and river sediments	Stationary mercury electrode	CSV ASV	Around 10^{-9} M	Locatelli and Torsi, 1999.
Cadmium Lead	Drinking water and mineral water	CPE bulk modified with bismuth oxide	ASV	5 μg l^{-1}- Cadmium 5 μg l^{-1}- Lead	Pauliukaite *et al.*, 2002.
Chromium	Tap and river water samples	HMDE	CAdSV	0.2 nM (10.4 ng l^{-1})	Grabarczyk and Korolczuk, 2003.

ASV: Anodic stripping voltammetry, **AdSV:** Adsorptive stripping voltammetry, **AdCSV:** Adsorptive cathodic stripping voltammetry, **CAdSV:** Catalytic adsorptive stripping voltammetry, **CCSCP:** Constant current stripping chronopotentiometry, **CSV:** Cathodic stripping voltammetry, **PSA:** Potentiometry stripping analysis, **CPE:** Carbon paste electrode, **HMDE:** Hanging mercury drop electrode, **GCE:** Glassy carbon electrode, **SMDE:** Static mercury drop electrode.

Of the analytical methods mentioned in the preceding section 1.4 for heavy metal detection, electrochemical stripping analysis due to practical and economic reasons, provides means of developing sensitive and robust sensing devices that are capable of screening and targeted analysis of metals. As sensors based on electrochemical transduction all follow conventional electrochemical practises, Section 1.5 introduces the principles behind electrochemical methods and measurements.

1.5 ELECTROCHEMICAL METHODS

Electrochemical methods in general concern the study of the chemical response of a system to an electrical stimulation. Measured parameters include voltage, current and charge and these are related to the activity or concentration of chemical moieties in solution. Voltage is the driving force behind an electrical reaction and it is sometimes referred to as electromotive force (emf) or potential difference (pd). It has units of volts (V) and the symbol *E*. Current is a flow of electrons and it has units of amperes (A) and the symbol *I* (or i). Resistance is the opposition to the voltage, preventing a flow of current. It has units of ohms (Ω) and the symbol *R*. The coulomb (C) is the unit of charge. An electron carries a charge of 1.60210 x 10^{-19} C. This can be related to the current by (Ahmad *et al.*, 2001):

$$Q = It \qquad (1.01)$$

Faraday's constant (F) is the charge carried by a mole of electrons. This has a value of

$$\begin{aligned} F &= 6.02252 \times 10^{-23} \text{ electrons mol}^{-1} \times 1.60210 \times 10^{-19} \text{ C/electron} \\ &= 96487 \text{ C mol}^{-1} \end{aligned} \qquad (1.02)$$

Electrochemical methods are well suited to sensing, monitoring and detection of organic and inorganic analytes. Electrochemical measurements can be classified according to the physical parameter (potential, current and time) involved in the transduction process (Table 1.4).

Table 1.4: Classification of some of the various standard electrochemical techniques.

Nature of Measuement Process	Controlled Parameter	Measured Parameter	Technique
Equilibrium measurements	Measurement at zero current	E	Potentiometry
Non-equilibrium measurements	E	I vs. E	Voltammetry Stripping voltammetry
	E	I vs. t	Chronoamperometry
	I	E vs. t	Chronopotentiometry Stripping chronopotentiometry

I = Current, **E** = Potential, **t** = Time

1.5.1 Potentiometry

A potentiometric probe like ISE has an output, which is proportional to the activity of a particular ion in solution. Ideally, the behaviour of such a system is governed by the following version of the Nernst equation:

$$E = E_a + \frac{(RT)}{zF} \ln a_i = E_o + 2.303\frac{(RT)}{zF}\log a_i \qquad (1.03)$$

where E is the potential (V), R the universal gas constant (8.134 J $mol^{-1}K^{-1}$), T the absolute temperature (K), z the ionic charge of the ion of interest and a_i its activity (M). The constant E_o depends on a number of factors influenced by the design of the electrodes. The plot of E against log a_i yields a straight line with an intercept of E_o and a slope of 2.303RT/zF. In practical terms, this means that, for a monovalent ion at 298 K, a slope of 59.1 mV per decade change in concentration occurs. This is often referred to as a Nernstian response (Ahmad *et al.*, 2001).

1.5.2 Voltammetry

The term voltammetry is used to classify the group of electrochemical techniques in which a potential (volt) is applied to an electrode immersed in an electrolyte solution containing a redox species, and the resulting flow of current (ampere) measured. The current arises from a heterogeneous electron transfer between the electrode and the redox couple. This electron transfer between the electrode and the redox species in solution near the electrode depends on the energy of electrons in the electrode and the energy of the lowest unoccupied orbitals or the highest occupied orbitals or the species in solution. The energy level of electrons in the electrode is referred to as the Fermi level. By applying a very negative potential to the electrode, the Fermi level is raised. When the energy of electrons in the metal equals or exceeds that of the lowest unoccupied orbital, electrons can transfer from the electrode to the species in solution. The species is reduced in the process and hence a reduction current flows. Similarly, the energy of the electrons can be lowered by imposing a more positive potential and at some point electrons on the species in solution will find a more favourable energy in the electrode and will transfer there. Their flow, from solution to electrode, produces an oxidation current response (McCarron, 1999; Bard and Faulkner, 2001).

By monitoring the current of an electrochemical cell at varying electrode potentials, it is possible to determine several characteristics of the solution species such as concentration, reaction kinetics and so forth. The graph of current versus potential obtained is called a voltammogram and it provides information about the redox reaction. It is important that the voltammogram reflect the analyte's reaction at the working electrode. Thus, it is important that the potentials measured be in a region where solvent, electrolyte and electrode are not reacting. This range of potentials is called the potential window.

The heart of a voltammetric system is the potentiostat. A potentiostat is a device, which will apply a potential (or voltage) across a pair of electrodes and simultaneously measures the current, which flows through a solution of an analyte. In voltammetry, experiments can be carried out in electrochemical cell with two electrodes immersed in a sample solution containing the electrochemically active analyte and excess amount of inert supporting electrolyte. The use of the two electrodes has the advantage of simplicity in both set-up and controls. The electrochemical reaction of interest occurs at the working electrode (WE) as it is used to monitor the response of analytes. The WE may be constructed from a wide variety of conduction materials, preferably of mercury and carbon (graphite, glassy carbon), or alternatively of gold and platinum. The primary characteristic of the other electrode in the electrochemical cell, the reference electrode (RE) is to maintain a stable voltage potential regardless of the current flow through it and the WE. However, changes in solution resistance or current flow, can directly affect the potential appearing at the WE. There are two electrodes, which are commonly used as reference electrodes for the precise control of the WE potential in aqueous media. These are the saturated calomel electrode (SCE) and the silver/silver chloride (Ag/AgCl) electrode in a solution of fixed chloride concentration.

Using a three-electrode arrangement for voltammetric measurements allows more accurate measurements of WE potential at the expense of more complex control electronics. A third electrode called a counter electrode (CE) is used to complete the electrical circuit. It is recommended that the area of this electrode is substantially larger than that of the WE. A platinum wire or foil is usually used as the CE but graphite-carbon rod can also be used (Gunzler and Williams, 2001; Scholz, 2002).

1.5.3 Processes at Electrode - Solution Interface

During voltammetric experiments, there are two types of processes that occur at the electrode - solution interfaces. These are faradaic and non-faradaic processes. Both these processes occur when electrode reactions take place. Although the faradaic processes are the primary interest, the transient currents due to nonfaradaic processes must be taken into account when using electrochemical data to obtain information about charge transfer and associated reactions (Bard and Faulkner, 2001).

1.5.3.1 Non-Faradaic processes

Non-faradaic processes do not involve charge transfer. They are usually adsorption or desorption processes that affect the structure of the electrode – solution interface and so can be observed to change with a change in applied potential or solution composition. Although electrons are not transferred across the electrode – solution interface, external currents can flow when the potential, electrode area, or solution composition changes. These currents flow to charge or discharge the interfacial region between the electrode and the solution. This region is known as the double layer (Bard and Faulkner, 2001).

The electrical properties of this layer are important, since they significantly affect the electrochemical measurements. In an electrical circuit used to measure the current that flows at a particular working electrode, the double layer can be viewed as a capacitor. To obtain a desired potential at the working electrodes, the double-layer capacitor must be first appropriately charged, which means that a charging current, not related to the reduction or oxidation of the electroactive species, flows in the electrical circuit. While this current carries some information concerning the double layer and its structure, generally, it interferes in electrochemical investigations by limiting the detectability of voltammetric techniques (Wang, 2000; Scholz, 2002).

1.5.3.2 Faradaic Processes

Processes in which charges are transferred across the metal – solution interface leading to the occurrence of oxidation or reduction, are faradaic processes. These processes follow Faraday's Law, which states that the amount of chemical reaction at an electrode is proportional to the current.

As the aim of voltammetric experiments is to obtain a current response that is related to the concentration of the target analyte, this objective is accomplished by monitoring the transfer of electron(s) during the redox process of the analyte:

$$O + ne^- \rightleftharpoons R \qquad (1.04)$$

where O and R are the oxidised and reduced forms of the redox couple. The Nernst equation gives the relationship between the potential and the concentrations of the oxidised and reduced form (C_o and C_R) of the redox couple at equilibrium (at 298 K):

$$E = E^o + \frac{2.3\,RT}{nF} \log \frac{C_O}{C_R} \qquad (1.05)$$

where E is the applied potential, E^o is the standard potential for the redox reaction, R is the universal gas constant (8.314 J K^{-1} mol^{-1}), T is the Kelvin temperature, n is the number of electrons transferred in the reaction in the reaction, and F is the Faraday constant (96,487 coulombs). The Nernst equation may or may not be obeyed depending on the system or the experimental conditions. The current resulting from a change in redox state of the redox couple is termed the faradaic current because it obeys Faraday's law (i.e. the reaction of 1 mole of substance involves a charge of n x 96487 coulombs). The magnitude of the observed faradaic current can provide information on the overall rate of the many processes occurring at the working electrode surface (Wang, 2000).

1.5.4 The Rate of Electrode Reaction

The electrode reaction rate i.e., the magnitude of the current flow, depend on the rate of the slowest step, which may be either the rate of mass transfer of the analyte to the electrode, rate of adsorption or desorption at the electrode surface, rate of the electrode transfer between the electroactive species and the electrode or rates of the individual chemical reactions which are part of the overall reaction scheme (McNaughtan *et al.*, 1999; Ahmad *et al.*, 2001; Bard and Faulkner, 2001).

Whether a given reaction is controlled by mass transfer or electron transfer, is usually determined by the type of compound being measured and by various experimental conditions (electrode material, media, operating potential, mode of mass transport, time scale, etc.). For a given system, the rate-determining step may thus depend on the potential range under investigation (Wang, 2000; Bard and Faulkner, 2001).

When the rate of electron transfer between the redox couple and the electrode is high compared with the rate of mass transfer, then the electrode reaction is reversible and hence controlled by the rate of mass transfer of the electroactive species to the electrode surface. Under these circumstances the concentration ratio for oxidised and reduced forms of the couple at the electrode surface is described by the Nernst equation (Equation 1.05).

If electron transfer between the redox species and the electrode is very slow (kinetical controlled) relative to the mass transfer of solution species to the electrode, then the observed current will not be a function of mass transfer. In this case, the low rate of electron transfer results in a concentration ratio of the two forms of the redox couple at the electrode surface that no longer conforms to the Nernst equation (Equation 1.05). Current – voltage curves of these so-called irreversible processes have the corresponding current peaks that are hugely separated, of different heights and of limited analytical utility.

An intermediate situation arises when the electron-transfer and mass-transfer rates are comparable. Such quasi-reversible electrode reactions are quite common, and their analytical utility depends to a large extent on careful control of the mass-transfer rate in the electroanalytical method used for their study (Gunzler and Williams, 2001).

1.5.4.1 Mass transfer

Mass transfer of electroactive species to the electrode surface occurs in three forms:

(i) *Migration* arises from the movement of charges by the action of electrostatic force on charged particles in solution when subjected to a potential gradient. It is the mechanism by which charge passes through the solution between two electrodes and without which there would be no electron flow in the electrical circuit.

(ii) *Convection* arises from the action of mechanical forces acting on the solution. This can take the form of natural convection due to thermal gradients or forced convection due to stirring of the solution.

(iii) *Diffusion* arises from the presence of a concentration gradient, which results in a flux of material diffusing to and from the electrode surface. In analytical applications, migration and convection can generally be controlled to ensure the current flow is limited by diffusion of the species to the electrode surface (McNaughtan *et al.*, 1999).

1.5.4.2 Electron transfer

Electron-transfer reactions can be either kinetically fast or slow. When most of the analyte react fast at the working electrode's surface, this type of reaction is reversible. For this reaction there is no advantage gained by increasing the applied potential. But for slow reactions, not all of the analyte reaching the working electrode's surface have time to react. So to drive the electrolytic reaction at a faster rate, a much higher potential or "overpotential" need to be used. Slow reactions are essentially irreversible. The more irreversible a reaction, the greater the applied potential must be. This results in greater noise and a poorer limit of detection.

1.5.5 Linear Sweep and Cyclic Voltammetry

1.5.5.1 Linear Sweep Voltammetry

Linear sweep voltammetry (LSV) (or it close relative, cyclic voltammetry) is the first technique employed in any electrochemical investigation since it is easy to perform and provides quick and useful information about the system under investigation (Fisher, 1996). LSV involves sweeping the potential at a scan rate (*v*) between an initial (lower limit, E_1) and a final potential (upper limit, E_2) as shown in Figure 1.1.

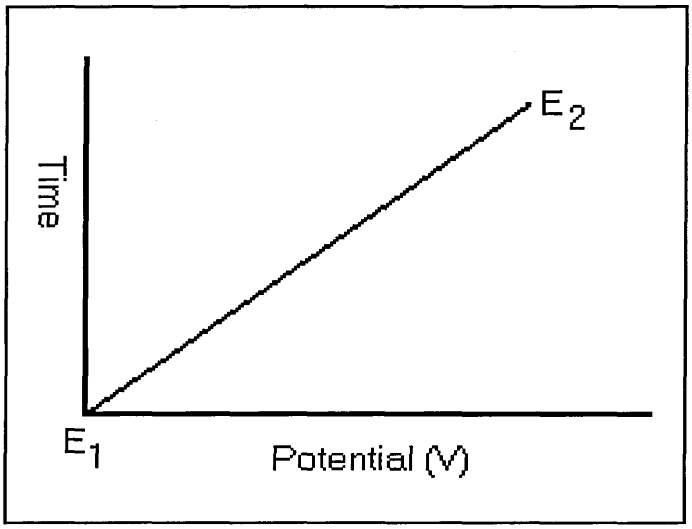


Figure 1.1: Potential – time excitation signal in linear sweep voltammetry experiment (adapted from Gunzler and Williams, 2001).

In LSV, it is typical to measure the peak current (i_p), the potential at the peak current (position of the current maximum, E_p) and the half-peak potential ($E_{1/2}$), which is the potential when the current is half of the peak current. For LSV measurements, the current response is plotted as a function of potential. For the Fe^{3+}/Fe^{2+} system

$$Fe^{3+} + e^- \rightleftharpoons Fe^{2+} \qquad (1.06)$$

the following voltammogram (Figure 1.2) is observed for a single potential scan.

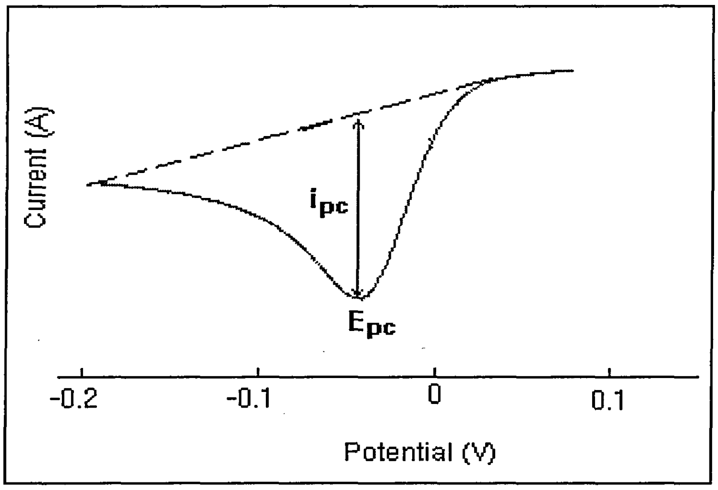


Figure 1.2: Typical linear sweep voltammogram showing the resulting current – potential curve.

The potential scan begins from the right hand side of the current - potential plot where no current flows. But as the potential is swept further to the left (to more negative values) a current begins to flow and eventually reaches a peak before dropping. To understand this behaviour the influence of applied potential on the equilibrium established at the electrode surface needs to be considered. If one considers the electrochemical reduction of Fe^{3+} to Fe^{2+}, the rate of electron transfer is fast in comparison to the potential sweep rate. Therefore at the electrode surface, equilibrium is established identical to that predicted by thermodynamics. From equilibrium electrochemistry, the Nernst equation (Equation 1.05) predicts the relationship between concentration and potential. So as the potential is swept from E_1 to E_2 the equilibrium position shifts from no conversion at E_1 to full conversion at E_2 of the reactant at the electrode surface.

The exact form of the voltammogram can be rationalised by considering the potential and mass transport effects. As the potential is initially swept from E_1 the equilibrium at the surface begins to alter and the current begins to flow. The current rises as the potential is swept further from its initial value as the equilibrium position is shifted further to the left hand side, thus converting more reactant. The peak occurs, since at some point the diffusion layer has grown sufficiently above the electrode so that the flux of reactant to the electrode is not fast enough to satisfy that required by the Nernst equation (Equation 1.05). In this situation the current begins to drop.

The voltammogram in Figure 1.2 was recorded at a single scan rate. If the scan rate is altered the current response also changes. Figure 1.3 shows a series of linear sweep voltammograms recorded at different scan rates for an electrolyte solution containing only **Fe**$^{3+}$

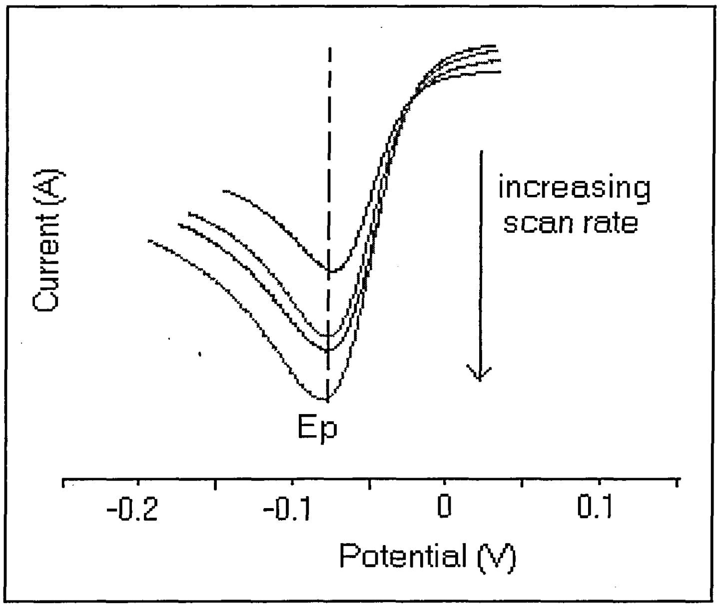


Figure 1.3: A series of linear sweep voltammograms recorded at different scan rates.

Each curve has the same form but it is apparent that the total current increases with increasing scan rate. This again can be understood by considering the size of the diffusion layer and the time taken to record the scan. Clearly the linear sweep voltammogram will take longer to record as the scan rate is decreased. Therefore the size of the diffusion layer above the electrode surface will be different depending upon the potential scan rate used. In a slow potential scan, the diffusion layer will grow much further from the electrode in comparison to a fast scan. Consequently the flux to the electrode surface is considerably smaller at slow scan rates than it is at faster rates. As the current is proportional to the flux towards the electrode, the magnitude of the current will be lower at slow scan rates and higher at high rates. This highlights an important point when examining linear sweep voltammograms (as well as cyclic voltammograms), although there is no time axis on the graph, the potential scan rate (and therefore the time taken to record the voltammogram) do strongly effect the behaviour seen.

Looking at the position of the current maximum in Figure 1.3, it is clear that the peak occurs at the same potential and this is a characteristic of electrode reactions, which have rapid electron transfer kinetics. These rapid processes are often referred to as reversible electron transfer reactions. When the electron transfer processes are 'slow' (relative to the potential scan rate), the reactions are referred to as quasi-reversible or irreversible electron transfer reactions. In this situation the potential applied will not result in the generation of the concentrations at the electrode surface predicted by the Nernst equation (Equation 1.05). This happens because the kinetics of the reaction is 'slow' and thus the equilibria are not established rapidly (in comparison to the potential scan rate) (University of Bath, 2003).

1.5.5.2 Cyclic Voltammetry

Cyclic voltammetry (CV) is very similar to LSV. In this case the potential is swept between two values at a fixed rate, however when the potential reaches $\mathbf{E_2}$, the scan is reversed and the potential is swept back to $\mathbf{E_1}$ (Figure 1.4).

A typical cyclic voltammogram recorded for a reversible single electrode transfer reaction is shown in Figure 1.5. E_{pa} and E_{pc}, are the peak positions for oxidation and reduction peak on the potential axis while i_{pa} and i_{pc} are the maximum currents for the oxidation and reduction peaks.

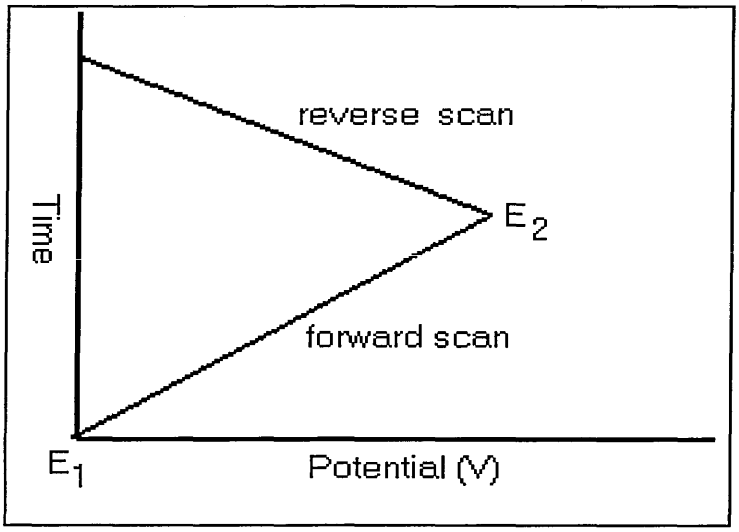


Figure 1.4: Variation of the applied potential as a function of time in a cyclic voltammetry experiment (adapted from Gunzler and Williams, 2001).

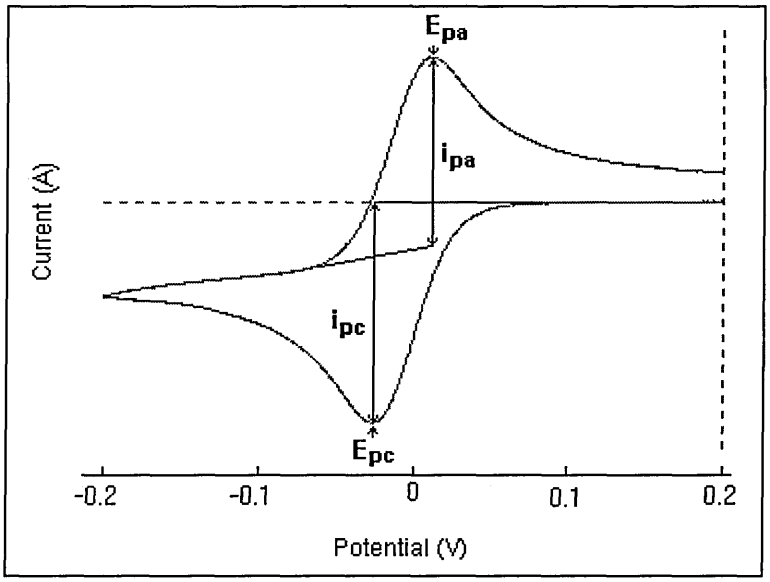


Figure 1.5: Cyclic voltammogram recorded for a reversible single electrode transfer reaction.

The forward sweep produces an identical response to that seen for the LSV experiment. When the scan is reversed we simply move back through the equilibrium positions gradually converting electrolysis product **Fe**$^{2+}$ back to reactant **Fe**$^{3+}$.

For a reversible electrochemical reaction, the CV recorded has certain well-defined characteristics (Wang, 2000; University of Bath, 2003):

(i) The separation between the peak potentials is given by

$$\Delta E = E_{pa} - E_{pc} = \frac{57}{n} \mathrm{mV} \qquad (1.07)$$

(ii) The positions of peak potential do not alter as a function of potential scan rate

(iii) The ratio of the peak currents is equal to one at all scan rates

$$\frac{i_{pa}}{i_{pc}} = 1 \qquad (1.08)$$

(iv) The peak currents (i_p) are proportional to the square root of the scan rate (v)

$$i_{pa} \text{ and } i_{pc} \propto \sqrt{v} \qquad (1.09)$$

(v) The electron transfer at the electrode (heterogeneous charge transfer, k^o) is very fast

$$k^o > 10^{-1}\ \text{cm s}^{-1} \qquad (1.10)$$

(vi) The peak current is given by the Randles-Sevcik equation:

$$i_p = (2.69 \times 10^5)\, n^{3/2} A D^{1/2} C^* v^{1/2} \qquad (1.11)$$

where n is the number of electrons, A is the electrode area (in cm^2), C is the concentration (in mol cm^{-3}), D is the diffusion coefficient (in $cm^2\ s^{-1}$), and v is the scan rate (in V s^{-1}).

The CV for cases where the electron-transfer is not reversible shows considerably different behaviour from their reversible counterparts. Again this may be rationalised in terms of the equilibrium at the surface is no longer establishing so rapidly. In these cases the peak separation is no longer fixed but varies as a function of the scan rate. Similarly the peak current no longer varies as a function of the square root of the scan rate. By analysing the variation of peak position as a function of scan rate it is possible to gain an estimate for the electron transfer rate constants (University of Bath, 2003).

For a quasi-reversible or irreversible electrochemical reaction, the CV recorded has certain defined characteristics (Wang, 2000):

(i) The peak current, given by

$$i_p = (2.99 \times 10^5)\, n(\alpha n_a)^{1/2} A C D^{1/2} v^{1/2} \qquad (1.12)$$

is still proportional to the bulk concentration, but will be lower in height (depending upon the value of α).

(ii) The peak potential shift with scan rate

$$E_p = E^o - \frac{RT}{\alpha n_a F}\left[0.78 - \ln\frac{k^o}{D^{1/2}} + \ln\left(\frac{\alpha n_a F v}{RT}\right)^{1/2}\right] \qquad (1.13)$$

where α is the transfer coefficient and n_a is the number of electrons involved in the charge transfer step. E_p occurs at potential higher than E^o, with the overpotential related to k^o and α. Independent of the value k^o, such displacement can be compensated by an appropriate change of the scan rate. The peak potential and the half-peak potential (at 25 °C) will differ by $0.048/\alpha n$ V. Hence, the voltammogram becomes more drawn-out as αn increases.

(iii) The heterogeneous charge transfer is extremely slow (e.g. due to large reorganisation energy) for irreversible process

$$k^o < 10^{-5}\ \text{cm s}^{-1} \qquad (1.14)$$

(iv) For quasi-reversible process

$$10^{-1}\ \text{cm s}^{-1} > k^o > 10^{-5}\ \text{cm s}^{-1} \qquad (1.15)$$

1.5.6 Pulsed Voltammetry

Pulsed voltammetric techniques (normal pulse voltammetry, differential pulse voltammetry and square-wave voltammetry) are aimed at suppressing the charging background current and so that the detection limits of voltammetric measurements can be lowered. Several waveforms are employed for improving the faradaic to charging current ratio, including normal pulse, differential pulse and square-wave. All are based on what is called chronoamperometry, the measurement of current as a function of time after applying a potential pulse (step). The duration of the pulse is about 50 ms. After the potential is stepped, the charging current decays very rapidly to negligible value, while the faradaic current decays more slowly as illustrated in Figure 1.6. Thus, by sampling the current in the last few milliseconds of the pulse, the current is almost largely faradaic (Wang, 2000). Due to their greatly improved performance, modern pulse techniques have largely supplanted classical polarography in the analytical laboratory.

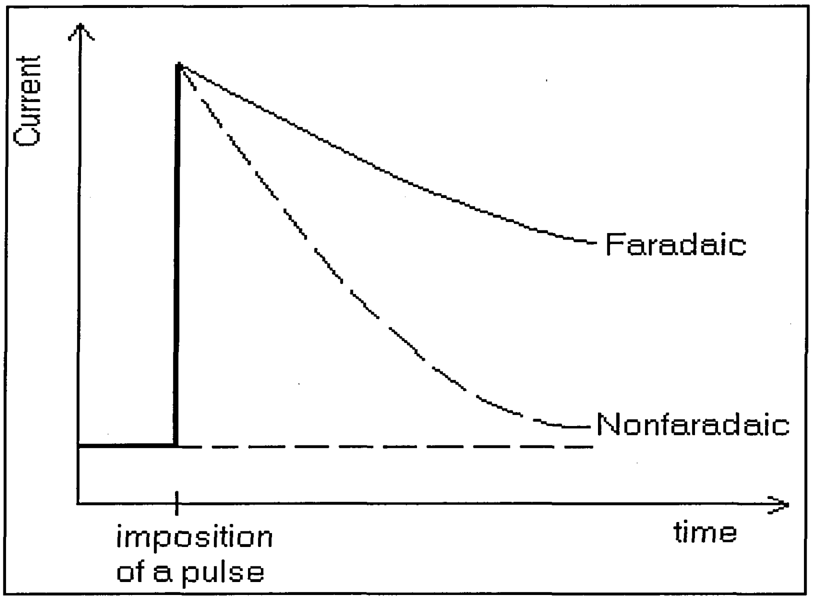


Figure 1.6: Faradaic and nonfaradaic currents flowing after the application of a potential pulse plotted vs. time (adapted from Scholz, 2002).

1.5.6.1 Square-wave voltammetry (SWV)

With SWV, the excitation signal consists of a symmetrical square pulse of amplitude E_{sw} superimposed on a ramped (staircase) waveform of step height ΔE. The current is sampled twice during each square-wave cycle, once at the end of the forward pulse (at t_1) and once at the end of the reverse pulse (at t_2). The difference between the two measurements is plotted against the base staircase potential (Wang, 1988; 2000). The technique is extremely rapid and several times more sensitive than differential pulse voltammetry (DPV) (Ahmad *et al.*, 2001).

1.5.7 Chronopotentiometry

In voltammetric studies, the current flowing through the working electrode is measured as a function of the applied voltage and the rate at which the voltage is scanned. In chronopotentiometry, the change of potential of the working electrode with time at constant current is followed. Chronopotentiometry has also been called "galvanostatic voltammetry" and is the most basic constant current experiment (Bott, 2000; Gunzler and Williams, 2001).

The basis of controlled current experiments is that a redox (electron transfer) reaction must occur at the surface of the working electrode in order to support the applied current. The potential of the electrode attains values that are characteristic of the electroactive couple changes at the electrode surface. One can think of the process as a titration of the electroactive reactant in the vinicity of the electrode by a continuous flux of electrons (Bard and Zoski, 2000).

Determinations are usually carried out under stationary conditions. The electrolytic processes occurring when current flows decide the ratio ($C_O : C_R$) of the analyte on the electrode surface, which leads to changes of potential according to the Nernst Equation (Equation 1.05). The current excitation signal and potential response are shown as a function of time in Figure 1.7. The time between the steep sections of the curve is called the transition time τ, which, in diffusion-controlled reversible electrode processes, is described by the Sand equation (Gunzler and Williams, 2001):

$$\tau^{1/2} = \frac{\pi^{1/2} n F D^{1/2} C}{2 jo} \qquad (1.16)$$

where *jo* is the current density (A cm^{-2}), and $\tau^{1/2}$ corresponds to the limiting diffusion current in DC polarography. At constant *jo*, the potential – time function is:

$$E = E_{\tau/4} + \frac{RT}{nF} \ln \frac{\tau^{1/2} - t^{1/2}}{t^{1/2}} \qquad (1.17)$$

with $E_{\tau/4} = E_{1/2}$ for a reversible reaction. Normal chronopotentiometry has little importance for analysis. Determinations are possible in the range $10^{-4} - 10^{-5}$ mol l^{-1} at best (Gunzler and Williams, 2001). However, the technique is of use in understanding the methods of stripping chronopotentiometry and potentiometric stripping analysis, which are important in trace analysis.

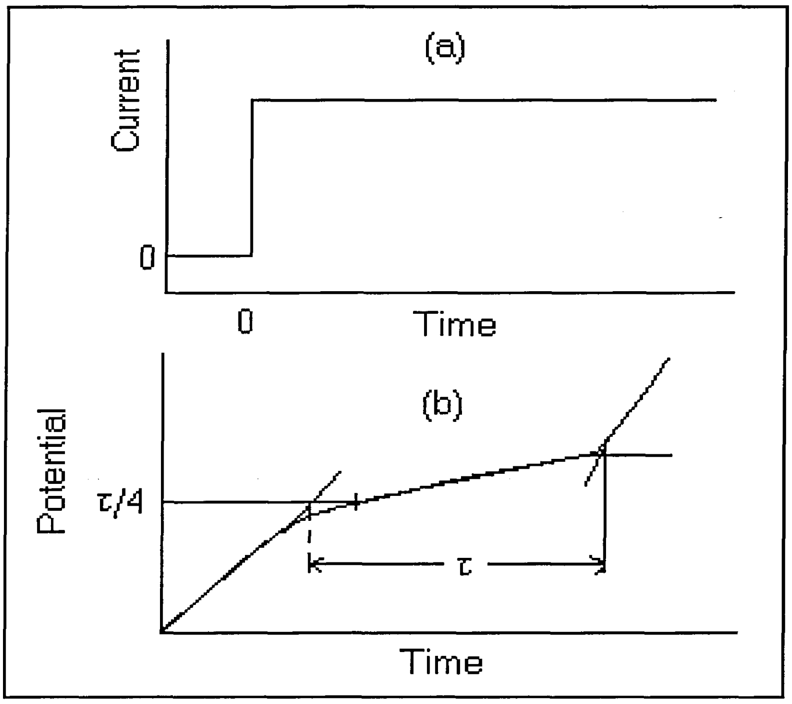


Figure 1.7: (a) Current excitation and (b) potential response for chronopotentiometry (adapted from Gunzler and Williams, 2001).

1.5.8 Chronoamperometry

Chronoamperometry involves poising the working electrode at a fixed value, such that a (chosen) oxidation or reduction occurs at the surface. The resultant current is determined by diffusion of reacting species to the electrode and is given by the Cottrell equation (Ahmad *et al.*, 2001):

$$I(t) = \frac{nFACD^{1/2}}{\pi^{1/2}\, t^{1/2}} \qquad (1.18)$$

The current is proportional to $t^{-1/2}$, which is termed Cottrell behaviour. Chronoamperometry is very common technique in the specialised field of biosensors. Its more common uses are for calculating the surface area of an electrode or for measuring the diffusion coefficient of an electroactive compound.

1.5.9 Stripping Analysis

The term stripping analysis would be better reserved as a generic name for the entire family of electrochemical techniques based on stripping procedures (Esteban and Casassas, 1994).

As a consequence, different classifications of stripping voltammetry (or stripping potentiometry) can be equally reasonable. In Table 1.5, a tentative classification of different schemes of stripping analysis is given. Two major groups are differentiated, depending on the type of preconcentration- electrolytic or non-electrolytic.

Table 1.5: A tentative classification of stripping electroanalytical schemes (Esteban and Casassas, 1994).

ELECTROLYTIC PRECONCENTRATION	SCHEME
By formation of amalgams.	Anodic stripping voltammetry Stripping potentiometry
The formation of sparingly soluble species.	Cathodic stripping voltammetry Anodic stripping voltammetry
By formation of sparingly soluble compounds with metal ions from the electrode.	Cathodic stripping voltammetry Stripping potentiometry
By formation of sparingly soluble compounds by co-electrolysis with another species.	Cathodic stripping voltammetry
NON-ELECTROLYTIC PRECONCENTRATION	**SCHEME**
Adsorptive stripping voltammetry.	Non-faradaic measurements/ faradaic measurements
Preconcentration on chemically modified electrodes.	By complexation with specific groups By ion-exchange reactions (ion-exchange voltammetry)

The several well-known schemes, such as anodic or cathodic stripping voltammetry (ASV or CSV), as well as other schemes such as stripping potentiometry (potentiometric stripping analysis (PSA) or constant current stripping chronopotentiometry (CCSCP)) which are not really stripping voltammetric approaches because a final potential versus time measurement is carried out, involve electrolytic preconcentration. Adsorptive stripping voltammetry (AdSV) and preconcentration on chemically modified electrodes (CME) are based on non-electrolytic preconcentration schemes. In Table 1.6 and 1.7, more detailed information regarding the basis of the different experimental approaches in stripping analysis is summarised.

Table 1.6: Different techniques of stripping analysis (Esteban and Casassas, 1994).

PRECONCENTRATION STEP	MEASURING STEP
Schemes of electrolytic preconcentration	
Anodic stripping voltammetry (ASV) $M^{n+} + ne \rightarrow M(Hg)$	$M(Hg) \rightarrow M^{n+} + ne$
Cathodic stripping voltammetry (CSV) $M + X^{m-} \rightarrow M_mX_n + ne$	$M_mX_n + ne \rightarrow M + X^{m-}$
Potentiometric stripping analysis (PSA) $M^{n+} + ne \rightarrow M(Hg)$ $M + X^{m-} \rightarrow M_mX_n + ne$ Constant current stripping chronopotentiometry (CCSCP) $M^{n+} + ne \rightarrow M(Hg)$ $M + X^{m-} \rightarrow M_mX_n + ne$	$M(Hg) + \text{Oxid.} \rightarrow M^{n+} + ne$ $M_mX_n + \text{Red.} \rightarrow M + X^{m-}$ $M(Hg) + \text{Oxid. Current} \rightarrow M^{n+} + ne$ $M_mX_n + \text{Red. Current} \rightarrow M + X^{m-}$
ASV or CSV with formation of sparingly soluble species, without reagent addition $M^{n+} + (n+1)OH^- \rightarrow M(OH)_{n+1} + e$ $M^{n+} + (n-1)OH^- + e \rightarrow M(OH)_{n-1}$	$M(OH)_{n+1} + e \rightarrow M^{n+} + (n+1)OH^-$ $M(OH)_{n-1} \rightarrow M^{n+} + (n-1)OH^- + e$
ASV or CSV with formation of sparingly soluble species, with reagent addition $M^{n+} + (n+1)L^- \rightarrow ML_{n+1} + e$ $M^{n+} + (n-1)L^- + e \rightarrow ML_{n-1}$	$ML_{n+1} + e \rightarrow M^{n+} (n+1)L^-$ $ML_{n-1} \rightarrow M^{n+} + (n-1)L^- + e$
CSV with formation of sparingly soluble species by co-electrolysis of another species $M_1^{n+}, M_2^{n+} + e \rightarrow M_1M_2$	$M_1M_2 + e \rightarrow M_1^{n+}M_2^{n+}$

M = Metal, **L** = Ligand, **Oxid.** = Oxidising, **Red.** = Reducing

Table 1.7: Other techniques of stripping analysis (Esteban and Casassas, 1994).

PRECONCENTRATION STEP	**MEASURING STEP**
Schemes of non-electrolytic preconcentration	
Adsorptive stripping voltammetry (AdSV) onto conventional electrodes $A \rightarrow A_{ads}$ $M^{n+} + L^{m-} \rightarrow (M_mL_n)_{ads}$	$A_{ads} \rightarrow A$ $A_{ads} \rightarrow A^* + ne$ $(M_mL_n)_{ads} \rightarrow M^{n+} + L^{m-}$ $(M_mL_n)_{ads} \rightarrow M^{n+} + L^{p-} + (m\text{-}p)e$ $(M_mL_n)_{ads} \rightarrow M + L^{m-} + ne$
Stripping voltammetry with preconcentration on chemically modified electrodes $M^{n+} + R(LH)_n \rightarrow R(LM)_n + nH^+$ $Org^{n+} + R(LH)_n \rightarrow R(LOrg)_n + nH^+$	$R(LM)_n + me \rightarrow M^{n-m)+}$ $R(LOrg)_n + me \rightarrow Org^{(n-p)+}$
Abrasive stripping voltammetry (AbrSV) Mechanical transfer of A	$A + e \rightarrow A^-$

M = Metal, **L** = Ligand, $\mathbf{R(LH)_n}$ = Ion exchangers, $\mathbf{Org^{n+}}$ = Organic cation, **A** = Absorbable species

Stripping analysis is a two-step electrochemical technique. The first step consists of an electrolytic deposition of the metal onto the working electrode. In the most common versions of stripping analysis, anodic stripping voltammetry (ASV) and stripping potentiometry (PSA or SCP), the preconcentration step involves reduction of metal ion to the metal, which then dissolves in the mercury electrode. For this purpose, a deposition potential (E_{dep}) is applied to the working electrode.

Preconcentration is followed by a stripping (dissolution) step, the measurement step-in which the metal is oxidised from the working electrode. In ASV, after the preconcentration step, the potential is scanned in the positive (anodic) direction, linearly, or according to some other potential - time waveform (e.g. differential pulse and square-wave). When the potential reaches the standard potential of a metal-metal ion couple, that particular metal is reoxidised back into solution and a current flow results. The potential – time waveform used in ASV experiment and the resulting response, is shown in Figure 1.8. The current-potential voltammogram recorded during the stripping step provides the desired analytical information. The peak potential (position), E_p, of each metal is characteristic of that metal and is related to the standard potential of its redox couple. Thus, it can be used for qualitative identification. The peak current (height), i_p and/or peak area is proportional to the concentration of the corresponding metal ion in the test solution (Estela *et al.*, 1995).

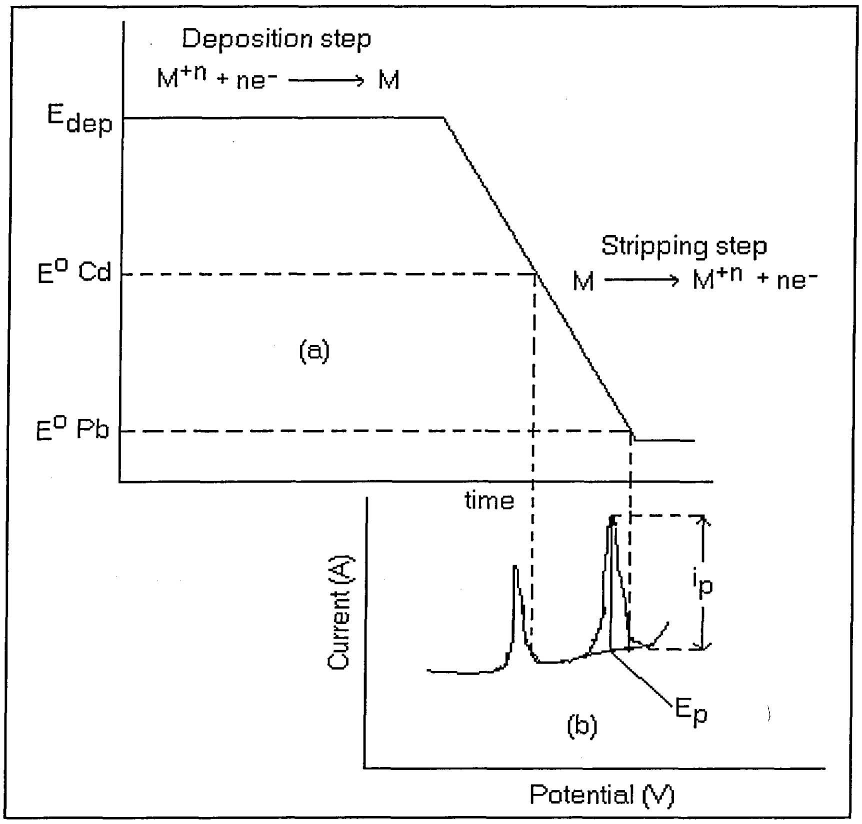


Figure 1.8: Anodic stripping voltammetry: (a) the potential - time waveform, (b) the resulting voltammogram (adapted from Wang, 2000).

In stripping potentiometry, the stripping mode differs in that, either an oxidant (for potentiometric stripping analysis); e.g. mercury (Hg^{2+}), oxygen (O_2), or a constant anodic current (for constant current stripping chronopotentiometry) is used to remove the analyte, and the potential is monitored as a function of time as shown in Figure 1.9a. No control is made of the potential of the working electrode during stripping because the potentiostat switches off once the deposition has taken place (Estela *et al.*, 1995; Wang, 2000). When the oxidation potential of a given metal is reached, the potential scan is slowed down as the oxidant or current is used for its stripping. A sharp potential step thus accompanies the depletion of each metal from the electrode. The resulting potentiogram consists of stripping plateaus, as in a redox titration curve. The transition time needed for the oxidation of a given metal, τ, is a quantitative measure of the sample concentration of the metal. Modern electrochemical analysers use microcomputers to register fast stripping events and to convert the wave-shaped response to a more convenient peak over a flat baseline. Such a differential display of dt/dE versus potential is shown in Figure 1.9b.

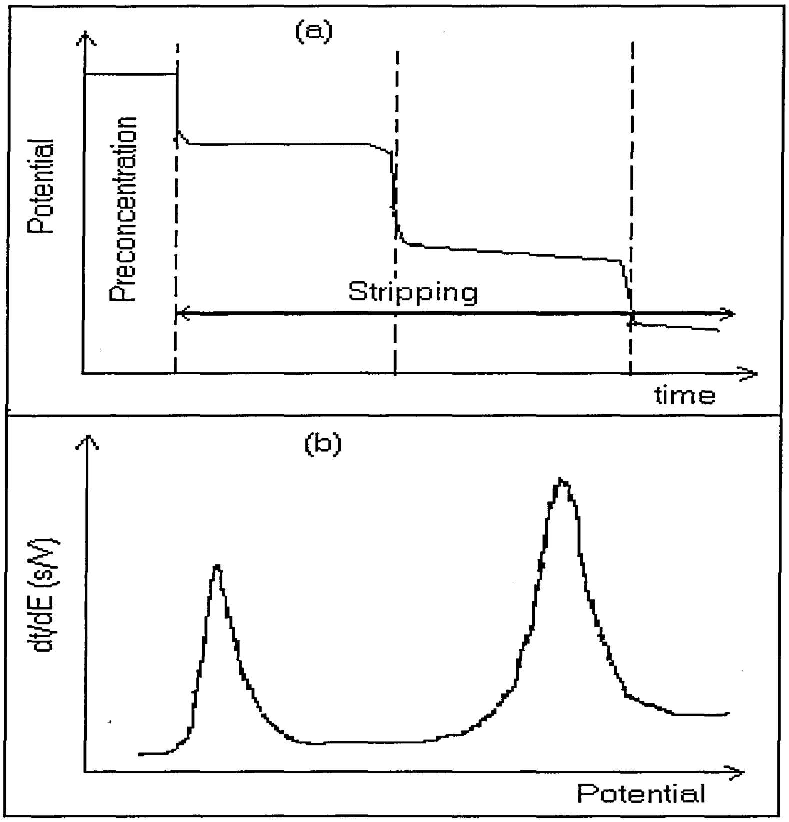


Figure 1.9: Stripping chronopotentiograms (a) original potential – time measurement display, (b) differential display of dt/dE vs. E (adapted from Estela *et al.*, 1995).

Cathodic stripping voltammetry (CSV) is the "mirror image" of ASV (Wang, 2000). It involves anodic deposition of the analyte, followed by stripping by a negative-going potential scan. Quantification is accomplished by measuring the height of the resulting reduction peak. Unlike ASV, the deposited analyte forms an insoluble film of the mercury salt on the mercury surface. As a result, it is not unusual for calibration curves to display non-linearity at high concentrations due to saturation of the electrode surface. CSV can also be used for direct determination of elements without the oxidation of Hg in an intermediate step, the decisive factor being the presence of a second species, which participates in the formation of an intermetallic compound or a salt. The redissolution of this compound by cathodic stripping allows the determination of the analyte (Esteban and Casassas, 1994).

Adsorptive stripping voltammetry (AdSV) is an approach aimed at extending the scope of stripping analysis toward numerous analytes. It is similar to conventional stripping analysis in that the analyte is preconcentrated onto the working electrode prior to its voltammetric measurement, but it differs from the conventional scheme in the way preconcentration is accomplished. Rather than using electrolytic deposition, adsorptive stripping voltammetry utilises controlled adsorptive accumulation for preconcentration. Hence, for a wide range of surface-active organic and inorganic species that cannot be preconcentrated electrolytically, the adsorption approach serves as an effective alternative. The analyte is allowed to accumulate interfacially on the electrode surface for a specified length of time, under conditions of maximum adsorption (electrolyte, pH, potential, mass-transport, etc.); then the adsorbed materials are determined by applying a negative- or positive-going potential scan (for reducible or oxidisable species, respectively).

Adsorptive collection of metals as their surface-active complexes on electrodes has been shown to be extremely useful for ultratrace measurements of metals such as aluminium, chromium, nickel, vanadium, uranium, iron and thallium that cannot be determined conveniently by conventional stripping analysis. AdSV also offers effective alternative procedures for metals such as copper, gallium, manganese and tin that are measurable with difficulty by conventional ASV. The formation of an appropriate surface-active complex of the metal, based on adding a suitable chelator, precedes the preconcentration step (Ewing, 1994).

1.6 PROJECT AIMS AND OBJECTIVES

The fact that environmental factors like changes in weather, the pH of the soil or water can mobilise bound metals and greatly increase their toxicity, sites contaminated with heavy metals must be monitored on regular basis. Also sites undergoing attempted remediation must be monitored frequently to assess progress in heavy metals cleanup. To be able to monitor and control toxic heavy metal ion contamination, there is urgent need for sensitive and robust sensing devices that are capable of on-site metal analysis. Incorporation of field methods into environmental monitoring processes decreases sample transportation and facilitates more rapid decision-makings.

Existing on-site heavy metal detection systems apart from electrochemical sensors are generally limited to spectrophotometric chemical test kits and field portable X-ray Fluorescence (XRF) spectroscopy. Spectrophotometric chemical kits measure the total amount of a specific metal in an environmental sample, but provide no information about metal speciation. XRF, even though is a rapid and inexpensive technique, has fairly high detection limit (e.g., in the low mg l^{-1} range). The use of stripping based sensors that are non-specifically inhibited would be a reasonable first step to tackling this problem.

The aim of the work presented in this thesis is concerned with the development of electrochemical sensors for heavy metal ions in contaminated soils and groundwaters. The envisaged device is a single-use sensor incorporating a three-electrode configuration directly transducing the response of metal ions from the application of a potential – time excitation waveform or current – potential excitation waveform.

To accomplish this aim, the following specific objectives outlined in the flow chart in Figure 1.10 were carried out:

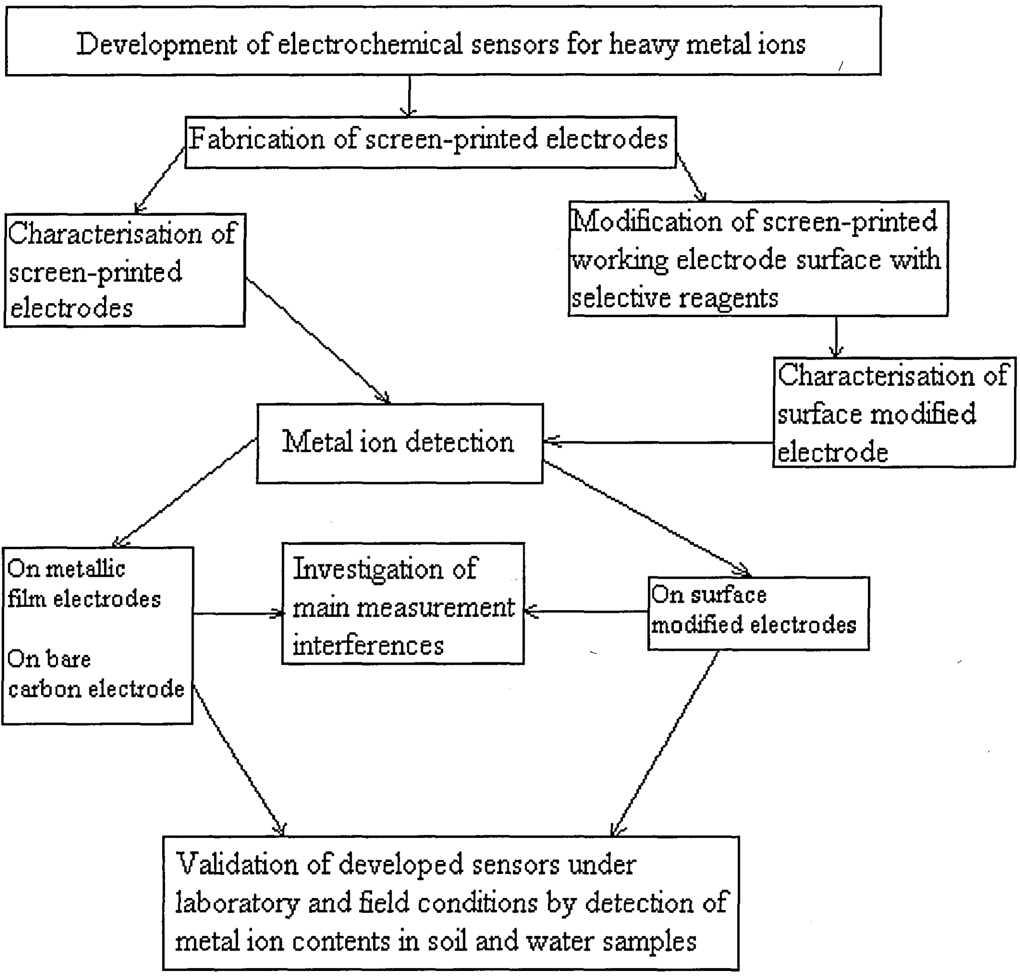


Figure 1.10: Flowchart outlining the objectives to be accomplished in this thesis.

CHAPTER 2

• 2 FABRICATION AND CHARACTERISATION OF SCREEN-PRINTED ELECTRODES

2.1 INTRODUCTION

There is an increasing demand for the development of electrochemical sensors with uniform properties and "user friendly" design. However, the overwhelming majority of the electrochemical cell assemblies commonly employed in research laboratories are not suitable for cost-effective mass fabrication (Gyurcsányi *et al.*, 2001).

Sensor microfabrication technologies like thick-film screen-printing and thin-film lithography can be used in the high volume production of electrochemical sensing devices with planar working and reference electrodes on plastic or silicon substrates. These strips can thus be considered as self-contained electrochemical cells onto which sample droplet is placed (Wang *et al.*, 1996b; 1999). As desired for decentralised testing, these easy-to-use miniaturised sensors obviate the need for traditional bulky electrodes and 'beaker-type' cells. Different electrode geometries can be fabricated using suitable patterned photomasks or stencils. Electrode structures with 0.5 µm dimensions can be produced with modern microlithographic processes, while larger (75-100 µm) structures can be achieved using screen-printing fabrication (Wang *et al.*, 1999).

Thick-film technology permits the construction of solid state, mechanically robust and planar sensors. The printing technology shows several significant advantages and these include flexibility of design, choice of materials, easy integration with electronic circuits, low cost in infrastructure and mass production, the possibility of automation of the fabrication process, a low barrier for technological transfer from thick-film to other technologies, and vice versa. Miniaturisation is feasible, along with a high degree of electrochemical activity of the devices deriving from high specific area and microporous structure. Although thick-film technology is an attractive option for fabrication of sensors, it is not free of drawbacks. Most of the problems presented by this technology are related to compatibility between the different materials, and between the materials and the manufacturing process. Thick-film technology has other shortcomings related to the sensor-fabrication process, such as the need for complicated curing cycles with high temperatures and long times, and low reproducibility of the constructed devices resulting, for example, from evaporation of solvents in paste formation (Galan-Vidal *et al.*, 1995).

The basic steps in the thick-film process involve forcing suitable inks through a patterned stencil or mask onto planar substrate materials, followed by a thermal curing. To accomplish this, there is a wide range of inks and substrate materials that can be used to mass-produce low cost sensor strips. Commercial carbon, platinum or gold ink formulations are commonly used for printing the working electrodes, whereas silver-based inks are used for obtaining the reference electrode (Wang *et al.*, 1996b; 1998b).

Many electrochemical devices rely on the use of carbon materials (Morrin *et al.*, 2003) because they lend electrodes desirable features, such as electrochemical inertness over a wide range of potentials, high hydrogen and oxygen evolution overvoltages, low background currents and high electrical conductivity (Osborne *et al.*, 1996). These carbon materials in the form of inks or pastes contain graphite particles, a polymer binder and other additives (for the dispersion, printing and adhesion tasks) with the exact ink or paste formulations regarded by the manufacturer as proprietary information (Wang *et al.*, 1996b; 1998b). For these reasons, the nature of the reactions at the electrode surface needs to be investigated so that valuable insights into the electroanalytical performance of the electrodes can be obtained. This is due to the fact that the electron transfer reactivity of the resulting carbon electrodes can be affected by the type, size and loading of graphite particles in the ink used.

The electrochemical characteristics of thick-film carbon electrodes have been studied and reported in a number of publications (Osborne *et al.*, 1996; Wang *et al.*, 1996b, 1998b; Cui *et al.*, 2001; Grennan *et al.*, 2001; Morrin *et al.*, 2003). Wang *et al.* (1998b) compared the electrochemical performance of screen-printed carbon electrodes (SPCEs) fabricated with four different commercially available carbon inks. These carbon inks are Acheson ink 49AB90 (Acheson Colloids, Ontario, CA), Ercon ink G-448-I (Ercon, Waltham, MA), Gwent ink C10903D14 (Gwent Electronic Materials, Gwent, U.K) and Dupont 7102 (Dupont Electronic Materials, Wilmington, DE). The SPCE sensors based on these inks exhibited a wide range of electrochemical reactivities (peak separation, hydrogen overvoltage and oxygen overvoltage) for benchmark redox systems. The data obtained, confirmed that the different compositions and preparations of the carbon strip electrodes have profound effect upon their electrochemical behaviour and electroanalyical utility.

Grennan *et al.* (2001) compared the electrochemical characteristics of a SPCE with that of a glassy carbon electrode (GCE) using the ferrocyanide redox couple. The peak separations (ΔE_p) obtained with the SPCEs, 100 – 120 mV, were higher than those obtained with GCEs, 66 –76 mV, using scan rates ranging from 10 to 100 mV s^{-1}. The heterogeneous electron transfer rate (k^o) for the ferrocyanide redox couple at the SPCE (2.2 x 10^{-3} cm s^{-1}) was much lower than that of GCE (1.2 x 10^{-2} cm s^{-1}) and this was attributed to the presence of the polymeric binder in the carbon ink, which serves to inhibit electron transfer rates.

The voltammetric studies of ferricyanide (potassium hexacyanoferrate (III)) on the carbon electrode were reported by Osborne *et al.* (1996) to be governed by the surface properties of the ink used for printing. Activation of the SPCEs yielded modified electrochemical responses. A ΔE_p of 823 mV using a scan rate of 100 mV s^{-1} was reported for an untreated Electrador ED5000 SPCE compared to ΔE_p of 140 mV for Electrador ED5000 SPCE, exposed to ultra-violet (UV) light from an excimer laser (laser energies of 50 mJ cm^{-2}). The calculated k^o were 2.75 x 10^{-6} cm s^{-1} for the untreated electrode and 2.49 x 10^{-3} cm s^{-1} for the treated electrode.

Comparison between commercially available SPCEs and electrodes fabricated in-house from various commercial inks was made by Morrin *et al.* (2003) in order to find the electrode most suited to amperometric sensor work. The commercially available electrodes yielded very poor reversibility for ferri/ferrocyanide couple with ΔE_p values of 471 ± 56 mV and 416 ± 37 mV for Euroflash™ and Ultra™ (Inverness Medical Ltd, Inverness, Scotland), respectively. This behaviour was attributed to poor charge transfer of the electrodes. Also the conducting path from working electrode to the contacts has got resistive properties that may be a significant limiting factor. The in-house produced electrode (Ultra-inH) designed with a silver-conducting path had the most preferable electrochemical properties. For the redox couple, a ΔE_p value of 264 ± 7 mV was obtained. The k^o was 3.09×10^{-4} cm s^{-1} for Ultra-inH while for Euroflash™ and Ultra™ (commercial screen-printed electrodes), it was 2.83×10^{-5} cm s^{-1} and 4.7×10^{-5} cm s^{-1}, respectively. The reported value for GCE was 5.9×10^{-2} cm s^{-1}.

In addition to the thick-film carbon working electrode, the characterisation of reference electrodes screen-printed with silver chloride based ink has also been reported (Desmond *et al.*, 1996; 1997; 1998; Erlenkötter *et al.*, 2000). The reproducibility and the stability over time of the silver/silver chloride (Ag/AgCl) electrode potentials were described to be very good, although the electrodes require constant chloride concentration in the solution under test in order to function correctly.

In this Chapter, work carried out in the development of an electrochemical sensor fit for heavy metal ion detection is described. Electrochemical methods were used to characterise the carbon- working electrode and the Ag/AgCl reference electrode fabricated using screen-printing techniques.

2.2 EXPERIMENTAL

2.2.1 Printing Materials/ Reagents

'Melinex' polyester sheets (228 x 350 mm), which accommodated the screen-printed electrodes was obtained from Cadillac Plastic (Swindon, U.K). The printing inks were E423-SS graphite-based ink from Acheson Colloids (U.K), a 15 % silver/silver chloride in silver paste ink from MCA Services (Cambridgeshire, U.K) and insulating ink from Agmet ESL (Reading, UK). The screen-printing screens (DEK 248 compatible) were manufactured to specification by DEK Precision Screen Division (Weymouth, U.K).

All chemicals were of analytical reagent grade and used as received. Hydrochloric acid (HCl), potassium chloride (KCl) and aluminium oxide (alumina powder) were obtained from BDH Ltd (Poole, England) while ammonium acetate was obtained from Merck (Darmstadt, Germany). Potassium hexacyanoferrate (II) (also known as potassium ferrocyanide) was obtained from Aldrich Chemical Company (Gillingham, England). All solutions were prepared using deionised water.

2.2.2 Equipments

A computer-controlled PGSTAT10, the Autolab Electrochemical Analyser with general-purpose electrochemical software operating system version 4.7 (Eco Chemie, Utrecht, The Netherlands) was used for electrochemical measurements (linear sweep voltammetric, cyclic voltammetric and chronoamperometric experiments). A specially adapted electrical edge connector obtained from Maplin (Milton Keynes, U.K) was used for connection of electrodes to the electrochemical analyser. Conventional electrodes (glassy carbon electrode, standard calomel electrode, silver/silver chloride reference electrode and platinum wire electrode) were obtained from Bioanalytical Sytems Ltd. (Cheshire, U.K).

A CC804 pH meter (Schott Gerate, Camlab, U.K) with a co-axial BNC lead was used for potentiometric measurements and a ASYC II MX 54C multimeter (Matrix, France) with two-point probe used to measure the electrical resistance of the electrodes.

2.2.3 Safety

Before 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.

2.2.4 Fabrication of electrodes

Screen-printed electrodes (SPEs) were mass-manufactured in-house by a multistage deposition process using a DEK 248-screen printer (DEK, Weymouth, UK) (Plate 2.1) and screens with appropriate stencil designs (100 per sheet) fabricated by DEK Precision Screen Division. The electrode devices were printed onto a 250μm thick 'Melinex' polyester sheet. The printer parameters were set to print at a 4 psi squeegee pressure at a 50 mm sec^{-1} carriage speed with a 2.5 mm print gap. The fabrication cycle started with the placement of E423-SS graphite ink onto the patterned screen, followed by it being forced through the screen mesh onto the polyester sheet with the aid of the squeegee as illustrated in Figure 2.1.

The first fabrication cycle produced the conducting basal track as illustrated in Figure 2.2 (100 sets were printed simultaneously on a polyester sheet). The printing of the reference layer was carried out in a similar fashion to the first cycle but this time, with a different patterned screen. Silver/silver chloride (Ag/AgCl) ink was printed over part of one of the carbon-graphite basal tracks (the left track). Each layer was allowed to dry for 2 hours at 60 °C before subsequent printing process took place. The insulating layer was printed last over the basal track, leaving a defined circular shaped area (electrochemical drop-on cell with an area of 3.2 mm^2) and the electrical contact area. This layer was cured by heat treatment at 120 °C for 2 hours and later left to dry. Each electrode set consisted of a working electrode, counter electrode and reference electrode.

Plate 2.1: A DEK 248 semi-automatic precision screen-printer.

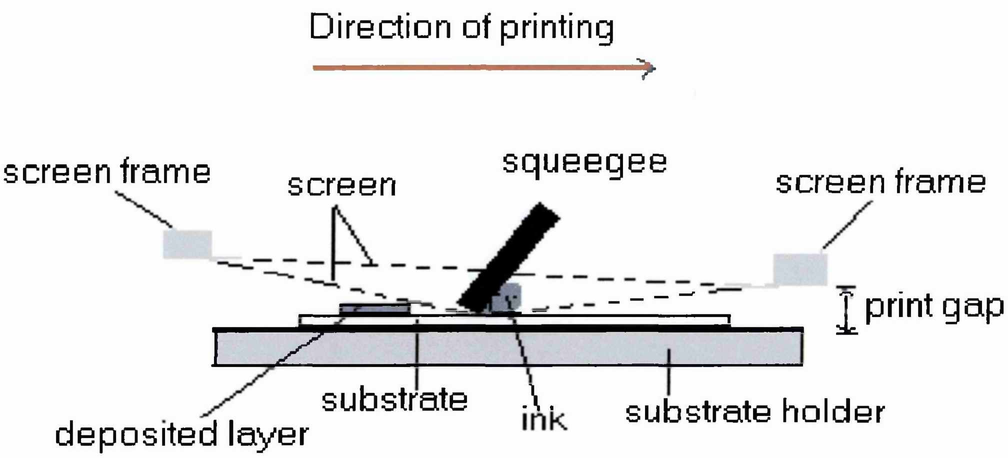
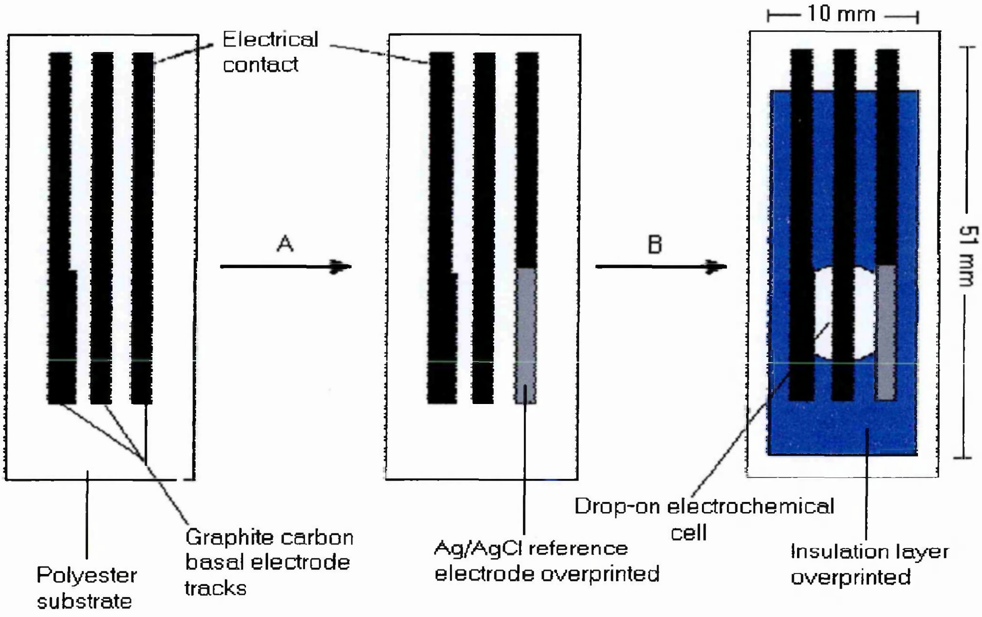


Figure 2.1: Schematic representation of the screen-printing process (adapted from Bachmann, 1999).



A = addition of reference layer   B = addition of insulating layer

Figure 2.2: Schematic representation of the fabrication steps for the screen-printed electrode system.

2.2.5 Electrochemical measurements

The electrochemical behaviours of the screen-printed electrodes were examined by cyclic voltammetry (CV), linear sweep voltammetry (LSV), chronoamperometry and potentiometry. All measurements were performed at room temperature.

2.2.5.1 Voltammetric studies

Evaluation of the polarization range on SPCE and GCE was obtained by recording the linear sweep voltammetric curve in 0.1 M HCl electrolyte solution and in a mixed medium solution of 0.1 M HCl and 0.5 M ammonium acetate. Before each experiment, the GCE was polished on slurries of alumina powder (0.3 μm) on a polishing cloth, and then cleaned with deionised water. The LSV measurements were performed from – 1.5 V to + 1.5 V relative to Ag/AgCl reference electrode using a scan rate of 50 mV s^{-1}.

The electrochemistry of the screen-printed carbon working electrode surface was investigated using CV and a benchmark compound, potassium ferrocyanide. CV was employed to determine the electroactive surface area of the working electrode (WE) as well as the electrochemical reversibility of potassium ferrrocyanide on the electrode. Cyclic voltammetric measurements of potassium ferrocyanide were carried out by scanning over a pre-set potential range (- 0.6 V to + 0.8 V relative to onboard Ag/AgCl reference electrode or saturated calomel electrode). Stock solution of potassium ferrocyanide (5 mM) was prepared in 0.1 M KCl and diluted to any appropriate concentration with 0.1 M KCl. The electrochemical behaviour of potassium ferrocyanide (0.5 mM) on the SPCEs was studied by varying the scan rate between 10 – 75 mV s^{-1}. Different concentrations of potassium ferrocyanide (0.5, 0.8, 1, 3 and 5 mM) were also studied at a fixed scan rate of 50 mV s^{-1}.

2.2.5.2 Chronoamperometric measurement

Chronoamperometric measurement of potassium ferrocyanide on SPCE was used for calculating the surface area of the WE. The measurements were carried out at an appropriate fixed potential (+ 0.8 V) over a period in unstirred solutions. The screen-printed carbon-working electrode was dipped into an electrochemical cell containing 10 ml of 0.1 M KCl (blank) and other electrodes (saturated calomel reference electrode (SCE) and platinum wire counter electrode). The chronoamperometric measurements involved taking out 100 µl of the 0.1 M KCl solution from the electrochemical cell after a steady state has been obtained for the response and then replacing it with 100 µl of 5 mM potassium ferrocyanide. The response is recorded over 2 minutes at constant potential.

2.2.5.3 Potentiometric measurement

Potentiometric measurements were carried out, by varying the concentration of KCl (1 mM – 3 M) using the screen-printed reference electrode (indicator electrode) in conjunction with a saturated calomel electrode (SCE) or Ag/AgCl reference electrode (conventional reference electrode). Both were connected to the CC804 pH meter with a BNC lead and the readings obtained using the mV function on the meter, after immersing the electrodes in the analyte solution for 15 minutes (readings taken at 1 minute interval).

2.2.5.4 Miscellaneous experiment

The electrical resistance measurement along the conducting path of the carbon-working electrode (for both the SPCE and GCE) was carried out using a multimeter. Plate 2.2 depicts how the surface resistance of the SPCEs were measured.

2.2.6 Data analysis

All experimental runs carried out in this work, was repeated three to five times with different screen-printed electrodes. Each response data point represents the mean value of three measurements and the error bar represents the standard deviation. The mean, standard deviation and relative standard deviation (R.S.D) were calculated for the measurements using Microsoft Excel 2000 and SigmaStat Statistical Software Version 2.03.

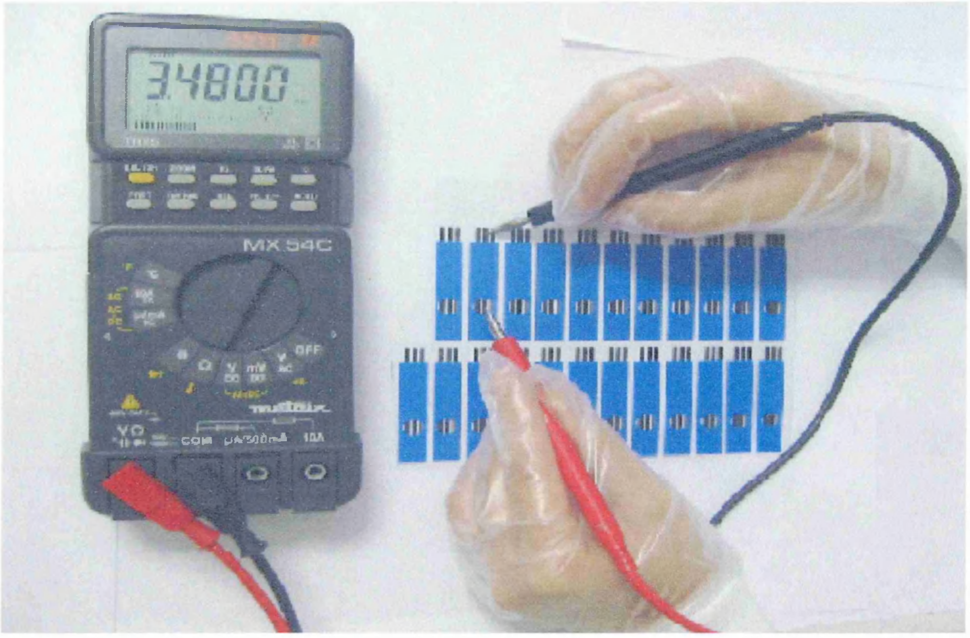


Plate 2.2: The measurement of electrical resistance along the carbon-working electrode conducting path using a multimeter with two- point probe.

2.3 RESULTS

2.3.1 Voltammetric characterisation of the working electrode

In order to assess the useful potential window of the screen-printed carbon- working electrode, initial experiments were undertaken with LSV. Comparison was made with GCE, a common working electrode with high hydrogen overpotential and wide potential window. The current response for two blank solutions of 0.1 M HCl and 0.5 M ammonium acetate + 0.1 M HCl was examined. The polarization range for the SPCE was examined since this parameter defines an electrode's negative and positive potential limits of operation.

When the SPCE was tested in 0.1 M HCl (pH 1.3), a smooth background response was obtained with a negative potential limit of approximately (~) – 0.7 V and a positive potential limit of ~ + 0.8 V. The polarization range obtained as shown in Figure 2.3a, was found to be much narrower compared to the GCE (~ - 1.1 V and ~ + 1.2 V) used. In a solution of 0.5 M ammonium acetate + 0.1 M HCl (pH 4.6), the commencement of hydrogen evolution on the SPCE shifted to a more negative value (Figure 2.3b). This is due to the increase in the pH of the medium. Apart from an interfering peak around – 0.65 V for SPCE, the polarization range obtained was sufficient for electrochemical detection of electroactive species on the working electrode surface. The nonfaradaic current obtained on the SPCE and GCE was negligible (< 1 x 10^{-8} A).

The characterisation of the working electrode (WE) was carried out by performing diagnostic electrochemical tests on the electrode surface using cyclic voltammetry and chronoamperometry. The fact that the sensor-to-sensor reproducibility of the WE area of the fabricated electrode assembly is a key issue for uniform stripping analysis operation, a benchmark compound, potassium ferrocyanide, was examined to determine the electrochemical nature of the SPCE surface.

The cyclic voltammograms obtained for 0.5 mM potassium ferrocyanide at a SPCE and a GCE (for comparison purpose) is depicted in Figure 2.4. At a low scan rate (20 mV s^{-1}), the peak-to-peak separation (ΔE_p) obtained for SPCE was 260 mV. A peak separation value of 95 mV was obtained using the GCE used in this work (Figure 2.4). Figure 2.5 shows the cyclic voltammograms obtained on SPCEs for potassium ferrocyanide at four different scan rates. The position of the oxidation peak potential (E_{pa}) in the forward sweep shifts slightly to the right with each increasing scan rate. This indicates that the redox reaction at the electrode is quasi-reversible.

The electrical resistance between the electrical contact point and the working electrode area was measured and a mean value of 3.53 ± 0.12 kΩ was obtained for the SPCE. For the GCE used for comparison in this work, a measured mean value of 5 ± 0.1 Ω was recorded.

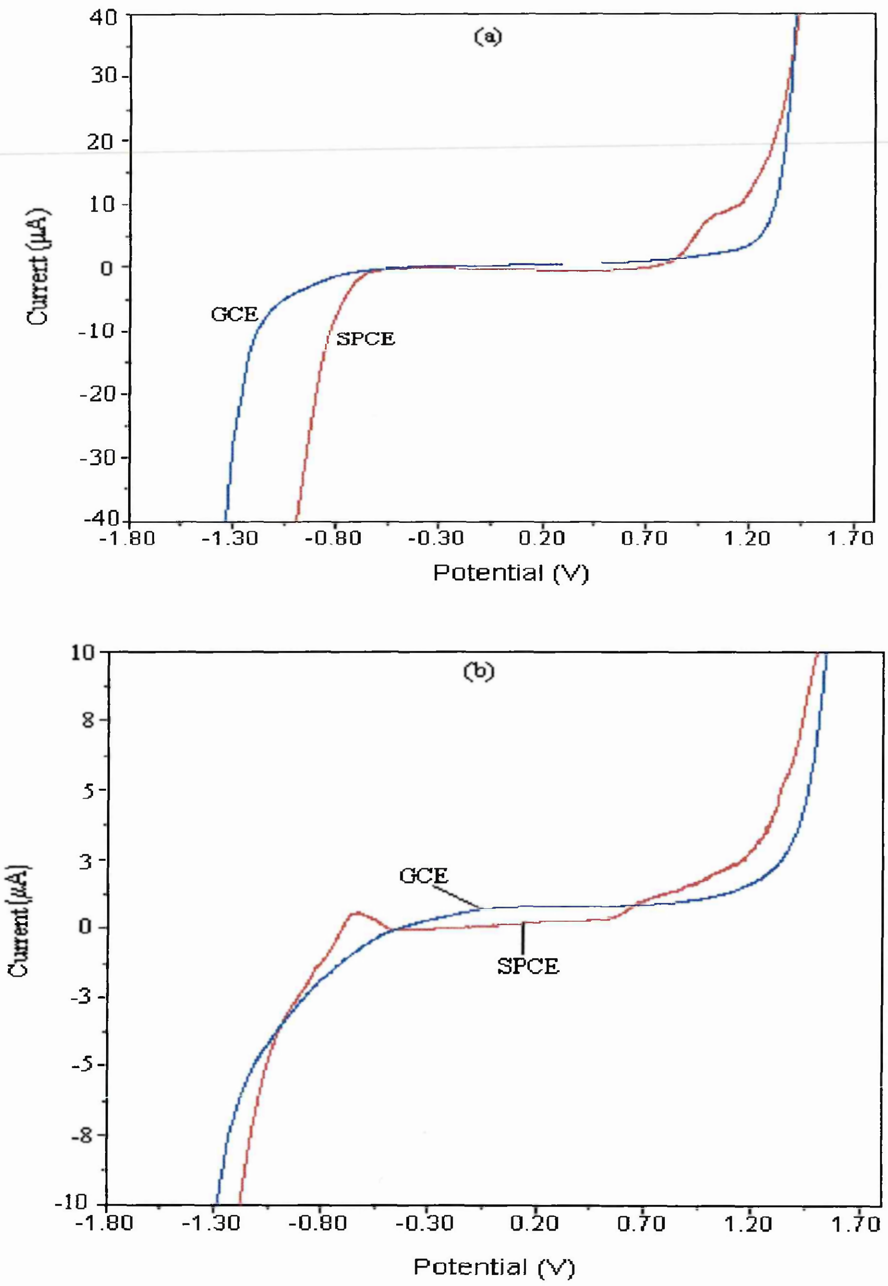


Figure 2.3: Linear sweep voltammograms of (a) 0.1 M HCl electrolyte solution and (b) 0.5 M ammonium acetate + 0.1 M HCl medium solution on different carbon electrodes. Scan range: – 1.5 V to + 1.5 V, scan rate: 50 mV s^{-1}.

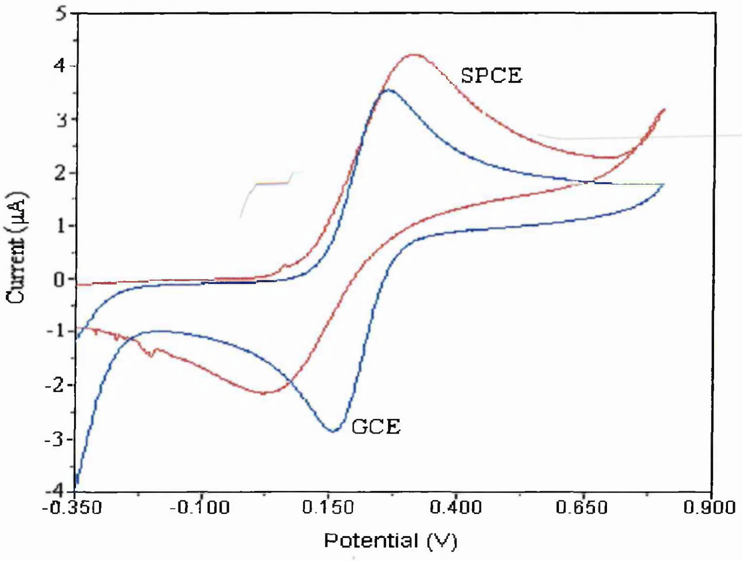


Figure 2.4: Cyclic voltammograms of 0.5 mM potassium ferrocyanide in 0.1 M KCl. SPCE (vs. screen-printed Ag/AgCl reference electrode) and GCE (vs. SCE, 3 M KCl). Scan range: – 0.6 V to + 0.8 V, scan rate: 20 mV s^{-1}.

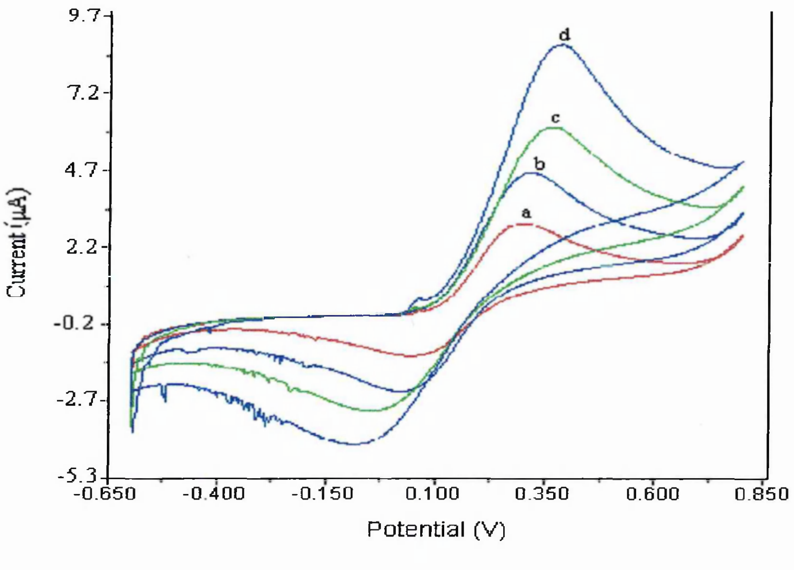


Figure 2.5: Cyclic voltammograms showing the variation in scan rates for 0.5 mM potassium ferrocyanide in 0.1 M KCl. (a) 10, (b) 25, (c) 50, (d) 75 mV s^{-1}. Scan range – 0.6 V to 0.8 V (vs. screen-printed Ag/AgCl reference electrode).

Investigating further the ease at which electron transfer take place on the SPCE surface, the heterogeneous electron transfer rate (k°) was assessed using the increase in peak separation (ΔE_p) obtained from the current – potential curves. The method described by Nicholson (1965), with the working curves that relate ΔE_p to a kinetic parameter (ψ) was used to determine the rate constant using the following equation (BAS Capsule 141, 1988a; Morrin *et al.*, 2003):

$$k^{o} = \psi \left(D_O \pi \nu \left(\frac{nF}{RT} \right) \right)^{1/2} \left(\frac{D_R}{D_O} \right)^{\alpha/2}$$

where ψ refers to a kinetic parameter, D_o is the diffusion coefficient for ferricyanide (7.6×10^{-6} cm^2 s^{-1}), D_R is the diffusion coefficient for the ferrocyanide (6.3×10^{-6} cm^2 s^{-1}), and α is the transfer coefficient, R is the universal gas constant (8.314 JK mol^{-1}), T is the absolute temperature (K), n is the number of electrons transferred, and F is Faraday's constant (96,485 C). The α value of 0.38 ± 0.005 was obtained from experiments with the equation (Galus, 1976):

$$\alpha n_a = 0.048 / E_p - E_{p/2}\ (V)$$

This value agrees with the slope value of 0.35 ± 0.015 obtained for α from plotting ΔE_p versus log v for 5 mM ferrocyanide in 0.1 M KCl (Figure 2.6). Using the α value of 0.38, a k° value of 1.36×10^{-4} cm s^{-1} was obtained for SPCE. For the glassy carbon electrode (GCE) used in this work, a k° value of 2.69×10^{-3} cm s^{-1} was obtained.

The electrochemical active area for the SPCE surface was deduced using the equation (Equation 1.12, Chapter 1):

$$i_p = (2.99 \times 10^{5})\, n(\alpha n_a)^{1/2} A\, C\, D^{1/2} v^{1/2}$$

Using the α value of 0.38 and the known diffusion coefficient (D) of 6.5×10^{-6} cm^2 s^{-1} for the ferrocyanide ion (Morrin *et al.*, 2003), values of 0.059 ± 0.006 cm^2 and

0.061 ± 0.002 cm^2 were calculated using slope values obtained from plot of anodic peak currents of 0.5 mM ferrocyanide versus square root of scan rates (10 – 75 mV s^{-1}, Figure 2.7) and plot of anodic peak currents versus various concentrations of potassium ferrocyanide at 50 mV s^{-1} (Figure 2.8).

The apparent geometrical area of the SPCE estimated by physical measurement was 0.130 cm^2 (0.65 cm x 0.2 cm). A value of 0.146 ± 0.017 cm^2 was generated with chronoamperometry using the slope of the linear region of the i_p versus $t^{1/2}$ plot for 0.05 mM ferrocyanide (Figure 2.9) and the Cottrell equation (Chapter 1, Equation 1.18) rearranged for area (A):

$$A = \frac{it^{1/2}\pi^{1/2}}{nFCD^{1/2}}$$

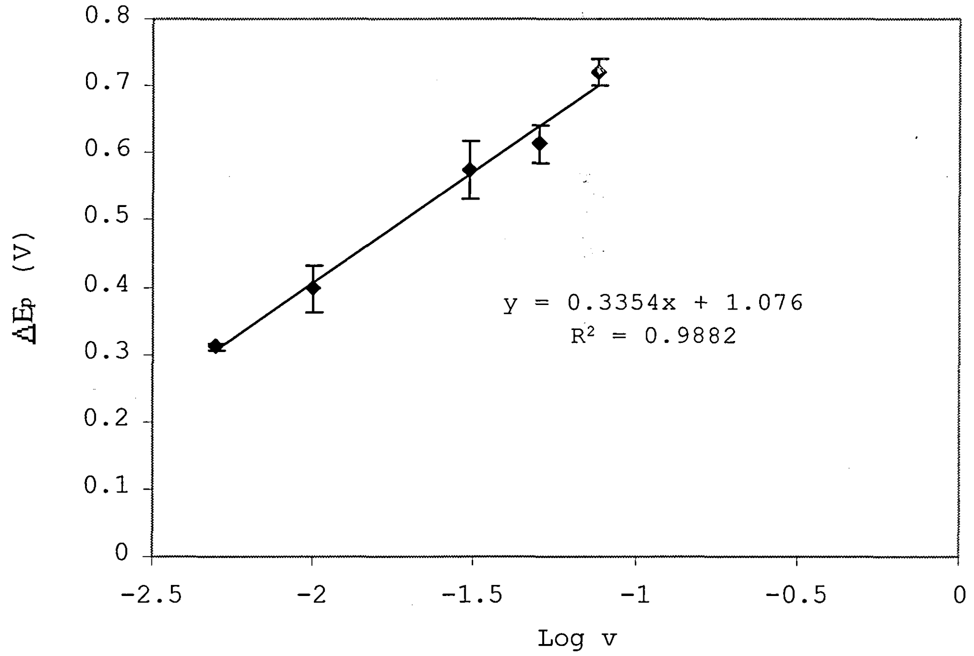


Figure 2.6: Plot of peak-peak separation (ΔE_p) versus *log v* for determining the transfer coefficient (α) of 5 mM ferrocyanide in 0.1 M KCl on SPCE. CV scan range = – 0.6 V to 0.8 V (vs. screen-printed Ag/AgCl reference electrode).

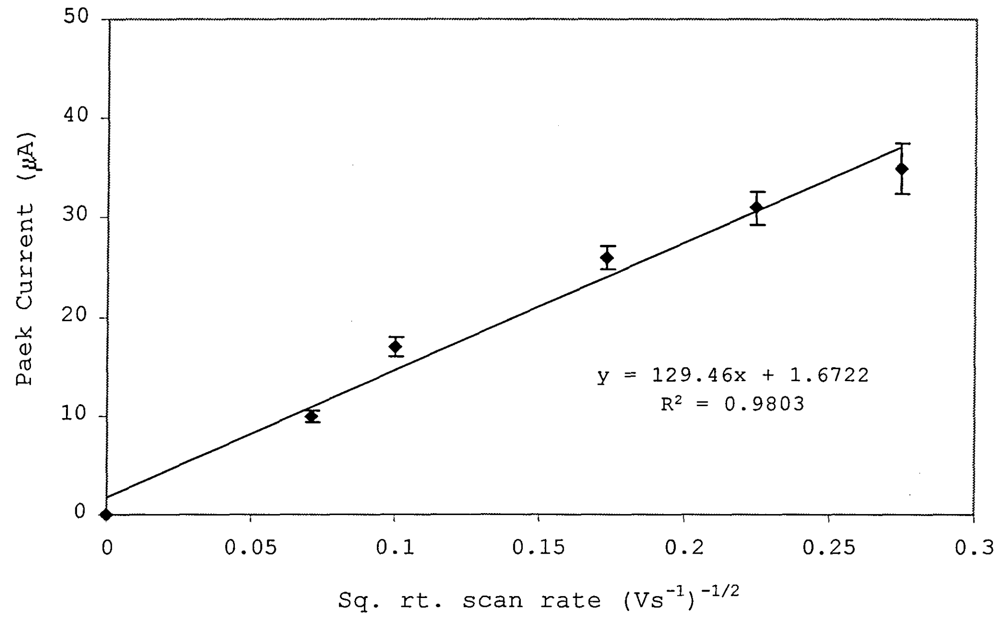


Figure 2.7: Plot of anodic peak currents versus square root of scan rates for the determination of the electrochemical active area of SPCE. CV scan range = – 0.6 V to 0.8 V (vs. screen-printed Ag/AgCl reference electrode).

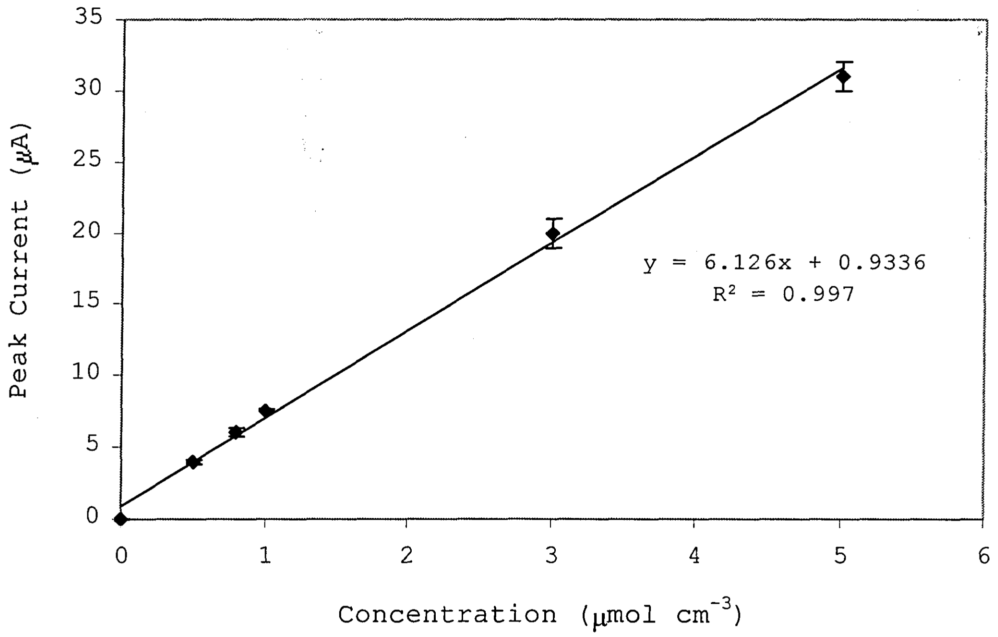


Figure 2.8: Plot of anodic peak currents versus different concentrations of ferrocyanide for the determination of the electrochemical active area of SPCE. CV scan range = – 0.6 V to 0.8 V (vs. screen-printed Ag/AgCl reference electrode).

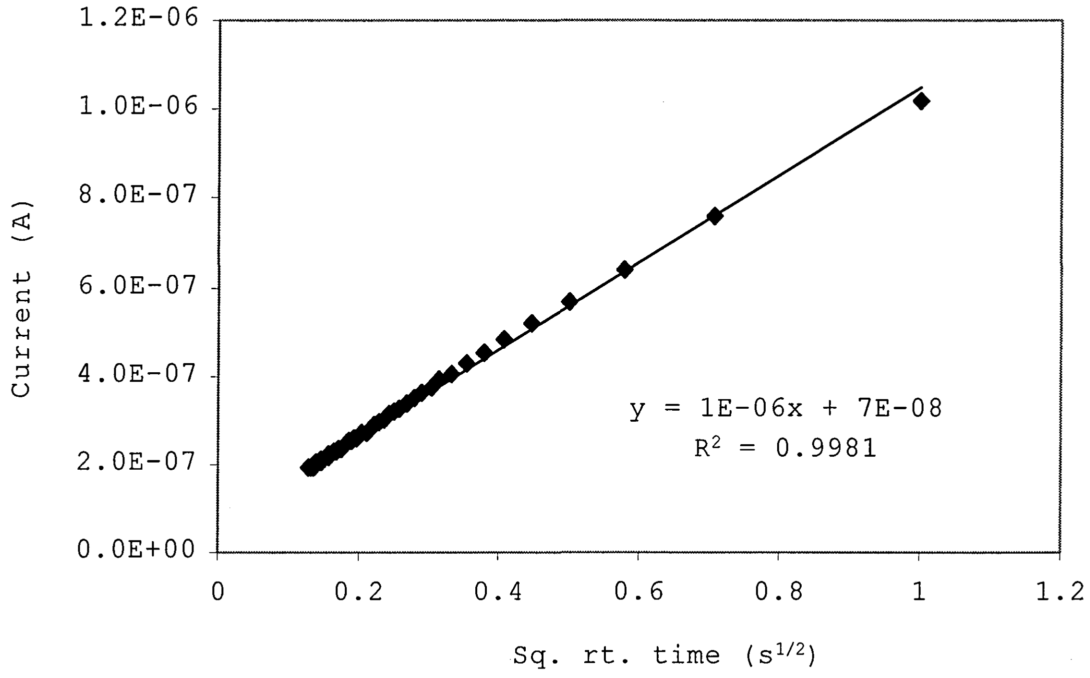


Figure 2.9: Typical plot of current response versus square root of time for 0.05 mM ferrocyanide in 0.1 M KCl. Measurement at + 0.8 V for 120 s (vs. screen-printed Ag/AgCl reference electrode).

2.3.2 Characterisation of the screen-printed reference electrode

The potential difference between the screen-printed reference electrode and the saturated calomel reference electrode (SCE) was measured in solutions of varying chloride concentrations (Figure 2.10). The readings for each concentration of chloride solution were taken 15 minute after immersing the electrode strip in the chloride solution. A logarithmic response was obtained between 1 mM and 1 M KCl with a slope value of 60.2 ± 1.2 mV per decade as illustrated in Figure 2.10b.

When the potential differences were measured between six screen-printed Ag/AgCl electrodes and a SCE, versus time in saturated potassium chloride as illustrated in Figure 2.11, a value of – 37.8 ± 4.8 mV was obtained. This value was the mean potential difference for 6 electrodes versus SCE after 15 minutes in a saturated KCl solution.

Measuring the potential difference between the screen-printed Ag/AgCl reference electrode and the convectional Ag/AgCl reference electrode (containing 3 M NaCl) in 0.1 M potassium chloride solution, led to a potential difference of – 67 ± 1.5 mV being obtained.

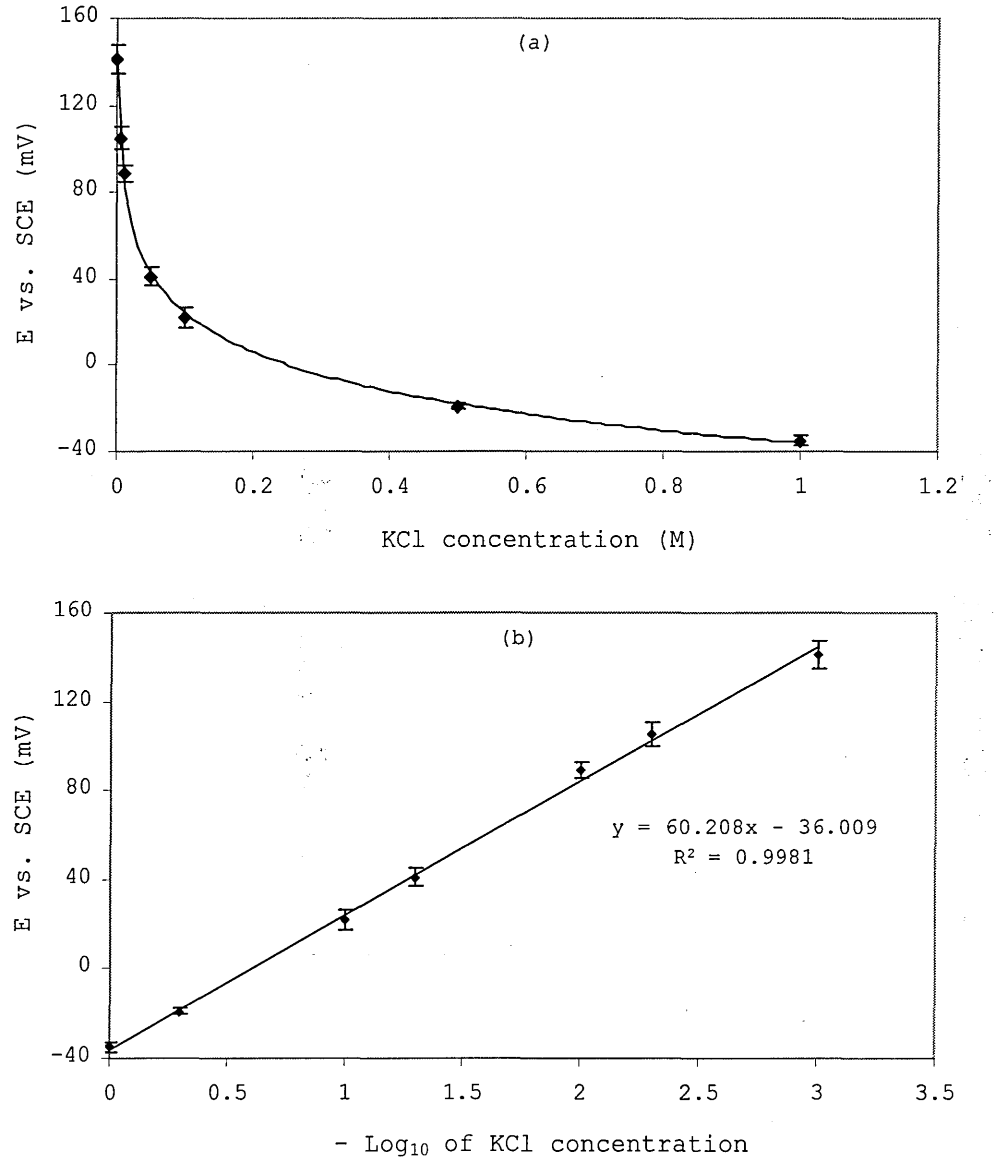


Figure 2.10: Potentiometric measurements of varying concentrations of potassium chloride. (a) normal plot, (b) logarithm plot. The data points represent mean values of 3 measurements ± standard deviation.

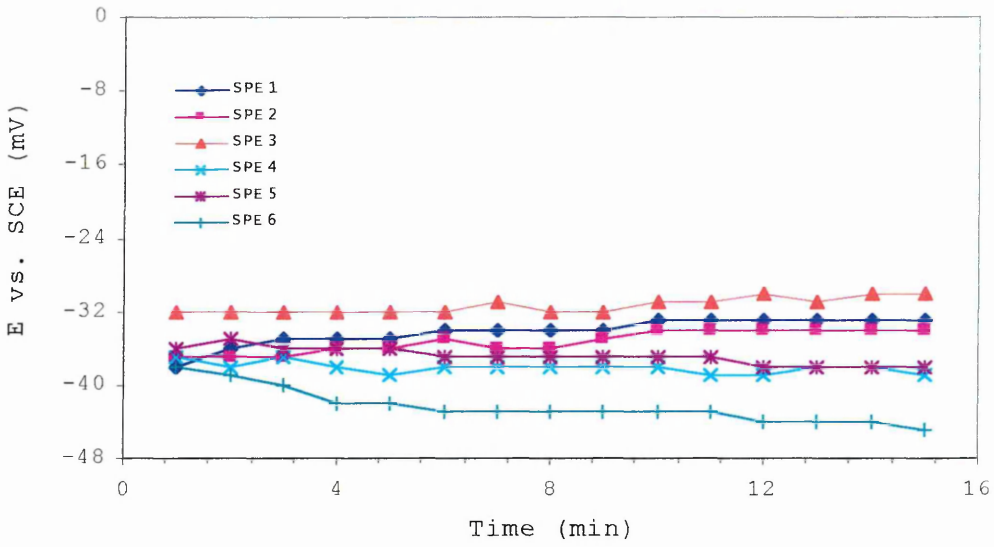


Figure 2.11: Plot of potential difference between six screen-printed Ag/AgCl reference electrodes and a SCE in saturated KCl.

2.4 CONCLUSION

In this chapter, the fabrication and characterisation of SPEs was presented. The electrodes fabricated from thick film inks form a convenient base for electrochemical metal ion sensors because they are cheap to produce. The characterisation of the SPEs showed that their surface structure and electrochemical activity are dependent on the electrode material. The SPCE did not display the ideal reversible behaviour of a fully conductive electrode (GCE) that might be expected for potassium ferrocyanide. This unattractive behaviour suggests a lower graphite-carbon loading compared to GCE. However, the inherent disposability of the SPCE removes the need for time-consuming surface polishing procedures necessary when using GCE for electrochemical measurements.

Although, the SPCE was not ideally suited for the study of electrochemical kinetics, this does not preclude their use in electroanalysis. Under acidic condition (pH 1.2), the SPCE is operable in the potential range – 0.7 V to ~ + 0.8 V, while in slightly acidic condition (pH 4.6), the SPCE is operable in the potential range – 1.1 V to ~ + 1.1 V.

The on-board screen-printed reference electrode was suitable for the precise control of the working electrode potential in aqueous media. The ink pigment of the screen-printed reference electrode is basically silver containing a small amount of silver chloride. Therefore, any medium solutions need to be supplemented with a constant concentration of chloride (0.1 M), as the potential of the screen-printed silver/silver chloride reference electrode is dependent on the concentration of chloride in the solution.

The integrated screen-printed electrodes (SPEs) characterised here in this chapter would be employed in the subsequent Chapters (3, 4 and 5) for the development of heavy metal ion sensors for use in synthetic aqueous solutions and in environmental samples.

CHAPTER 3

• 3 HEAVY METAL IONS DETECTION ON SCREEN-PRINTED ELECTRODES

3.1 INTRODUCTION

The analyses of trace heavy metals have been reported in many publications. These include examples where electrodes in conjunction with electrochemical stripping techniques, have either shown promise or have been successfully applied to the determination of heavy metals in different matrices. The different types of electrodes used include conventional bulk electrodes such as carbon paste electrode, dropping mercury electrode, glassy carbon electrode, gold disc electrode, silver electrode, etc. (Arrigan *et al.*, 1994a; 1994b; Jagner *et al.*, 1996; Kopanica and Novotny, 1998; Locatelli and Torsi, 1999; Bonfil and Kirowa-Eisner, 2002; Misiego *et al.*, 2003). Others such as microelectrodes, screen-printed electrodes (thick-film electrodes) and thin-film electrodes have also been used (Kovacs *et al.*, 1995; Daniele *et al.*, 1997; Honeychurch *et al.*, 2000; Silva *et al.*, 2001).

Due to demand for electroanalytical stripping methodologies for on-site monitoring of heavy metals, particular attention has been given to the replacement of bulky electrodes and cumbersome cells with self-contained test strips. Initially, field based stripping analysis experiments were undertaken with the electrodes commonly used in the laboratory, i.e. hanging mercury electrodes or mercury plated thin-film glassy carbon electrodes (Bond *et al.*, 1997). However, maintenance of the electrodes in the field is often difficult and in the last 11 years a number of reports describing the development and application of screen-printed disposable electrodes have appeared (Wang and Tian, 1992; 1993a; 1993b; Wang *et al.*, 1993; Desmond *et al.*, 1998; Reeder and Heineman, 1998; Palchetti *et al.*, 1999; Honeychurch *et al.*, 2000; Zen *et al.*, 2002; Masawat *et al.*, 2003; etc). These strips rely on planar carbon, silver or gold working electrodes and sometimes a silver reference electrode printed on an inexpensive plastic or ceramic support (Wang *et al.*, 1999).

The interest in screen-printed electrodes (SPEs) as electrochemical transducers is due to the possibility of coupling these electrodes with compact and user-friendly electrochemical analyser (Wang, 1994). The surfaces of these electrodes can be employed as a support for the deposition of metallic films. Generation of the metallic film on the working electrode surfaces is mostly performed *in situ* by reduction of mercury (II) (or silver (I), gold (III), bismuth (III)) added to the supporting electrolyte during the preconcentration step of the stripping analysis. The preplating or *ex situ* plating of the metallic film on the electrode surface in mercury (II) (or silver (I), gold (III), bismuth (III)) solution before analysis takes place can also be used. A metallic film can also be obtained by modifying the working electrode surface using a selected metal precursor which is electrochemically reduced during the conditioning or preconcentration step.

Mercury is among the most widely used electrode material in electrochemical stripping analysis. This is because of its many advantageous features with respect to stripping applications. These include a readily renewable and reproducible surface, a high hydrogen overpotential and the ability to dissolve many metals that aids the preconcentration process (Khoo and Guo, 2002). However, the growing concern over the toxicity of mercury plated on electrode surface and the need to apply stripping analysis for decentralised screening and on-site monitoring has prompted the search for mercury free electrode sensors (Honeychurch *et al.*, 2002b).

In the following sub sections (3.1.1, 3.1.2 and 3.1.3), a review of published work on the applications of SPEs as electrochemical metal sensors is presented. Detections on these electrode surfaces, i.e., mercury film screen-printed carbon electrodes (MFSPCEs), unmodified bare screen-printed carbon electrodes (SPCEs) and other metallic film coated screen-printed electrodes are highlighted.

3.1.1 Detection with mercury film screen-printed carbon sensors

Wang and Tian, (1992) employed screen-printed ExacTech glucose electrode strips (MediSense Inc.) for the determination of lead by means of anodic stripping voltammetry (ASV) and potentiometric stripping analysis (PSA). The carbon contact area (2 × 8 mm) of the electrode strip served as the substrate onto which the mercury film was plated, with a second strip providing the Ag/AgCl reference electrode. The working mercury film electrode was prepared by pre-plating from a nondeaerated, stirred solution containing 80 mg l^{-1} mercury in 0.02 M hydrochloric acid. The deposition potential and time were – 1.15 V and 15 minutes for ASV and – 0.90 V and 15 minutes for PSA respectively. The stripping voltammograms obtained were similar to those recorded with mercury-coated glassy carbon electrode and hanging mercury drop electrode, with no compromise in the stripping performance, i.e., sensitivity and signal to background. The optimum sensitivity, using a 10 minute preconcentration from stirred solution, was obtained using differential pulse anodic stripping voltammetry (DPASV) with detection limits of 0.03 and 0.05 μg l^{-1} for lead and cadmium, respectively. Wang *et al.*, (1993) also described mercury film screen-printed electrode arrays for on-site stripping analysis of lead, copper and cadmium. These disposable composite electrodes were fabricated by a combination of thick-film printing and laser machining technologies. The stripping performances were not compromised by placing non-deaerated and unstirred sample drops on the strip, as desired for field monitoring of trace metals. The calibration linear range obtained with square-wave anodic stripping voltammetry (SWASV) for lead and cadmium was 10 – 80 μg l^{-1} following 2 minute preconcentration. The detection limit was 0.1 μg l^{-1} for analogous measurements of 0.5 μg l^{-1} lead following 4 minutes preconcentration. The precision was good with a relative standard deviation of 4.7 % for ten repetitive measurements of 40 μg l^{-1} lead. For potentiometric stripping analysis (PSA), the stripping response is linear over the entire 2.5 – 25 μg l^{-1} range ($r = 0.999$). The limit of detection ($S/N = 3$) was found to be 0.3 μg l^{-1} for 2 minute preconcentration. Twelve repetitive PSA measurements of a 15 μg l^{-1} lead solution yielded a reproducible response with a relative standard deviation of 4.2 %. The method was applied to assays of drinking water.

A portable environmental monitoring system based on stripping voltammetry and SPEs was described by Desmond *et al.* (1998) for trace metal analysis. These SPEs consisted of working electrode, reference electrode and counter electrode. The mercury film was plated *in situ* onto the working electrode surface. Concentration ranges of between 0 and 1000 μg l^{-1} gave reasonably linear calibration curves with limits of detection being 55, 71, 64 and 123 μg l^{-1} for zinc, cadmium, lead and copper, respectively, using a 300 s preconcentration time.

Palchetti *et al.* (1999) reported the characterisation of disposable SPEs for detection of heavy metals. The graphite surface of the working electrode was used as a substrate for preplating a thin mercury film (TMF), which allowed the electrochemical preconcentration of heavy metals. With the measurement parameters optimised, detection limits of 0.4, 1 and 8 μg l^{-1} were obtained from the analysis of 10 μg l^{-1} of cadmium (II), lead (II) and copper (II) respectively for 3 minute preconcentration time, using SWASV. The precision (relative standard deviation) obtained for the analysis of 10 μg l^{-1} of lead (II) was 15 % with a three-electrode system containing conventional reference and counter electrodes. In order to have a more practical device that excludes the use of a conventional reference electrode; the performance of single working electrode strips was compared to two-electrode strips using PSA. The detection limits for 5 μg l^{-1} of lead, cadmium and copper were 0.6, 0.4 and 0.8 μg l^{-1} respectively, using a two-electrode strip with a preconcentration time of 75 s. The relative standard deviation (RSD) of 9 repetitive measurements on the same strip was 7 % (14 % on different strips) for 5 μg l^{-1} of lead (II). Under the conditions applied, the PSA technique was found to be more sensitive and more rapid than SWASV. The method was applied to assays of tap water

A hand-held electrochemical analyser designed and developed by Yarnitsky *et al.* (2000) was described for field screening of trace lead (II). The stripping-based instrument was reported to meet the size, weight and power-consumption requirements for decentralised lead analysis while also maintaining the high sensitivity and reliability of centralised stripping operations. A SPE, which serve as a disposable cell is inserted into the side of the meter. Potentiometric stripping measurements of trace lead were carried out by placing a 300 μl sample drop on the electrode strip to cover the working and reference electrodes and plating the mercury film *in situ*. When the hand-held instrument was coupled with the screen-printed disposable sensors, it was reported that the units' response varied linearly with the lead concentration between 20 - 300 μg l^{-1} (50 s deposition) using cadmium (100 μg l^{-1}) as a pilot ion. The performance of the hand-held instrument was tested on on-site assays of drinking water. The supporting electrolyte was 0.18 M HCl solution, which contained 3 mg l^{-1} and 50 - 100 μg l^{-1} mercury (II) and cadmium (II) ions, respectively. Data obtained compared favourably with those obtained with an established laboratory-based stripping instrument.

A copper/mercury-plated screen-printed electrode, (CMSPE), was described by Zen *et al.* (2000) for trace lead (II) determination by SWASV. The intermetallic property of lead with copper in mercury was utilised to increase the sensitivity of lead (II) detection. The calibration curve was linear in the range 0 – 100 $\mu g\ l^{-1}$ lead (II) with a detection limit (S/N = 3) of 0.81 $\mu g\ l^{-1}$ for 60 s preconcentration. Calcium (II), manganese (II), nickel (II), zinc (II) and mercury (II) were reported to have little effect on the lead (II) response but bismuth (III), thallium (III) and tin (III) showed an adverse effect on the lead (II) response. The analytical utility of the CMSPE was demonstrated by applying to various water samples.

Choi *et al.* (2001) described a screen-printed disposable sensor prepared by integrating a mercury oxide (HgO) as a built-in mercury precursor into the working electrode matrix for stripping analysis of heavy metals. The disposable sensor consisted of the modified working electrode, a carbon counter and a silver/silver chloride reference electrode printed onto a polycarbonate substrate. The HgO-modified working electrode was evaluated using lead and cadmium as probe metals. Repetitive stripping analysis of 30 $\mu g\ l^{-1}$ lead and cadmium solution gave relative standard deviations of less than 2.5 % (n = 15) for peak current responses. Simultaneous analyses were performed using the certified reference material (CRM) obtained from high-purity standards company.

3.1.2 Detection with unmodified bare screen-printed sensors

Reeder and Heineman (1998) described a thick-film carbon sensor (screen-printed) for trace determination of lead. The sensor consisted of an electrode pair of graphite carbon black working electrode and Ag/AgCl reference electrode printed onto a polycarbonate surface. Lead solution in the 2.5 – 20 μM (0.5 – 4 mg l^{-1}) concentration range using linear sweep anodic stripping voltammetry (LSASV) with identical deposition times (90 s) resulted in a linear relationship between peak current and analyte concentration. SWASV was reported to offer the advantage of rapid determination of lead in oxygen containing solutions compared to LSASV. Multi-elemental analysis at bare carbon sensor provided stripping analysis, which was comparable to that of mercury film electrode for the determination of lead. Good precision was obtained for both the sensor/sensor variability (5.8 %) and repetitive analysis upon a single carbon sensor (5.1 %).

Honeychurch *et al.* (2000), have reported the use of SPCEs as disposable sensors for the measurement of trace levels of lead without any chemical modification of the sensor surface. The anodic peak of greatest magnitude was obtained in 0.1 M HCl using cyclic voltammetry (CV). Deposition potential of - 1.4 V was used in conjunction with DPASV. Calibration plots were found to be linear in the range 6.3 to 24 μg l^{-1} and 24 to 50 μg l^{-1} using a deposition time of 150 s. The limit of detection was 2.5 μg l^{-1} and the coefficient of variation determined on one single electrode at a concentration of 12.6 μg l^{-1} was calculated to be 2.4 % (n = 5). The effect of copper, cadmium, and zinc on the lead stripping peak was examined and, under the conditions employed, no significant change in current was found. The sensors were evaluated by carrying out lead determinations on spiked and unspiked pond water. The recovery was calculated to be 97.4 % and the coefficient of variation was 3.2 % at a concentration of 12.6 μg l^{-1}.

SPCE, without chemical modification, was used by Honeychurch *et al.* (2002a) as disposable sensor for the measurement of trace levels of copper. The anodic peak of greatest magnitude was obtained in 0.1 M malonic acid using CV. Studies with DPASV showed that the copper response is linear from 8 – 450 μg l^{-1} using a preconcentration potential and time of – 1.0 V and 150 s, respectively. The detection limit was calculated to be 8.2 μg l^{-1}. The effect of bismuth, cadmium, iron, lead, mercury, antimony and zinc on the copper stripping peak was examined with only mercury found to have significant effect under the conditions employed. The proposed SPCE was evaluated by carrying out copper determinations on bovine serum, tap water, river water and seawater.

Zen *et al.* (2002) investigated the use of screen-printed silver electrodes (AgSPEs) as disposable sensors for the measurement of trace levels of lead. The AgSPE was printed onto the flexible polypropylene film using a conducting silver-gel followed by coating of thin gel layer and cured under ultraviolet (UV) irradiation for 1 hour.

Under the optimised analytical conditions, the obtained linearity and detection limit are 5 – 80 μg l^{-1} (r = 0.9992) and 0.46 μg l^{-1} (S/N = 3), respectively. The interference effect was thoroughly studied with various metals and no significant change in current was found in the determination of 5 μg l^{-1} lead. The utility of the electrode was demonstrated by applying it to the determination of lead in pond water and groundwater.

3.1.3 Detection with other metallic-film coated screen-printed electrodes

Wang and Tian (1993a) reported a mercury-free disposable lead sensor based on PSA at gold-coated SPEs. ExacTech blood glucose strips (MediSense Inc.) were also used as electrodes. The gold film was pre-plated from a non-deaerated, stirred 50 mg l^{-1} gold solution in 0.25 wt % hydrochloric acid by holding the carbon strip electrode at – 0.40 V for a 20 minute period. The potential was then switched to + 0.70 V and held for 5 minutes. Gold film prepared *in situ*, by co-deposition with the target lead ions was also carried out but such operation, while yielding a well-defined lead response, required 5 - 6 deposition and stripping cycles before the peak is stabilised. Subsequent work was carried out with pre-plated gold films using a pH 4.5 acetate buffer. Detection limits of 0.1 and 0.6 μg l^{-1} were deduced following 20 and 4 minute deposition, respectively from stirred solutions, whilst a value of 3 μg l^{-1} was obtained in unstirred solution for 10 minute deposition. The gold-coated electrode was reported to function in a highly stable manner over a 2-week period, performing 60 solution runs of 10 μg l^{-1} lead with no apparent loss of the response (RSD = 2.8 %). The electrode-to-electrode reproducibility was good (n = 6, RSD = 6.1 %). There was a linear correlation between the stripping response and lead concentration in the range: 5 – 25 μg l^{-1} (240 s, stirred), 50 – 250 μg l^{-1} (30 s, stirred) and 50 – 600 μg l^{-1} (30 s, unstirred). Also the same authors (Wang and Tian, 1993b) used the same screen-printed carbon strip coated with a thin gold film for potentiometric stripping measurements of trace levels of mercury. A detection limit of 0.5 μg l^{-1} was obtained for 4 minute deposition. The precision was good with 20 repetitive measurements of 25 μg l^{-1} of mercury yielding a relative standard deviation of 2.5 %.

Wang *et al.* (2001a) described the use of bismuth- coated SPEs for the measurement of lead. The bismuth-coated SPCEs offered a reliable quantitation of trace lead in conjunction with ASV. The bismuth film was preplated onto the electrode surface using a solution containing 100 mg l^{-1} bismuth (III). The stripping response was linear over the 10 – 100 μg l^{-1} lead range examined (120 s preconcentration time), with a detection limit of 0.3 μg l^{-1} (600 s preconcentration time) and good precision (RSD = 7.4 % at 20 μg l^{-1} lead).

Masawat *et al.* (2003) used a disposable sputtered gold sensor, which allows underpotential lead preconcentation in combination with a specially fabricated thin-layer flow cell for stripping analysis. The optimised flow injection (FI) system allowed the convenient monitoring of micrograms per litre lead concentrations following short preconcentration times. The detection limit was 0.8 μg l^{-1} for 120 s preconcentration. The method was evaluated by determining lead in spiked drinking and tap water samples.

In the following Sections, work carried out towards electrochemical stripping analysis of lead, cadmium and copper using in-house fabricated electrodes is described. Experimental results including the optimisation procedures for mercury film SPCE, bismuth film SPCE and bare SPCE are presented.

3.2 EXPERIMENTAL

3.2.1 Apparatus and reagents

A computer-controlled Autolab PGSTAT10 Electrochemical Analyser with general-purpose electrochemical software operating system GPES version 4.7 or 4.8 (Eco Chemie B.V., Utrecht, The Netherlands) was used for constant current stripping chronopotentiometric and square-wave stripping voltammetric measurements. The in-house fabricated screen-printed electrodes (see Section 2.2.4 in Chapter 2) were used for the electrochemical measurements with connection made to the electrochemical analyser using a specially adapted electrical edge connector from Maplin (Milton Keynes, UK). Glassy carbon electrode (GCE, 0.07 cm^2), silver/silver chloride (Ag/AgCl) reference electrode and platinum wire electrode, obtained from BAS (Chesire, UK) were used for comparative studies.

All chemicals were of analytical reagent grade and used as received. Hydrochloric acid (HCl) and potassium chloride (KCl) were obtained from BDH Ltd (Poole, England) while ammonium acetate was obtained from Merck (Darmstadt, Germany). Potassium hexacyanoferrate (III) (also known as potassium ferricyanide), sodium dodecyl sulphate (SDS), bovine serum albumin (BSA), Triton X-100 and atomic spectroscopic standard stock solutions (1000 mg l^{-1} in 1 wt. % HNO_3 or HCl) of cadmium (II), lead (II), copper (II), mercury (II), chromium (III), iron (II) and bismuth (III) were obtained from Aldrich (Gillingham, Dorset, UK).

3.2.2 Risk assessments and codes of practice

Experiments were performed in compliance with Departmental codes of practice and C.O.S.H.H regulations; waste was disposed of by approved routes.

3.2.3 Procedure

Studies were carried out by placing a 100 µl sample drop on the three-electrode strip. Each electrochemical measurement was carried out in triplicates with a new SPE strip in non-dearated and unstirred solution. Metal working standard solutions were prepared by diluting the standard stock solution (1000 mg l^{-1}) with deionised water and supporting electrolyte. Other reagents were prepared by dissolving in deionised water. Studies with conventional electrodes (GCE – working electrode, Ag/AgCl reference electrode and platinum wire - counter electrode) were carried out in an electrochemical cell using a total volume of 10 ml. Between each set of experiments, the GCE was mechanically polished with alumina powder (0.3 µm) and rinsed thoroughly with deionised water.

3.2.3.1 Stripping voltammetry

Square-wave anodic stripping voltammetric (SWASV) measurements were carried out by depositing the target metal ions (lead, cadmium or copper) on the screen-printed carbon electrode surfaces plated *in situ* with metallic films of mercury. A sufficiently negative deposition potential (vs. screen-printed Ag/AgCl reference electrode) was applied to preconcentrate the analytes for a fixed time onto the surface of the working electrode. After the deposition period, the preconcentrated analytes are stripped from the surface of the working electrode until a limit of 0 V (unless otherwise stated). A square-wave frequency of 50 Hz, a step potential of 5 mV and square-wave amplitude of 25 mV were used for the measurements. The background signal for the supporting electrolyte was also measured in the same manner. The analytical signal is subtracted from the background signal.

As part of the work carried out the influence of mercury (II) concentration on the stripping responses of lead, cadmium and copper was examined in the mercury ion concentration range of 0 to 120 mg l^{-1}. The effect of deposition potential was examined in the potential range – 0.7 to – 1.2 V while the effect of deposition time was studied in the time range 0 to 300 s. The concentration dependence for each of the metal ions was investigated between 0 and 300 or 500 μg l^{-1}.

3.2.3.2 Stripping chronopotentiometry

Constant current stripping chronopotentiometric (CCSCP) measurements were carried out by depositing the target metal ions (lead, cadmium and/or copper) on the bare screen-printed carbon electrode or on the screen-printed carbon electrode surfaces plated *in situ* with metallic films of mercury or bismuth. A sufficiently negative deposition potential (vs. screen-printed Ag/AgCl reference electrode) was applied to preconcentrate the analytes. After the deposition period, a constant current was applied to strip the preconcentrated analytes until a limit of 0 V (unless otherwise stated) using a measurement maximum time of 3 or 10 s (unless otherwise stated). The background signal for the supporting electrolyte was also measured in the same manner. The analytical signal is subtracted from the background signal.

The influence of mercury (II) concentration on the stripping responses of lead, cadmium and copper was examined in the mercury ion concentration range of 0 - 100 mg l^{-1}. The influence of bismuth (III) concentration on the stripping responses of lead and cadmium was examined in the bismuth ion concentration range of 0 - 1000 μg l^{-1}. The influence of pH on the stripping responses of lead and cadmium was studied between pH 1 and 6 by altering the pH of 0.1 M HCl with different concentrations of ammonium acetate. The effects of the supporting medium (HCl, KCl and ammonium acetate + HCl) and interfering substances (co-existing ions and surface-active substances) on the stripping responses of lead, cadmium and copper were investigated. The studies on the effect of the surface-active substances (Triton X-100, SDS and BSA) were carried out at 5 and 50 mg l^{-1} concentration levels. The effect of deposition potential was also examined in the potential range – 0.7 to – 1.2 V while the effect of deposition time was studied in the time range 0 to 360 s. The concentration dependence for each of the metal ions was investigated between 0 and 1000 or 2000 μg l^{-1}.

3.2.4 Data analysis

All experimental runs carried out in this work, were repeated three to five times with different screen-printed electrodes. Each response data point represents the mean value of three measurements and the error bar represents the standard deviation. The mean, standard deviation and relative standard deviation (R.S.D) were calculated for the measurements using Microsoft Excel 2000 and SigmaStat Statistical Software Version 2.03.

The detection limits were evaluated with the criterion, 3 x S_b/m, where S_b is standard deviation of the intercept and m, the slope of the linear plot.

3.3 RESULTS

3.3.1 Mercury film screen-printed carbon electrode – Optimisation of experimental variables for stripping voltammetric detection of lead, cadmium and copper

SWASV studies were carried out with either 0.1 M HCl or 0.5 M ammonium acetate + 0.1 M HCl as supporting medium. The effect of mercury (II) concentration on the stripping responses of lead, cadmium and copper, the effect of deposition potential, the effect of deposition time and the concentration dependence for each of the metal ions were investigated. Studies on the batch-to-batch variation of the screen-printed electrodes were also evaluated. This can be found in the Appendix section (see Appendix 1.1)

3.3.1.1 Influence of mercury (II)

Figure 3.1, 3.2 and 3.3 show the influence of mercury (II) concentration on the square-wave anodic stripping voltammetric response of lead (II), cadmium (II) and copper (II). Lead and cadmium stripping responses levelled off above a mercury concentration of about 50 mg l^{-1} after increasing rapidly at much lower mercury (II) concentration. Hence a mercury (II) concentration of 80 mg l^{-1} was used for subsequent stripping voltammetric work. For copper, the increase in stripping response levelled off above a mercury concentration of 40 mg l^{-1} after an increase from 10 to 40 mg l^{-1}. Hence a mercury concentration between 40 and 80 mg l^{-1} was used for subsequent stripping voltammetric work.

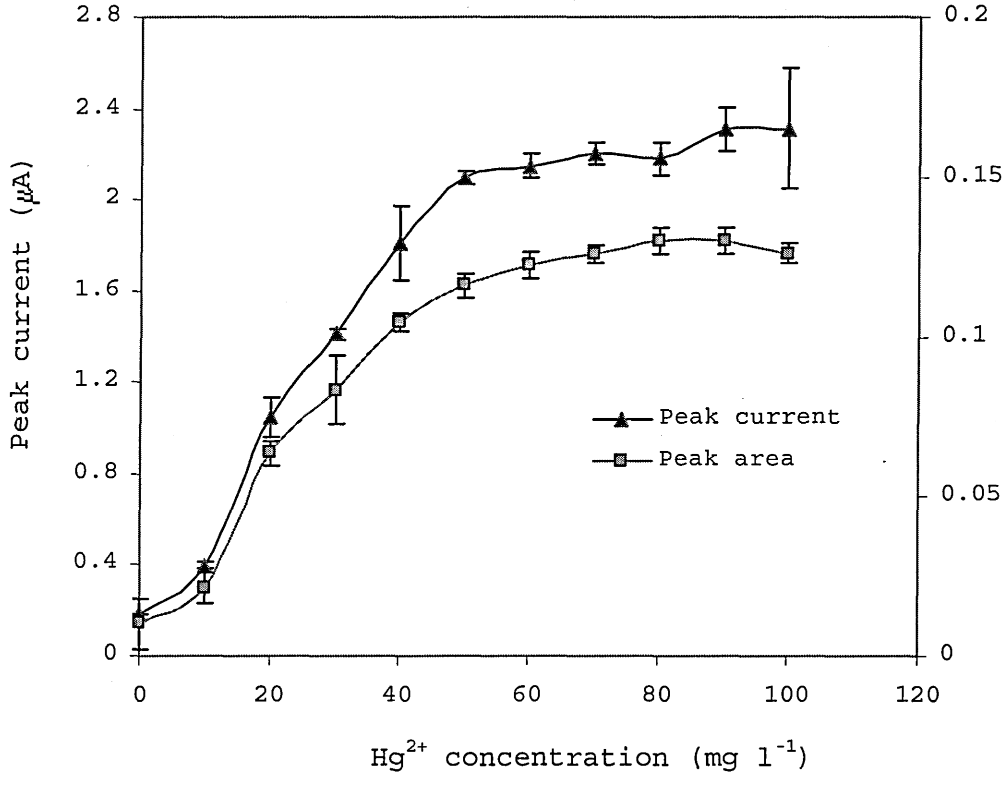


Figure 3.1: Effect of mercury concentration upon the stripping voltammetric response of 50 μg l^{-1} lead (II) in 0.1 M HCl. Experimental conditions: deposition potential = - 0.9 V, final potential = - 0.2 V, deposition time = 120 s, square-wave (SW) frequency = 50 Hz, step potential = 5 mV and SW amplitude = 25 mV.

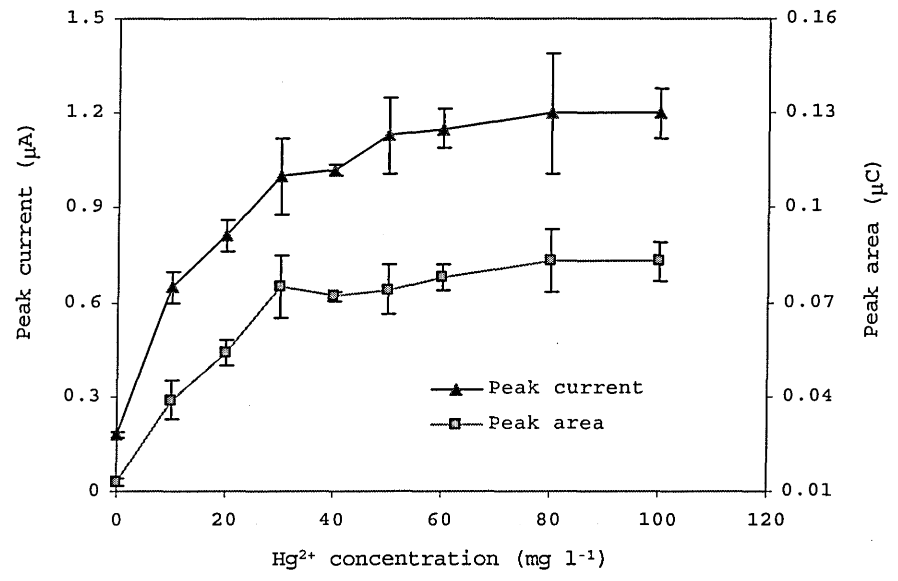


Figure 3.2: Effect of mercury (II) on the voltammetric stripping response of 50 μg l^{-1} cadmium (II) in 0.1 M HCl + 0.5 M ammonium acetate solution. Experimental conditions: deposition potential = - 1.1 V, final potential = - 0.2 V, deposition time = 120 s, SW frequency = 50 Hz, step potential = 5 mV, SW amplitude = 25 mV.

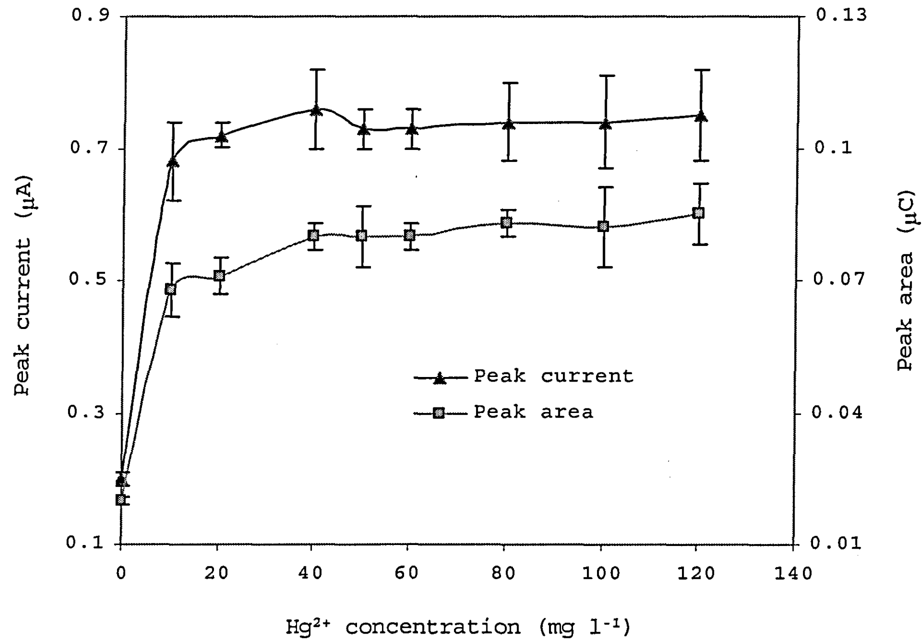


Figure 3.3: Effect of mercury (II) on the voltammetric stripping response of 50 μg l^{-1} copper (II) in 0.1 M HCl + 0.5 M ammonium acetate solution. Experimental conditions: deposition potential = - 0.9 V, final potential = 0.05 V, deposition time = 120 s, SW frequency = 50 Hz, step potential = 5 mV, SW amplitude = 25 mV.

3.3.1.2 Effect of deposition time

Figure 3.4 presents the effect of deposition time on the stripping voltammetric responses of cadmium (II) and lead (II). As can be seen, the stripping response increased rapidly with deposition time from 30 to 300 s. A deposition time of 120 s was used for subsequent experiments, as this is sufficient for obtaining well-defined stripping peaks for lead and cadmium (as well as copper, but not shown) at microgram per litre concentrations. Also the saturation of the mercury film electrode due to unduly long deposition time is avoided.

3.3.1.3 Effect of deposition potential

For the effect of deposition potential on the stripping response, the SWASV signals for lead and cadmium increased when the deposition potential proceeded to more negative potentials (Figure 3.5). At potentials more negative than – 1.1 V, the reproducibility of the stripping responses were affected by increase in the background response, therefore, a potential of – 0.9 V was chosen for subsequent lead (as well as copper) measurements while a deposition potential of – 1.0 V was used for cadmium measurements. A deposition potential of – 1.0 V was also employed for simultaneous detection of cadmium, lead and copper. The chosen deposition potentials provided the best compromise between sensitivity of the main peak and the background response.

3.3.1.4 Calibration curves for lead, cadmium and copper

3.3.1.4a Lead (II)

Figure 3.6 shows the stripping voltammograms obtained for increasing concentrations of lead (II) in the range 0 to 150 μg l^{-1}. The calibration plot obtained for lead was found to be bimodal with linear ranges between 0 and 150 μg l^{-1} (correlation coefficient (r^2) = 0.9869) and 150 and 300 μg l^{-1} (r^2 = 0.9945) with sensitivities of 1.4 ± 0.07 nC l μg^{-1} and 0.3 ± 0.05 nC l μg^{-1}, respectively for 120 s deposition (Figure 3.7). The precision (relative standard deviation) of the measurements were determined by performing 6 replicate measurements on solutions containing 25 and 150 μg l^{-1} (Table 3.1) with the calculated relative standard deviation values being 6.7 and 9.5 %, respectively. A detection limit of 1 μg l^{-1} was estimated using a deposition time of 120 s.

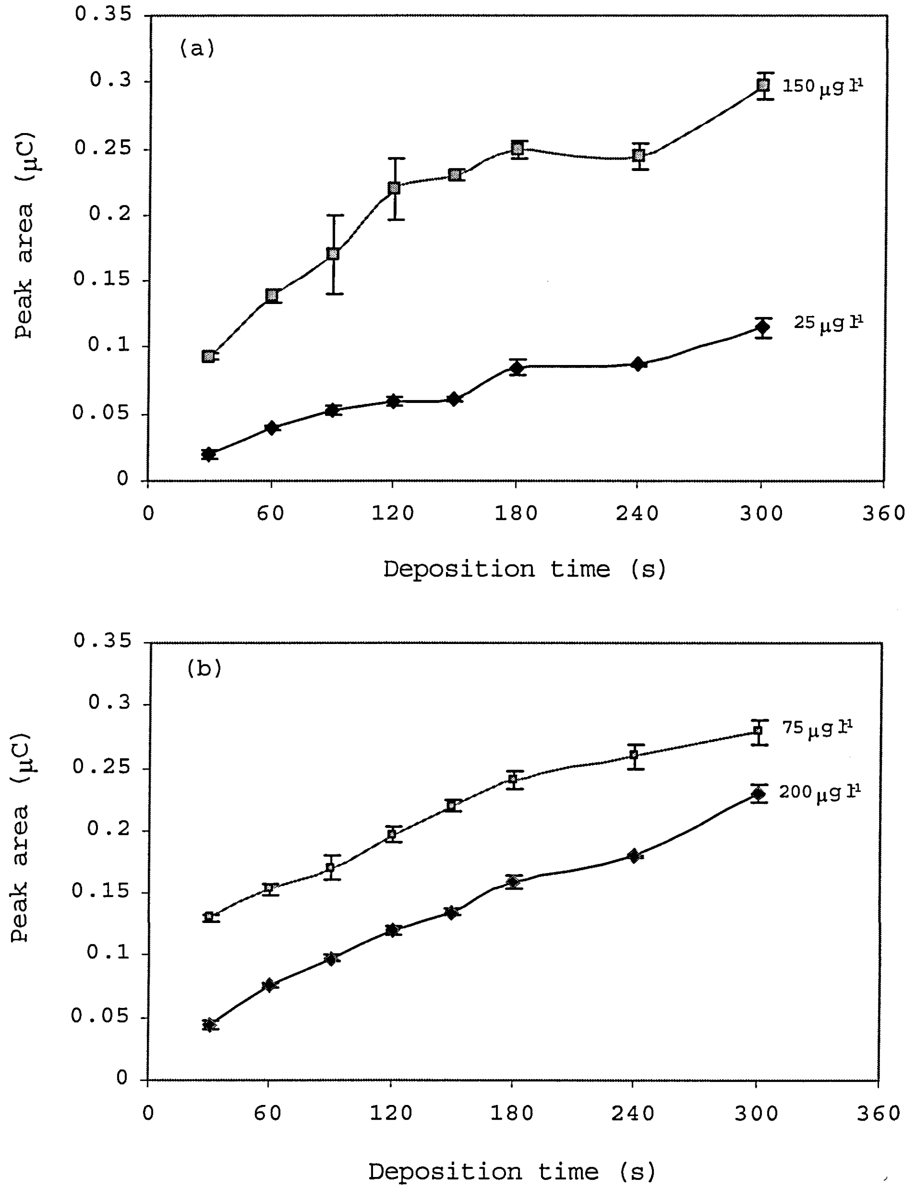


Figure 3.4: Effect of deposition time on the stripping voltammetric responses of (a) cadmium (II) and (b) lead (II). Deposition potential = - 1.0 V, SW frequency = 50 Hz, step potential = 5 mV, SW amplitude = 25 mV, Hg^{2+} = 80 mg l^{-1}.

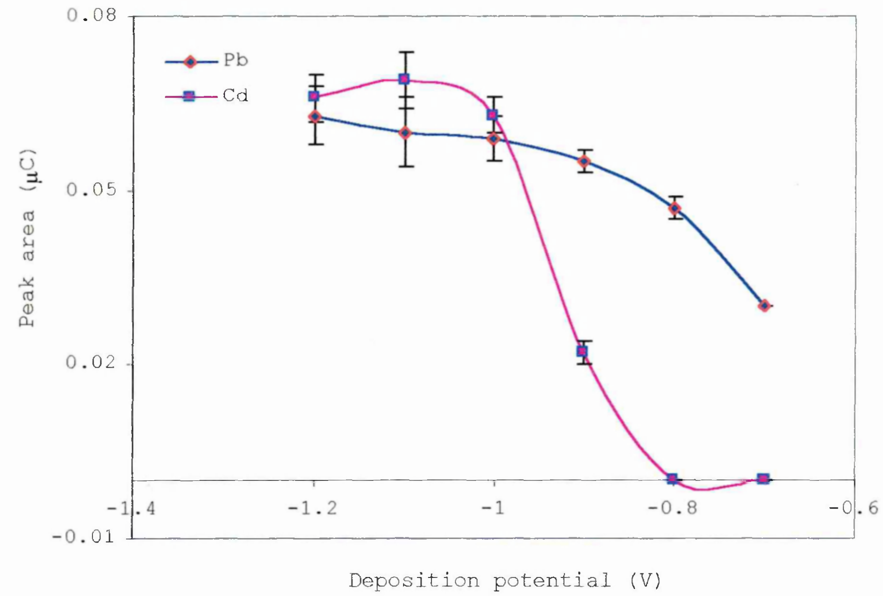


Figure 3.5: Effect of deposition potential on the stripping voltammetric responses of 25 μg l^{-1} lead (II) and cadmium (II). Deposition time = 120 s, SW frequency = 50 Hz, step potential = 5 mV, SW amplitude = 25 mV, Hg^{2+} = 80 mg l^{-1}.

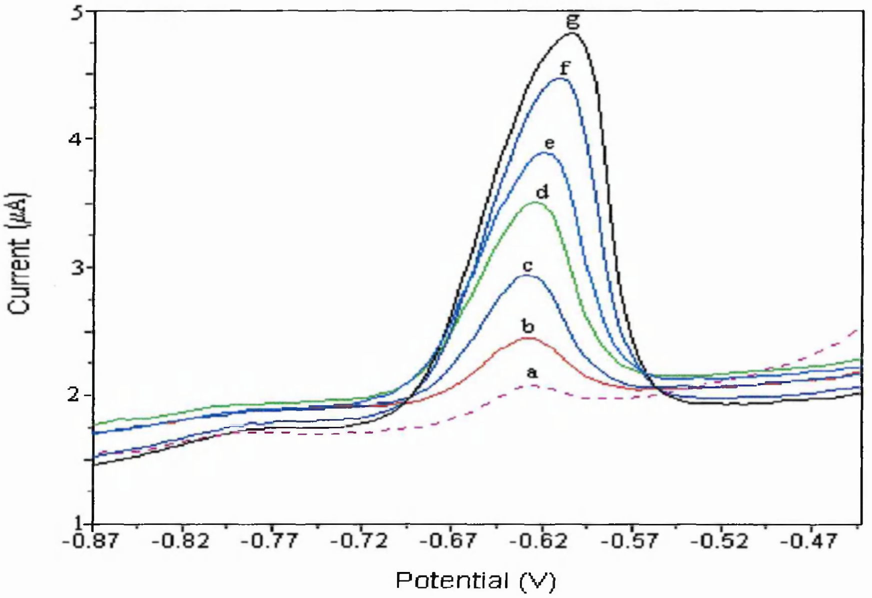
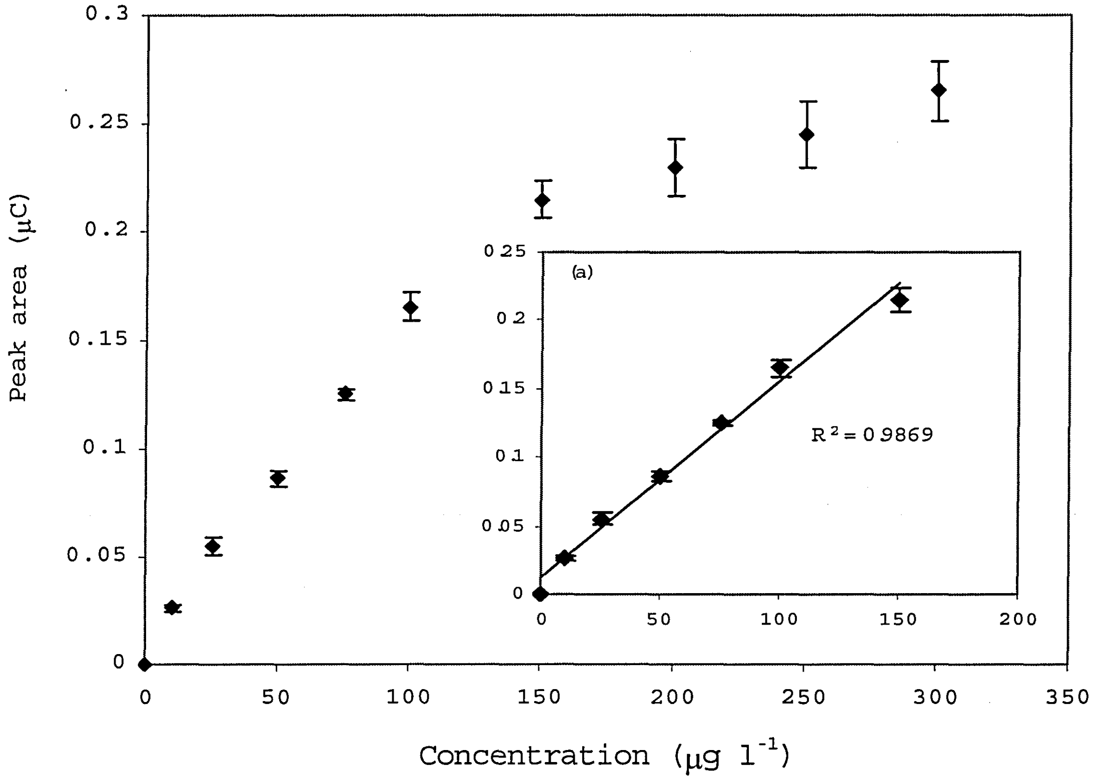


Figure 3.6: Stripping voltammograms for increasing concentrations of lead (II) in 0.1 M HCl + 0.5 M ammonium acetate. (a) Blank, (b) 10, (c) 25, (d) 50, (e) 75, (f) 100, (g) 150 μg l^{-1}. Hg^{2+} = 80 mg l^{-1}, deposition time = 120 s, deposition potential = - 0.9 V, frequency = 50 Hz, SW amplitude = 25 mV, step potential = 5 mV.



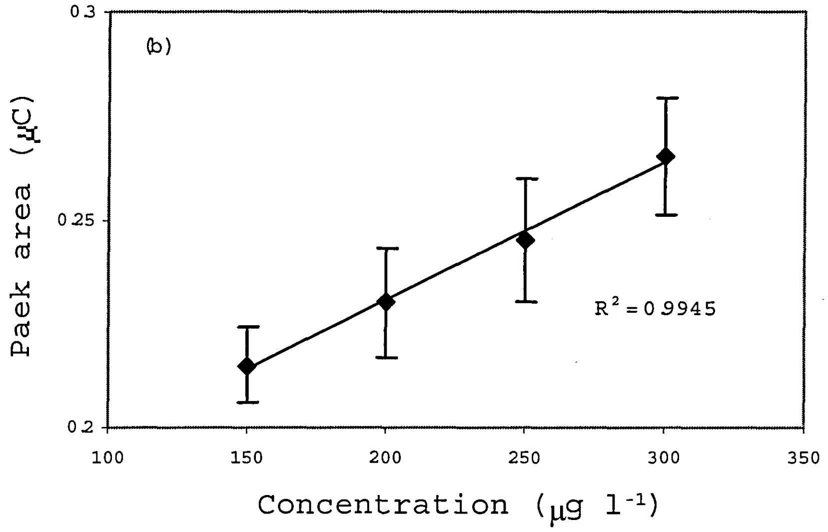


Figure 3.7: Calibration curves for lead (II) in 0.1 M HCl + 0.5 M ammonium acetate. (a) Inset and (b) below: Linear ranges for lead (II) at mercury-coated SPCE. Hg^{2+} = 80 mg l^{-1}, deposition time = 120 s, deposition potential = - 0.9 V, final potential = 0.05 V, frequency = 50 Hz, SW amplitude = 25 mV, step potential = 5 mV.

Table 3.1: Response for replicate measurements of lead on mercury film SPCE using conditions shown in Figure 3.7.

Experiment run	**25 µg l^{-1} Lead (µC)**	**150 µg l^{-1} Lead (µC)**
1	0.0514	0.261
2	0.0544	0.229
3	0.0598	0.223
4	0.0498	0.200
5	0.0552	0.210
6	0.0518	0.214
Mean	0.0537	0.223
Standard deviation	0.0036	0.0212

3.3.1.4b Cadmium (II)

The stripping voltammograms obtained for increasing concentrations of cadmium (II) in the range 0 to 100 µg l^{-1} is depicted in Figure 3.8a. For the calibration plot presented in Figure 3.8b, a linear correlation was obtained between the stripping response (peak area) and cadmium concentration in the range 0 to 100 µg l^{-1} (correlation coefficient, $r^2 = 0.9842$), with sensitivity of 1.9 ± 0.1 nC l μg^{-1} for 120 s deposition. Above a concentration level of 100 µg l^{-1}, there was deviation in the linearity of the calibration plot. The precision of the measurements (R.S.D) determined by performing 6 replicate measurements of 5 µg l^{-1} cadmium was 6.9 %. A detection limit of 2 µg l^{-1} was estimated for 120 s deposition.

3.3.1.4c Copper (II)

The stripping voltammograms and calibration plot obtained for increasing concentrations of copper are depicted in Figure 3.9. There was a linear correlation between the stripping peak area and copper concentration in the range 0 to 200 µg l^{-1} ($r^2 = 0.9812$) with sensitivity of 0.71 ± 0.04 nC l μg^{-1} for 120 s deposition. There was a levelling off of the calibration curve at higher concentrations (> 200 µg l^{-1}) due to the saturation of the *in situ* plated mercury film SPCE. The precision of the measurements (R.S.D) determined by performing 6 replicate measurements of 50 µg l^{-1} copper was 13.5 %. A detection limit of 6 μg l^{-1} was estimated for 120 s deposition.

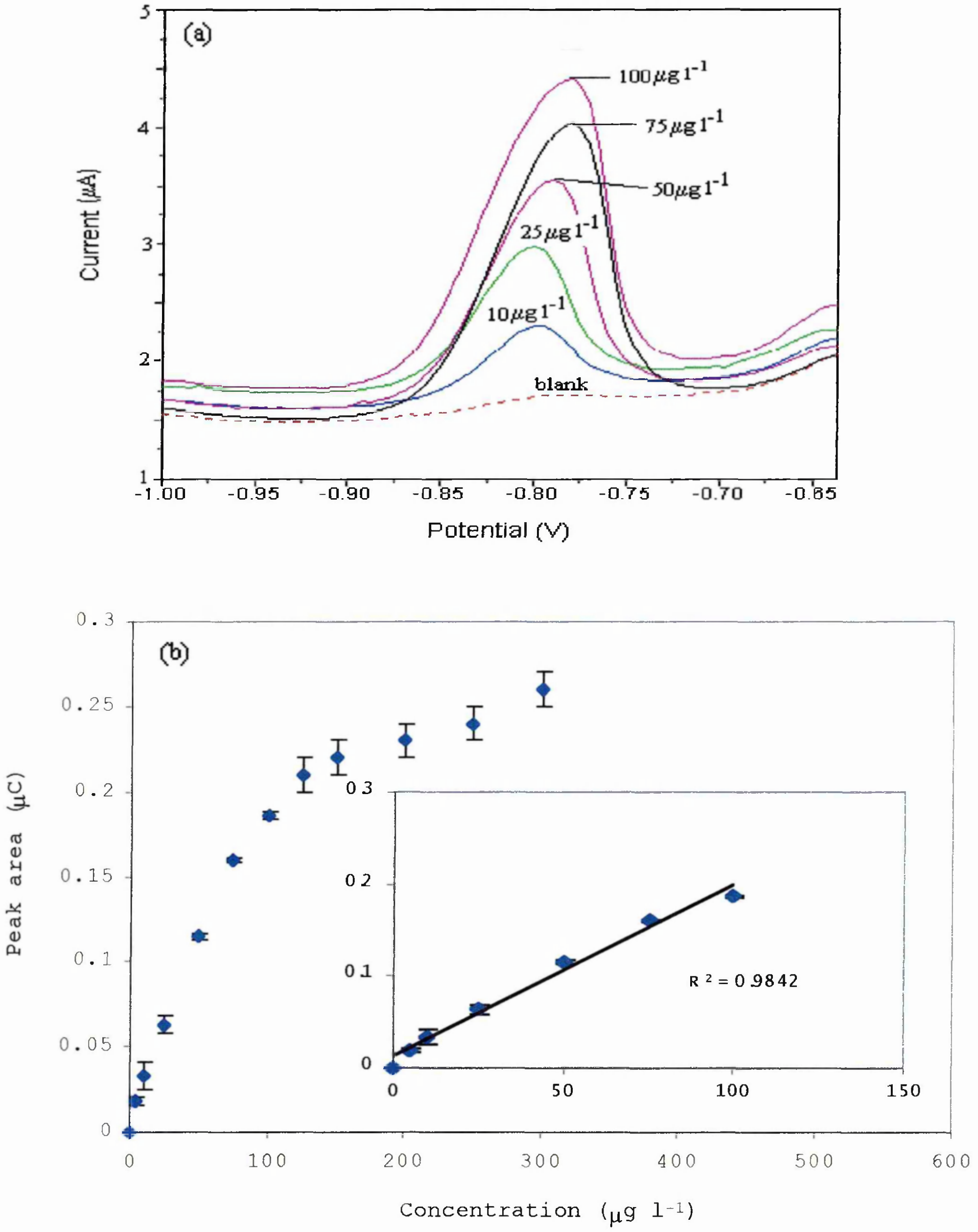


Figure 3.8: (a) The stripping voltammograms and (b) calibration plot for increasing concentrations of cadmium (II) in 0.1 M HCl + 0.5 M ammonium acetate along with the blank response. Inset (a): Linear range for cadmium (II). Hg^{2+} = 80 mg l^{-1}, deposition time = 120 s, deposition potential = - 1.1 V, frequency = 50 Hz, step potential = 5 mV, SW amplitude = 25 mV.

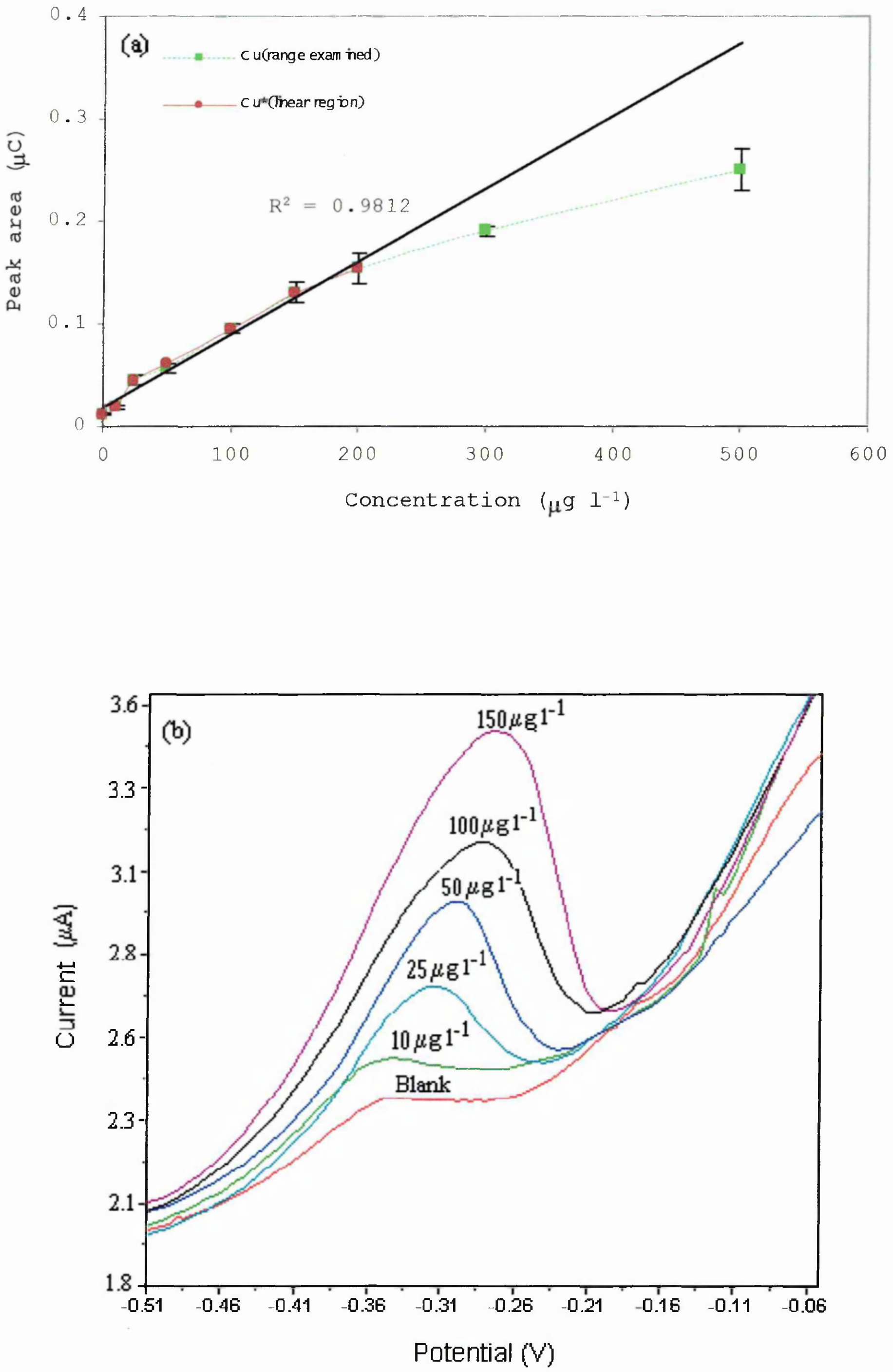


Figure 3.9: The calibration plot (a) and stripping voltammograms (b) for increasing concentrations of copper (II) in 0.1 M HCl + 0.5 M ammonium acetate along with the blank response. Hg^{2+} = 80 mg l^{-1}, deposition time = 120 s, deposition potential = - 0.9 V, frequency = 50 Hz, SW amplitude = 25 mV, step potential = 5 mV.

3.3.2 Mercury film screen-printed carbon electrode - Optimisation of experimental variables for stripping chronopotentiometric measurement of lead and cadmium

In order to detect lead (II) and cadmium (II) using constant current stripping chronopotentiometry (CCSCP), conditions of the parameters which most influence the measurement process were optimised: concentration of mercury (II) co-deposited *in situ* with the target metals (lead and cadmium), deposition time (t_{dep}), deposition potential (E_{dep}), pH and concentration range of the target analyte. The effects of coexisting metal ions and surface-active compounds were also evaluated. Preliminary study on the batch-to-batch variation of the screen-printed electrodes was also carried out and this can be found in the Appendix section (see Appendix 1.1)

3.3.2.1 Influence of mercury (II) concentration on stripping process

The concentration of mercury ions for co-deposition was evaluated by varying the mercury (II) concentration along with a fixed concentration of lead (50 $\mu g\ l^{-1}$) and cadmium (50 $\mu g\ l^{-1}$). The Hg^{2+} was added *in situ* so that a metallic film of mercury is plated on the screen-printed carbon surface. The graphical plot and stripping chronopotentiograms in Figure 3.10 show that the concentration of mercury governs the oxidation rate of the amalgamated analyte (in this case, lead), and thus the sensitivity of the stripping signals obtained. The stripping time for lead decreased upon raising the mercury concentration and this was due to a fast oxidation rate at high mercury (II) concentrations. Similar result was obtained for cadmium. A mercury ion concentration of 10 mg l^{-1} was chosen for co-deposition.

3.3.2.2 Effect of pH

Figure 3.11 and 3.12 depict the mercury film electrode's stripping chronopotentiometric responses to lead and cadmium over the pH range 1.3 to 5.7. The pH of the test solution was altered, by adding different concentrations of ammonium acetate to 0.1 M HCl. HCl was chosen as the medium to use in this study because chloride ion is needed in the test solution for stabilising the potential of the on-board screen-printed Ag/AgCl reference electrode (Desmond *et al.*, 1997; 1998; Choi *et al.*, 2001) and also because it is normally free from metal ion impurities compared to potassium chloride (KCl) (Kadara *et al.*, 2003).

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3.3.2.2 Effect of pH

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As can be observed from Figure 3.12, the sensitivity for lead and cadmium depends on the pH of the test solution in which they are analysed. The chronopotentiometric stripping response for lead was highly sensitive at low pH (1.3 – 3.1), while cadmium response was more sensitive at mildly acidic pH (pH 4 – 5). Hence, subsequent experiments were carried out in 0.1 M HCl (pH 1.3) for single analysis of lead (II) and in 0.1 M HCl + 0.5 M ammonium acetate medium (pH 4.6), for analyses involving cadmium (II).

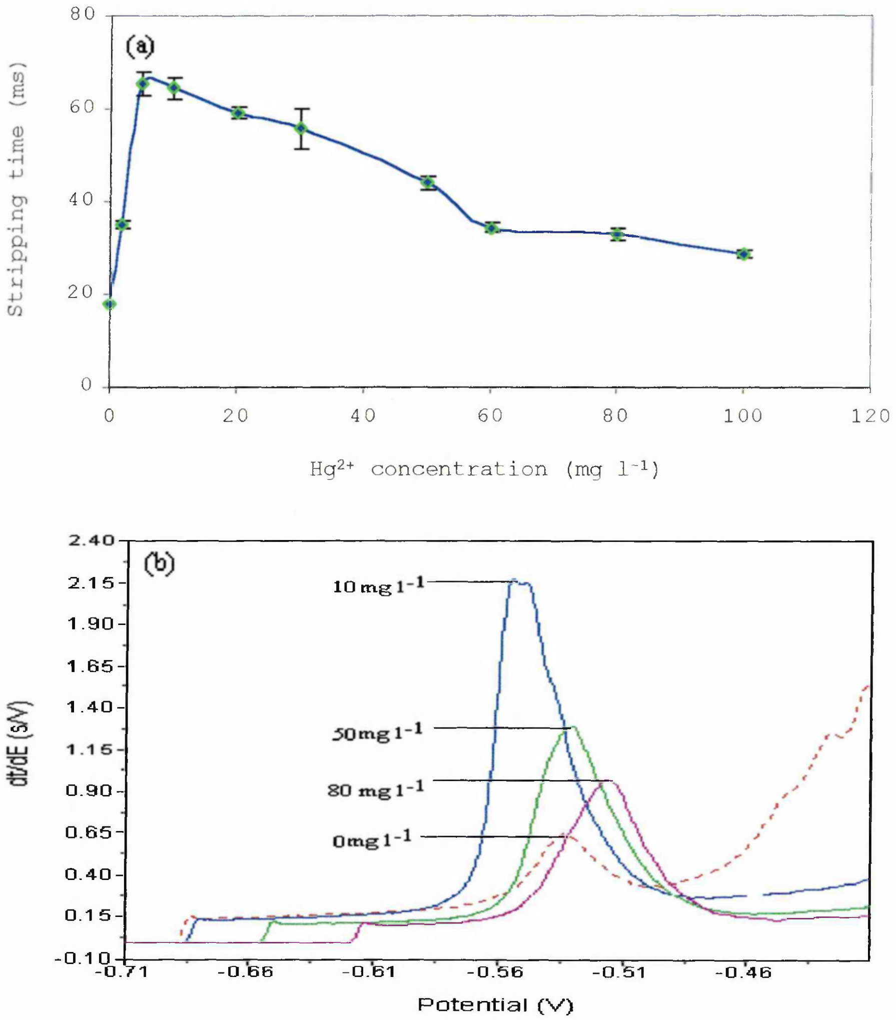


Figure 3.10: Effect of mercury (II) concentration on stripping peak response of lead (II). (a): Graphical summation of the effect of mercury (II). (b): Stripping chronopotentiograms for 50 $\mu g\ l^{-1}$ lead (II) in 0.1 M HCl with the various mercury (II) concentrations Deposition potential = - 0.9 V, final potential = - 0.2 V, deposition time = 120 s, constant current = 1 μA and maximum time for measurement = 3 s.

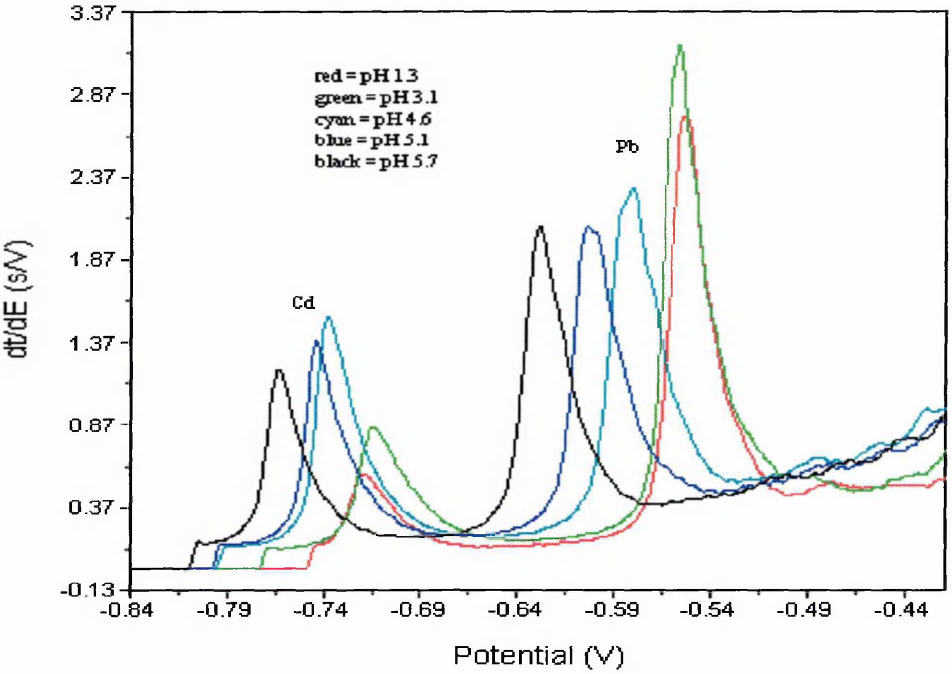


Figure 3.11: Stripping chronopotentiograms showing the effect of pH on the stripping responses of 50 μg l^{-1} of cadmium (II) and lead (II) in 0.1 M HCl with varying concentrations of ammonium acetate. Deposition potential = - 0.9 V, Hg^{2+} = 10 mg l^{-1}, deposition time = 120 s, constant current = 1 μA.

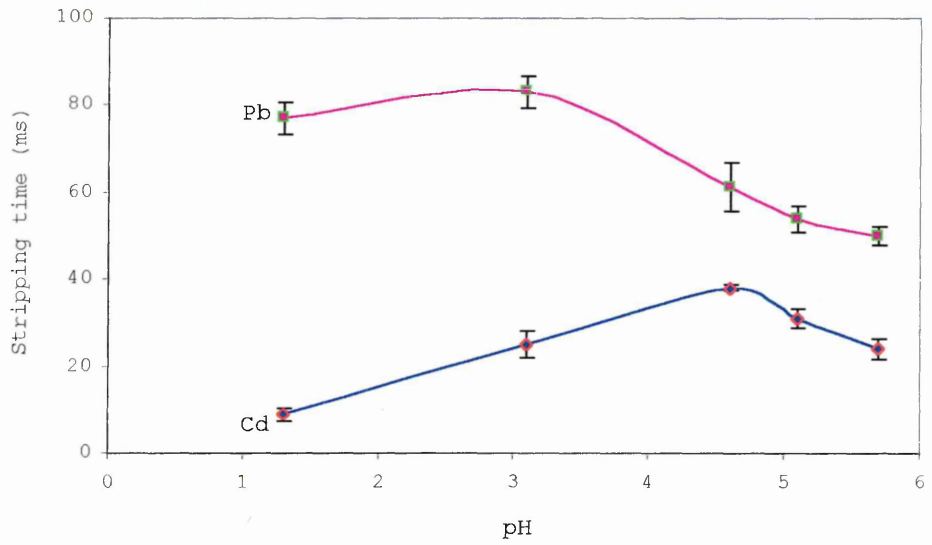


Figure 3.12: Effect of pH on stripping responses of 50 μg l^{-1} of cadmium (II) and lead (II). Deposition potential = - 0.9 V, Hg^{2+} = 10 mg l^{-1}, deposition time = 120 s, constant current = 1 μA.

3.3.2.3 *Effect of deposition potential*

Deposition potential (E_{dep}) is an important parameter for stripping chronopotentiometry and has significant influence on the sensitivity of determination. The effect of deposition potential on the stripping chronopotentiometric peak responses of lead (II) and cadmium (II) is shown in Figure 3.13. The negative shifts of electrode potential obviously improve the reduction of lead (II) and cadmium (II) on the mercury film screen-printed carbon electrode and increase the stripping peak responses. However, the peak response changes little when deposition potentials were more negative than – 0.9 V for lead and – 1.1 V for cadmium. On the basis of sensitivity, resolution and reproducibility, a deposition potential of - 0.9 V was employed for subsequent lead measurements and – 1.0 V for simultaneous detection of cadmium and lead.

3.3.2.4 *Effect of deposition time*

The dependence of the stripping peak response on deposition time (t_{dep}) for 25 and 50 µg l^{-1} lead was investigated (Figure 3.14). The stripping peak response increased with increase in the deposition time in the range examined, 30 to 360 s. As the ultimate choice of deposition time to apply for measurements depends on the concentration of metal ion in the solution, a deposition time of 120 s was chosen for lead (II) (as well as cadmium (II)) measurements as a compromise between length of analysis (reduction in the experimental timescale), increase hydrogen evolution and sensitivity.

3.3.2.5 *Interference studies*

Interference studies were carried out in order to check the selectivity of the proposed method. The interference from coexisting ions and surface-active compounds on the responses for lead (II) and cadmium (II) were investigated. The effect of copper and cadmium on the stripping peak response of 50 µg l^{-1} lead (II) was evaluated and the result is presented in Figure 3.15. Suppression of the lead stripping peak response is observed in the presence of high concentration (1000 µg l^{-1}) of copper (II) but the effect of 1000 µg l^{-1} cadmium (II) on 50 µg l^{-1} lead (II) was negligible.

Figure 3.16 depicts the effect of copper and lead on the stripping response of 50 µg l^{-1} cadmium (II). There is a slight increase in the stripping response for cadmium in the presence of lead while in the presence of copper; there is a decrease in the cadmium stripping response. The effect of SDS, Triton X-100 and BSA on the stripping chronopotentiometric response of lead was also evaluated. The effect of these surface-active compounds on 50 μg l^{-1} lead (II) is shown in Figure 3.17. A huge increase in the response for lead was observed in the presence of Triton X-100 while in the presence of SDS and BSA, there was a slight increase.

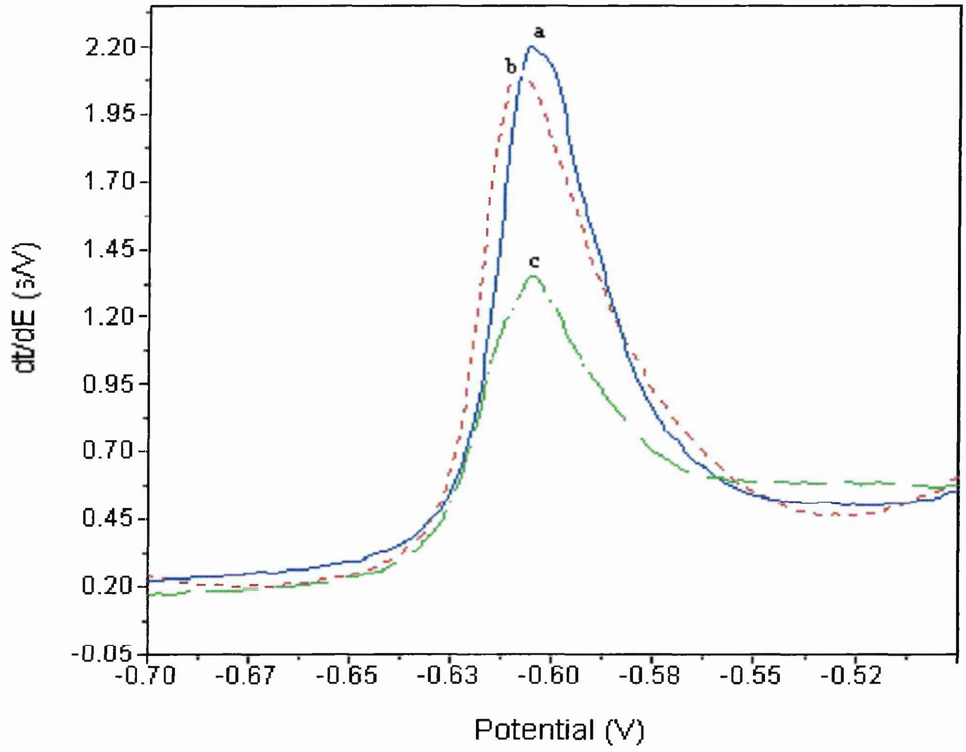


Figure 3.13: Stripping chronopotentiograms showing the effects of cadmium (II) and copper (II) on the stripping response of lead (II) in 0.1 M HCl + 0.125 M ammonium acetate. (a) 50 μg l^{-1} lead (II), (b) 50 μg l^{-1} lead (II) + 1000 μg l^{-1} cadmium (II), (c) 50 μg l^{-1} lead (II) + 1000 μg l^{-1} copper (II). Deposition potential = - 0.9 V, Hg^{2+} = 10 mg l^{-1}, deposition time = 120 s, constant current = 1 μA.

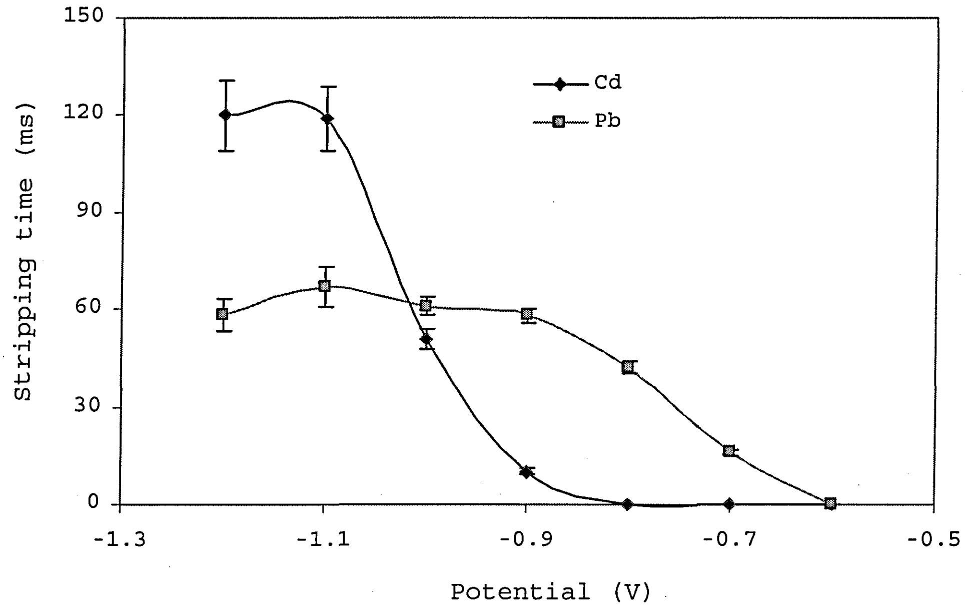


Figure 3.14: Effect of deposition potential on stripping response of 50 μg l^{-1} lead (II) and 50 μg l^{-1} cadmium (II) in 0.1 M HCl + 0.5 M ammonium acetate. Deposition time = 60 s, Hg^{2+} = 10 mg l^{-1}, constant current = 1μA.

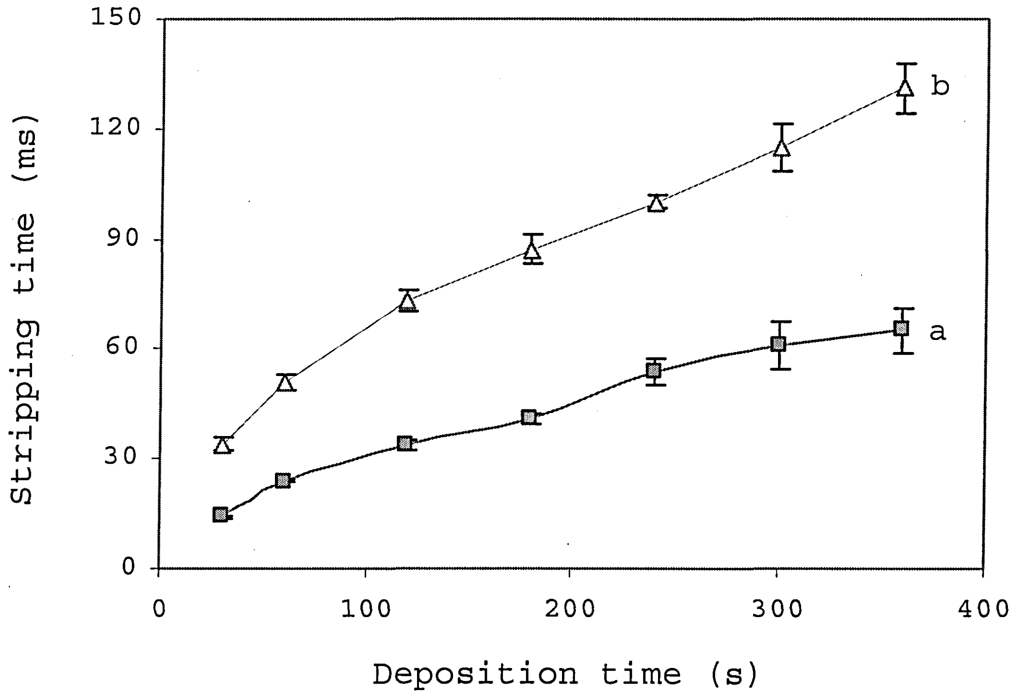


Figure 3.15: Effect of deposition time on stripping response of (a) 25, (b) 50 μg l^{-1} lead (II) in 0.1 M HCl. Deposition potential = - 0.9 V, Hg^{2+} = 10 mg l^{-1}, constant current = 1μA.

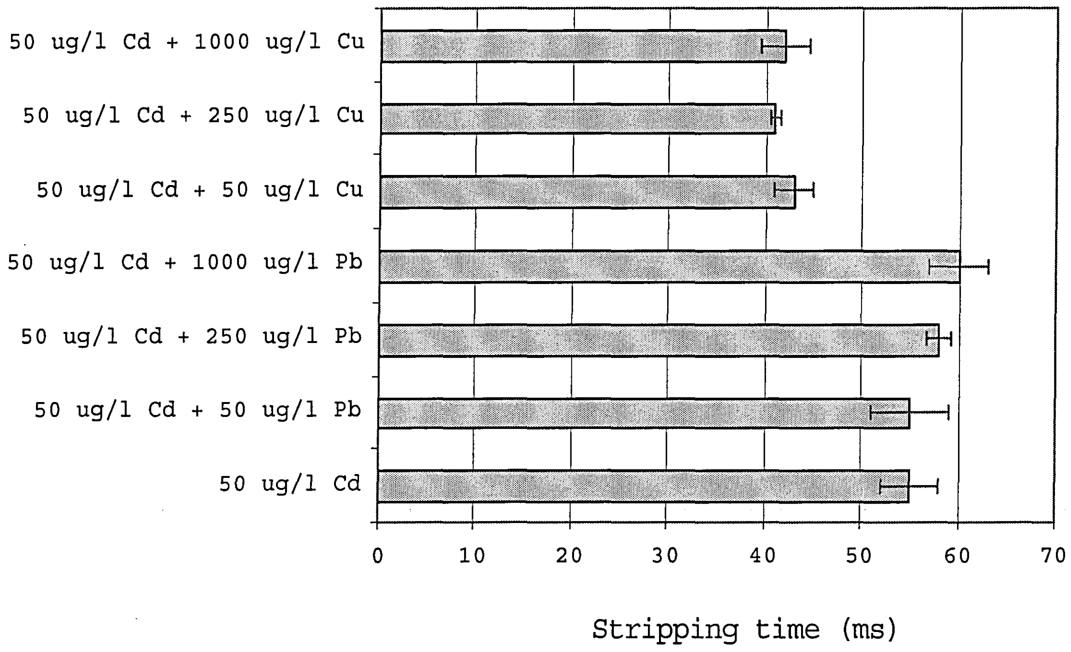


Figure 3.16: Effects of lead and copper on the stripping response of cadmium (II) in 0.1 M HCl + 0.5 M ammonium acetate. Deposition potential = - 1.0 V, Hg^{2+} = 10 mg l^{-1}, deposition time = 120 s, constant current = 1 μA.

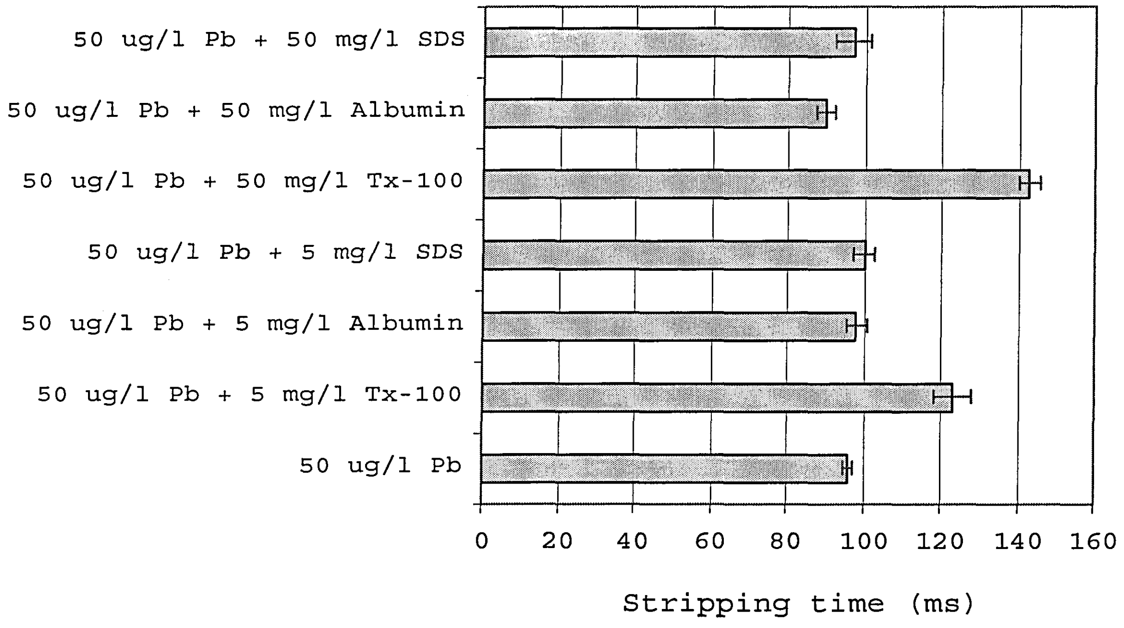


Figure 3.17: Effects of surface-active compounds on the stripping responses of lead (II). Deposition potential = - 0.9 V, Hg^{2+} = 10 mg l^{-1}, deposition time = 120 s, constant current = 1 μA.

3.3.2.6 Calibration curves for lead and cadmium

To evaluate the analytical performance of the *in situ* mercury coated screen-printed carbon electrode, some analytical parameters such as linear range, precision and detection limit were determined for the different metal ions. The detection limit for each metal ion was estimated as three times the signal to noise ratio (S/N = 3).

3.3.2.6a Lead (II)

A calibration plot was obtained for lead (II) in the range examined, 0 to 1000 μg l^{-1} (Figure 3.18). The stripping peak responses (stripping times or stripping peak areas) obtained in 0.1 M HCl increased proportionally with the lead concentration to yield a linear calibration plot, with sensitivity of 1.54 ± 0.02 ms l μg^{-1} (r^2 = 0.9991). A detection limit (S/N = 3) of 1 μg l^{-1} was estimated following 120 s deposition. The stripping response was also reproducible with ten repetitive measurements of 10 μg l^{-1} lead (120 s deposition) yielding a relative standard deviation (R.S.D) of 5.8 %.

3.3.2.6b Cadmium (II)

A calibration plot was obtained for cadmium (II) in 0.1 M HCl + 0.5 M ammonium acetate (Figure 3.19). The calibration plot was linear in the range examined, 0 to 1000 μg l^{-1}, with sensitivity of 1.09 ± 0.003 ms l μg^{-1} (r^2 = 0.9999). A detection limit (S/N = 3) of 3 μg l^{-1} was estimated following 120 s deposition. The stripping response was also reproducible with a series of eight repetitive measurements of 10 μg l^{-1} cadmium (120 s deposition) yielding a relative standard deviation of 6.9 %.

3.3.2.6c Lead (II) and cadmium (II)

Following the individual metal ion studies, the simultaneous detection of both lead and cadmium was also attempted. Typical stripping chronopotentiograms for the simultaneous detection in 0.1 M HCl + 0.5 M ammonium acetate supporting medium are depicted in Figure 3.20. The peak area responses (stripping time) obtained were proportional to lead and cadmium concentration in the microgram per litre range using a deposition time of 120 s.

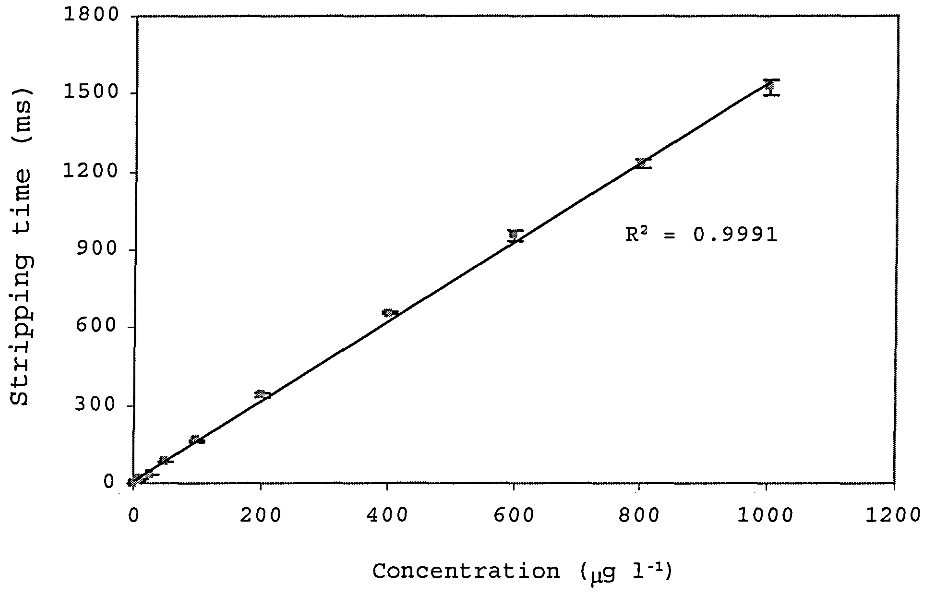


Figure 3.18: Calibration plot for increasing concentrations of lead (II). Hg^{2+} = 10 mg l^{-1}, deposition potential = - 0.9 V, deposition time = 120 s, constant current = 1 µA.

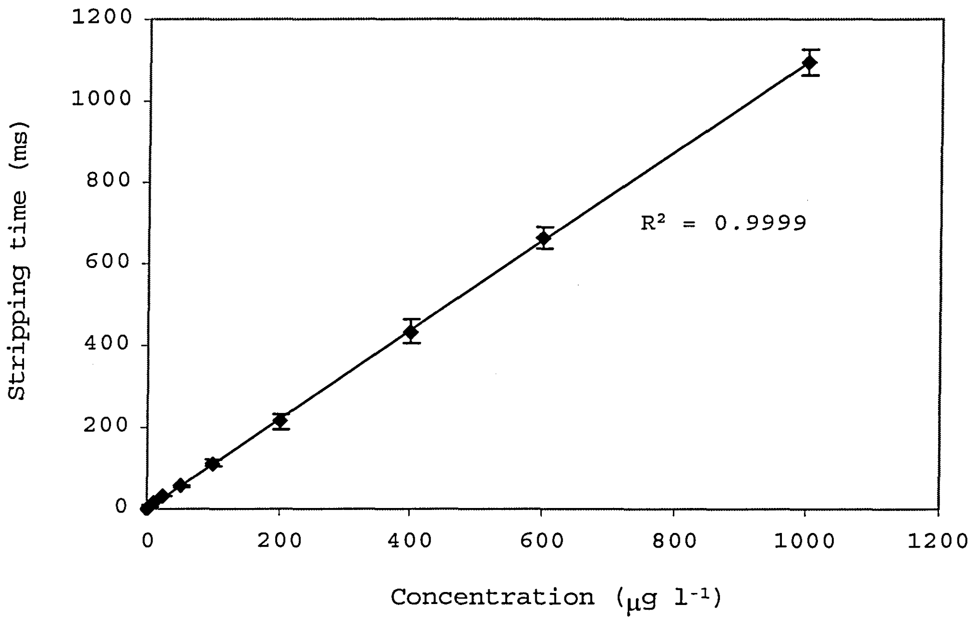


Figure 3.19: Calibration plot for increasing concentrations of cadmium (II). Hg^{2+} = 10 mg l^{-1}, deposition potential = - 1.0 V, deposition time = 120 s, constant current = 1 µA.

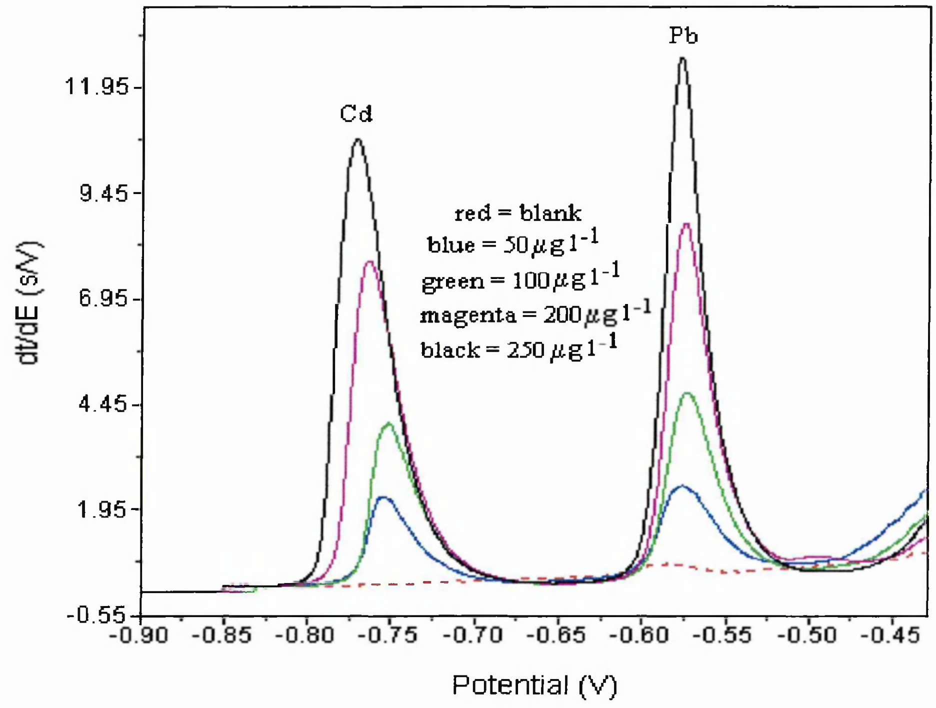


Figure 3.20: Stripping chronopotentiograms for increasing concentrations of lead (II) and cadmium (II) along with the blank response. Hg^{2+} = 10 mg l^{-1}, deposition potential = - 1.0 V, deposition time = 120 s, constant current = 1 µA.

3.3.3 Mercury-film screen-printed carbon electrode - Optimisation of experimental variables for stripping chronopotentiometric measurement of copper

Most of the work presented in this section has been published in the Analytical Chimica Acta Journal by Kadara *et al.*, 2003 (see publication).

Using the same concentration of mercury (II) (10 mg l^{-1}) as used previously for lead (II) and cadmium (II), for generating mercury film on the bare SPCE surface, the effects of the supporting medium and the constant stripping current on the stripping response of copper (II) were evaluated. Also the concentration dependence of the stripping response was examined.

3.3.3.1 Effect of medium

The use of 0.1 M HCl and 0.1 M KCl were considered as supporting electrolytes for the detection of copper (II). The two were chosen because chloride ion is needed in the test solution for stabilising the potential of the on-board screen-printed Ag/AgCl reference electrode (Desmond *et al.*, 1997; 1998; Choi *et al.*, 2001). The two electrolytes at 0.1 M concentration level were found to be inadequate for reliable detection of copper. Figure 3.21 shows the stripping response for copper (II) obtained on the mercury film SPCE (MFSPCE) and mercury film glassy carbon electrode (MFGCE) using 0.1 M HCl as supporting medium. The stripping peak obtained for copper at MFSPCE using the onboard screen-printed reference electrode was poorly defined and obscured by the large background signal for the blank supporting electrolyte solution in the potential range in which copper is detected (Figure 3.21a). Similar response was obtained using screen-printed carbon-working electrode with conventional-sized Ag/AgCl (containing 3 M sodium chloride as the internal electrolyte) reference electrode and platinum wire counter electrode (Figure 3.22). With MFGCE, a well-defined and reproducible chronopotentiometric stripping signal was obtained for copper (II) and the blank supporting medium (Figure 3.21b). Figure 3.23 shows the response obtained using MFSPCE coupled to ASV. The stripping voltammetric signal for the blank supporting medium did not obscure the copper stripping peak in the same way as it did using the MFSPCE coupled to CCSCP (Figure 3.21a). It seems the insufficient coverage of mercury film on the screen-printed working electrode surface and the inherent sensitivity of the CCSCP technique to the surface reactions on the working electrode are more or less the likely reason for this.

To solve this problem of the large background signal and the ill-defined copper stripping peak obtained on the MFSPCE (10 mg l^{-1} mercury plated *in situ*), the effect of increasing concentrations of HCl and KCl on the copper stripping chronopotentiometric response was investigated instead of increasing the concentration of mercury (II) plated *in situ*. The overlaid stripping chronopotentiograms obtained in Figure 3.24 and 3.25 show that the copper-stripping peak becomes more defined as the concentration of the chloride ion mediums exceed 1 M even though the magnitude of the stripping signal decreases as well. Hence, a chloride concentration of 2 M was used for subsequent experiments with HCl as the choice, because it is normally free from metal ion impurities.

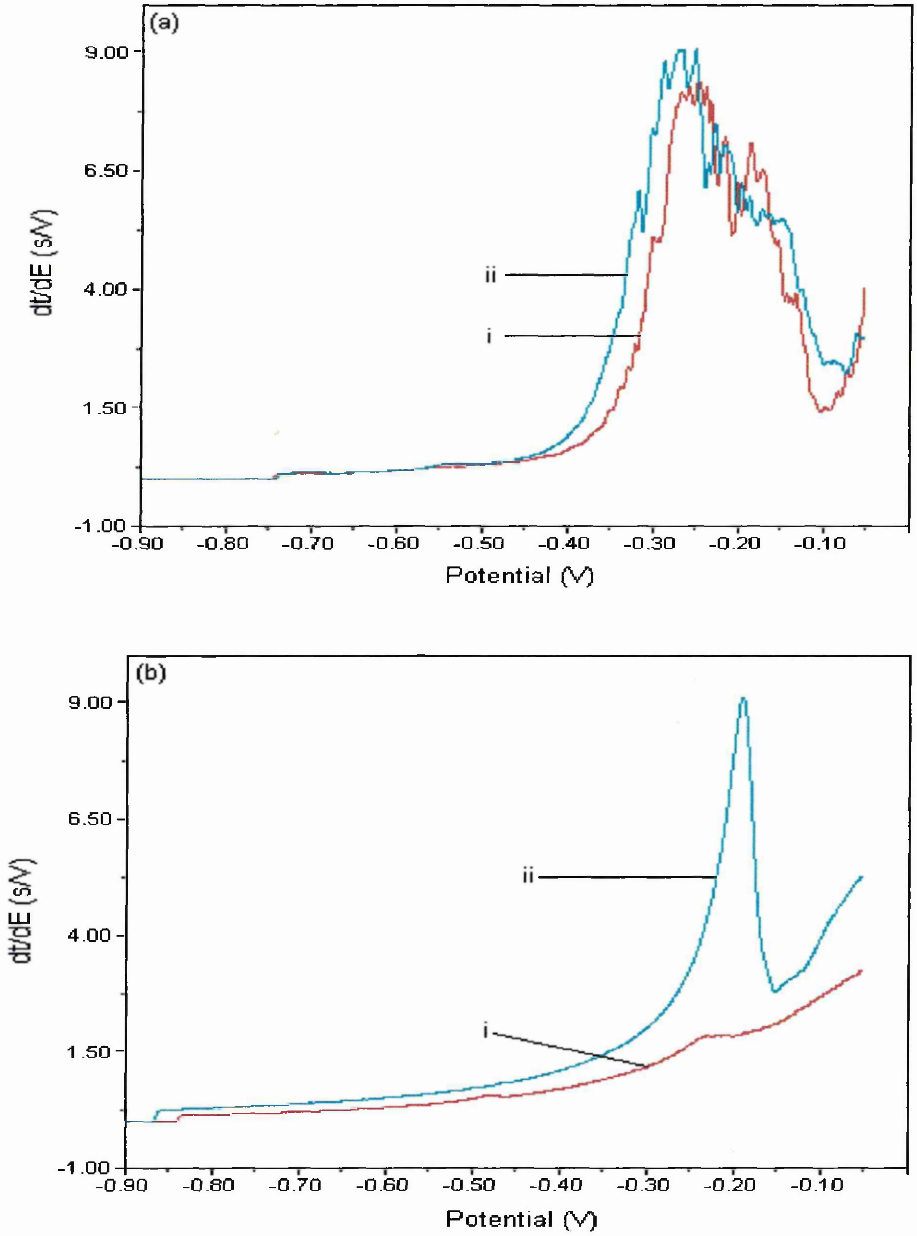


Figure 3.21: Stripping chronopotentiograms for (i) blank and (ii) 50 μg l^{-1} copper (II) on (a) MFSPCE and (b) MFGCE. Supporting electrolyte = 0.1 M HCl. Hg^{2+} = 10 mg l^{-1}, deposition time = 120 s, deposition potential = - 0.9 V, constant current = 1 μA.

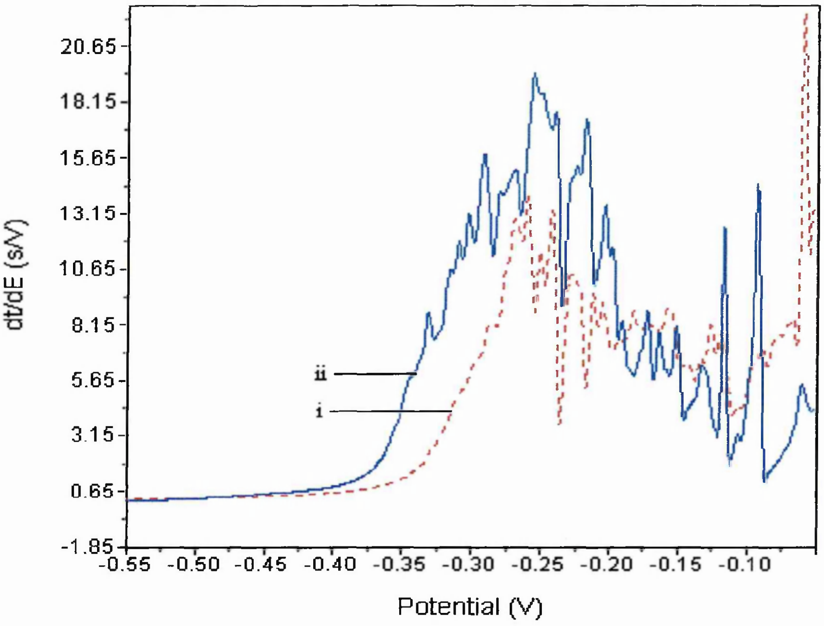


Figure 3.22: The response of the mercury film SPCE + conventional Ag/AgCl reference electrode to (i) 0.1 M HCl and (ii) 50 µg l^{-1} copper (II) in 0.1 M HCl. Hg^{2+} = 10 mg l^{-1}, deposition time = 120 s, deposition potential = - 0.9 V, constant current = 1 µA.

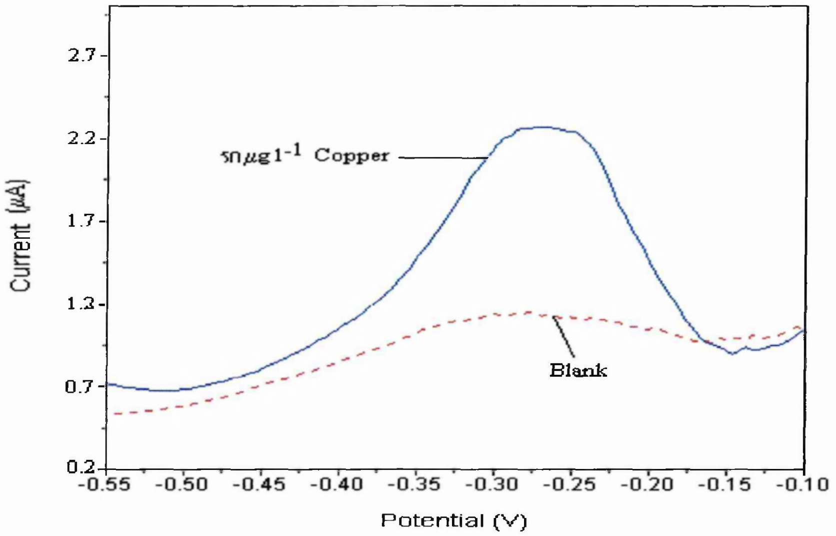


Figure 3.23: Stripping voltammetric responses on SPCE plated *in situ* with 10 mg l^{-1} mercury (II). Supporting electrolyte = 0.1 M HCl, deposition time = 120 s, deposition potential = - 0.9 V, final potential = 0.05 V, frequency = 50 Hz, SW amplitude = 20 mV, step potential = 5 mV.

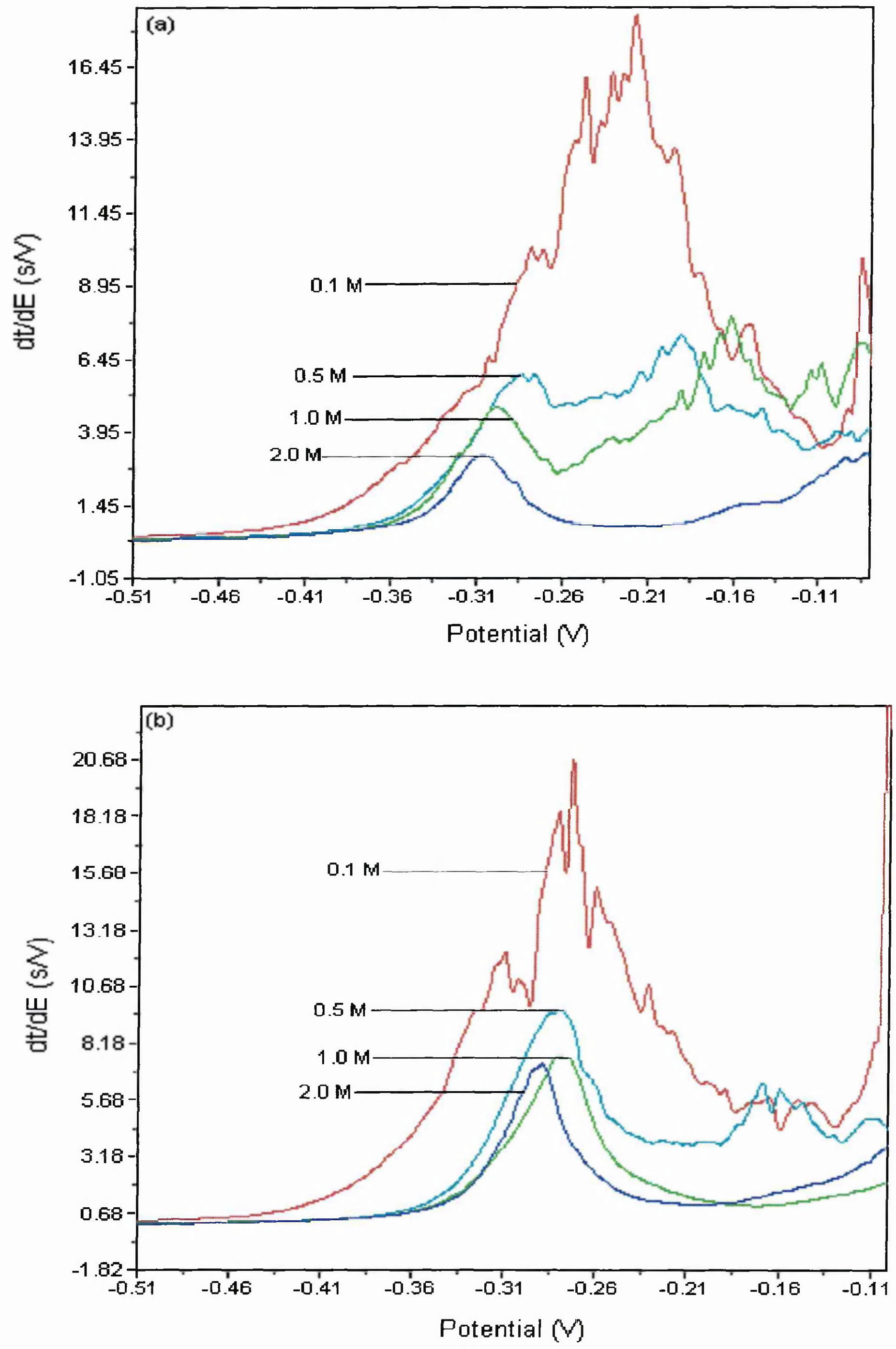


Figure 3.24: Stripping chronopotentiograms for (a) 50 and (b) 200 µg l^{-1} copper (II) in solutions containing HCl at different concentration using MFSPCE. Hg^{2+} = 10 mg l^{-1}, deposition time = 120 s, deposition potential = - 0.9 V, constant current = 1 µA.

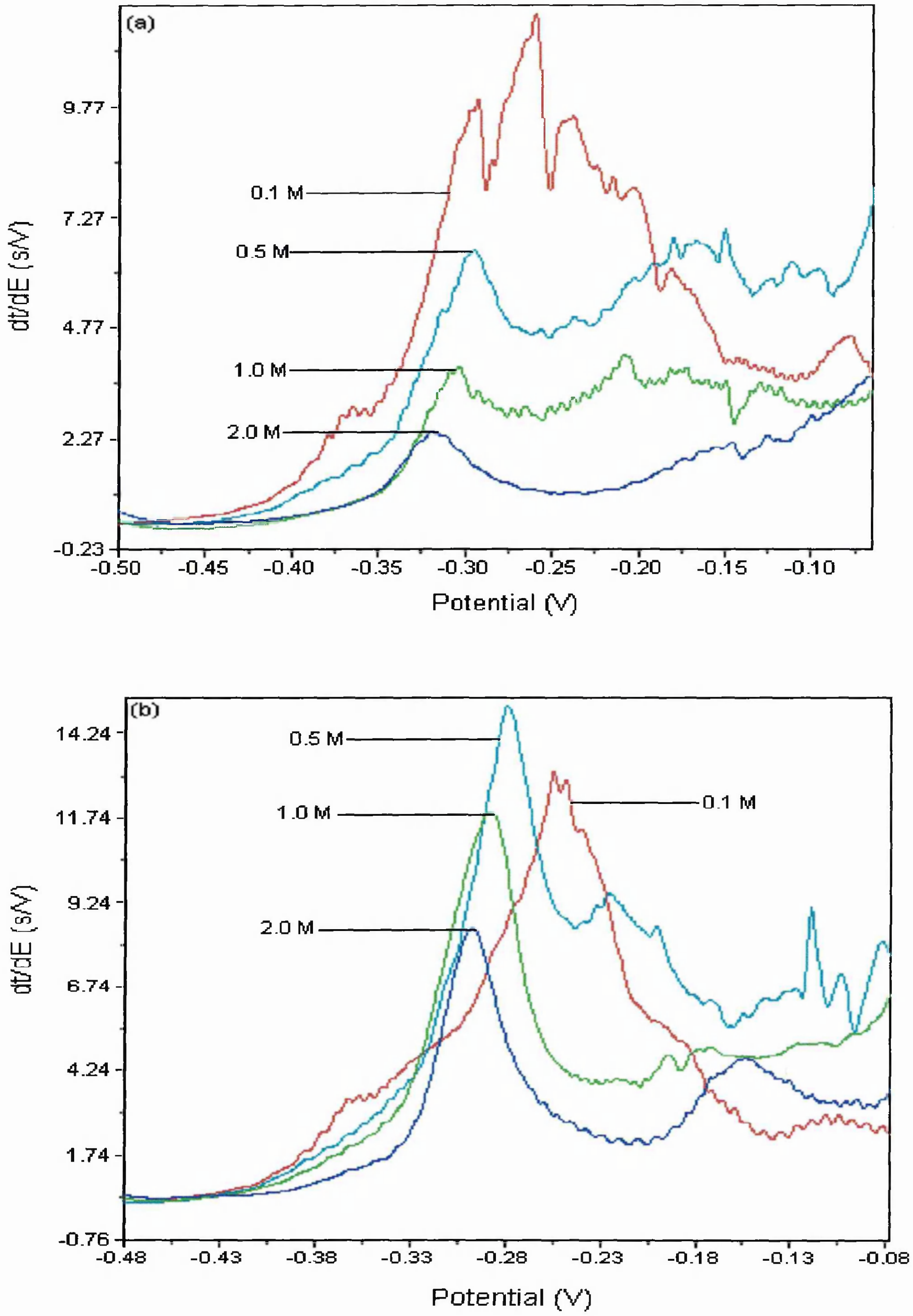


Figure 3.25: Stripping chronopotentiograms for (a) 50 and (b) 200 μg l^{-1} copper (II) in solutions containing KCl at different concentration using MFSPCE. Hg^{2+} = 10 mg l^{-1}, deposition time = 120 s, deposition potential = - 0.9 V, constant current = 1 μA.

Other medium compositions were also examined because it would be desirable to be able to carry out the simultaneous detection of copper with other metals (cadmium and lead) on MFSPCE (10 mg l^{-1} mercury (II) plated *in situ*) in a less concentrated chloride based medium and in a medium with higher pH (pH range 3 – 6). Hence, studies were carried out with 0.5 M ammonium acetate + 0.1 M HCl because the pH of the medium composition (~ pH 4.6) is well suited to the simultaneous detection of copper, lead and cadmium.

The chronopotentiometric stripping response of copper obtained using this medium (Figure 3.26a) was not as well defined or reproducible as ones generated with 2 M HCl and 2 M KCl (Figure 3.24 and 3.25). Hence the concentrations of HCl and ammonium acetate in the medium were increased to 0.5 M and 1 M, respectively. The stripping peak obtained for copper using 1 M ammonium acetate + 0.5 M HCl was much more stable and well-defined (Figure 3.26b) compared to one generated at a lower concentration (Figure 3.26a).

3.3.3.2 Effect of applied constant stripping current

The applied stripping current has significant influence on the sensitivity and reproducibility of response obtained using CCSCP. The profound effect of the applied stripping current upon copper stripping response and peak potential (E_p) is illustrated in Table 3.2.

The stripping response decreased when the applied stripping current was high, because of the fast stripping of the deposited copper and increased when the stripping current was low due to the increased stripping time of the stripping step. The E_p also shifts to more positive values as the stripping current increases. This shift has been assigned (Beinrohr *et al.*, 1994; 1996; 1999) to an ohmic potential (*iR*) drop between the working and reference electrode.

For CCSCP measurements, inaccurate results can be obtained (Beinrohr *et al.*, 1996; 1999) if proper attention is not paid in applying the appropriate stripping current. The only way to circumvent this is, by careful optimisation of the stripping current applied. From studies carried out with 2 M HCl medium, a stripping current of + 1 μA was chosen as a compromise between signal stability and sensitivity even though a higher response was obtained with a lower stripping current, 0.1 μA (Table 3.2).

The reproducibility data for stripping response of 50 μg l^{-1} copper (shown in Table 3.2) was highest when using an applied stripping current of 0.1 μA due to the heavy stripping step and this is the case when too low a stripping current is applied. For 1 M ammonium acetate containing 0.5 M HCl, the application of a stripping current between + 3 and + 5 μA was considered to be the most appropriate choice because of the better background baseline response (Figure 3.27).

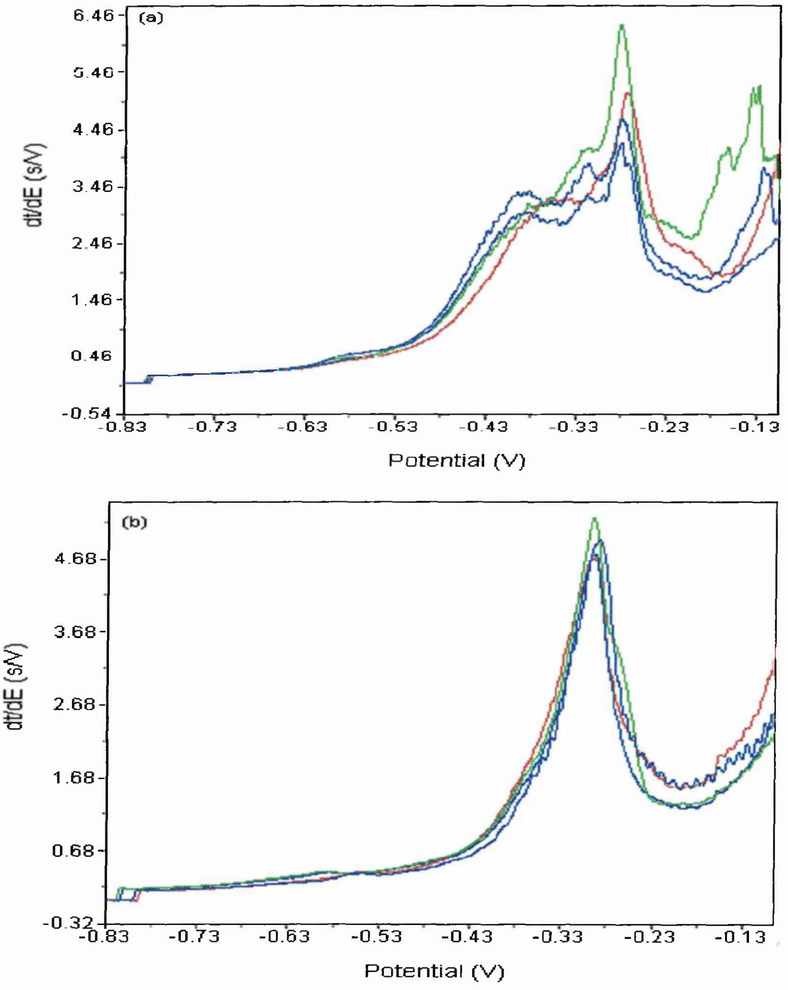


Figure 3.26: Four successive stripping chronopotentiograms of 50 μg l^{-1} copper (II) in solutions containing (a) 0.1 M HCl + 0.5 M ammonium acetate and (b) 0.5 M HCl + 1 M ammonium acetate. Hg^{2+} = 10 mg l^{-1}, deposition time = 120 s, deposition potential = - 0.9 V, constant current = 1 μA.

Table 3.2: Effect of applied stripping current on the stripping response of 50 μg l^{-1} copper.

	Stripping current (μA)					
	0.1	1.0	3.0	5.0	8.0	10.0
Peak potential (V)	-0.315	-0.295	-0.250	-0.235	-0.200	-0.170
*Stripping time (ms)	233	115	63	44	31	31
R.S.D (%)	20.0	7.7	6.5	7.7	7.1	7.4

*mean for 6 measurements

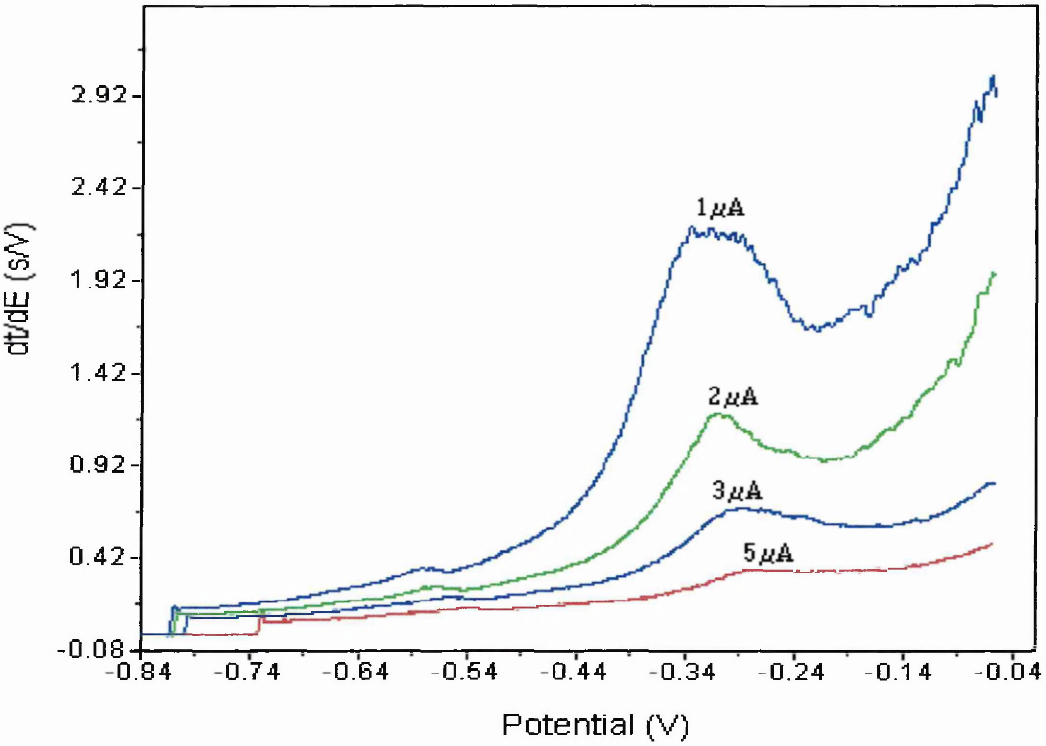


Figure 3.27: Effect of stripping current on the stripping chronopotentiomeric response of blank solution of 1 M ammonium acetate containing 0.5 M HCl. Hg^{2+} = 10 mg l^{-1}, deposition time = 120 s, deposition potential = - 0.9 V.

3.3.3.3 *Calibration curve, detection limit and precision*

Figure 3.28 displays the calibration plot and also the stripping chronopotentiograms for increasing concentrations of copper (II) in 2 M HCl. The calibration plot was linear in the range examined, 10 – 2000 µg l^{-1}, with sensitivity of 1.40 ± 0.02 ms l μg^{-1} ($r^2 = 0.9988$).

For 1 M ammonium acetate containing 0.5 M HCl, a linear plot was obtained in the range 25 – 1000 µg l^{-1} with sensitivity of 2.35 ± 0.04 ms l μg^{-1} ($r^2 = 0.9980$). The calibration curve and stripping chronopotentiograms for increasing concentrations of copper (II) in 1 M ammonium acetate containing 0.5 M HCl are depicted in Figure 3.29 and 3.30.

The detection limits (120 s deposition) based on the use of S/N = 3 criterion were 6 µg l^{-1} for 2 M HCl as supporting medium and 20.5 µg l^{-1} for 1 M ammonium acetate + 0.5 M HCl as supporting medium. The stripping response was also reproducible with a series of eight repetitive measurements of 50 µg l^{-1} copper (II) in 2 M HCl and 1 M ammonium acetate containing 0.5 M HCl yielding relative standard deviations of 6.9 and 7.9 %, respectively.

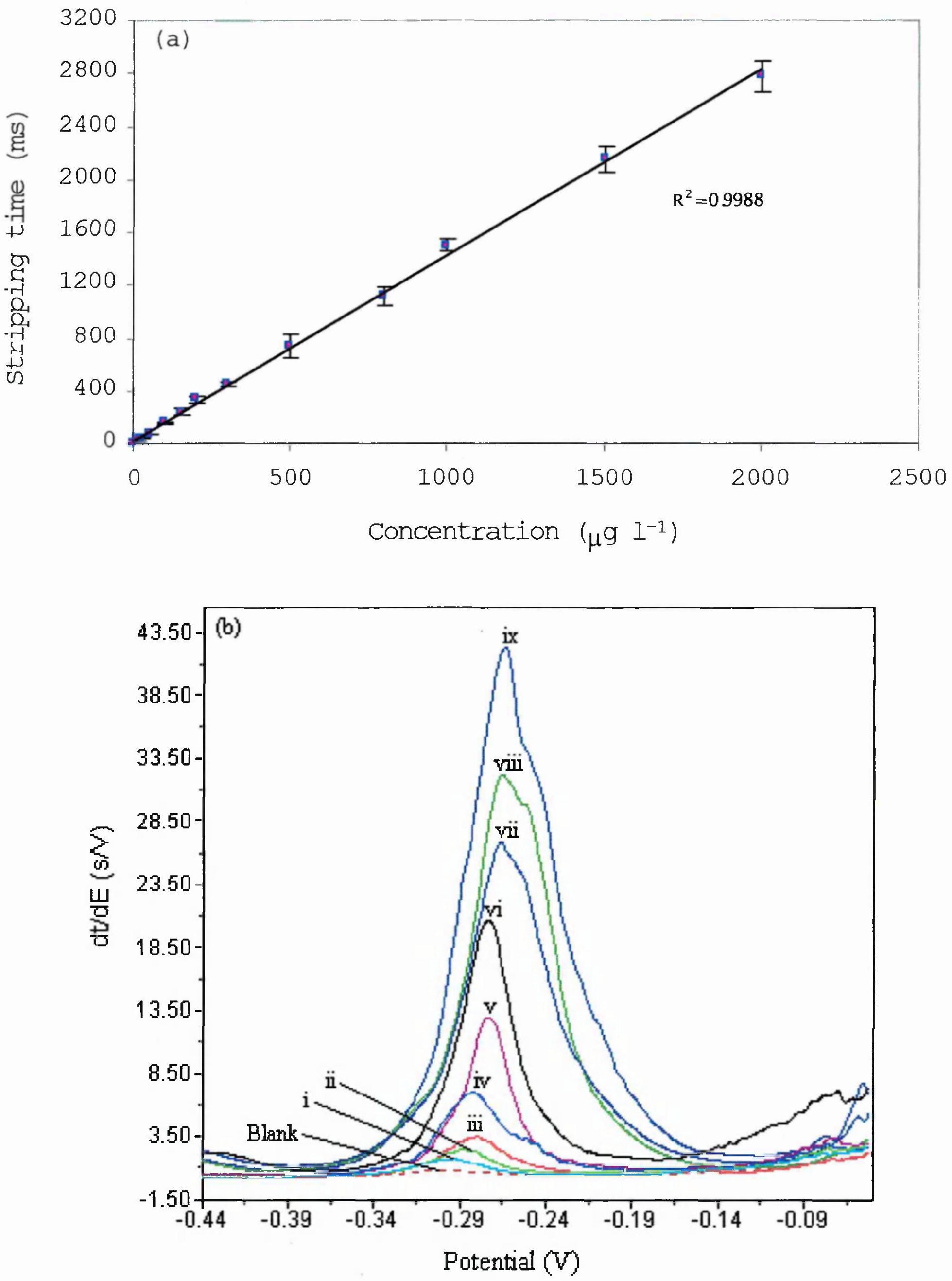


Figure 3.28: (a) Calibration plot and (b) stripping chronopotentiograms for increasing concentrations of copper (II) in 2 M HCl. (i) 25, (ii) 50, (iii) 100, (iv) 200, (v) 300, (vi) 500, (vii) 1000, (viii) 1500 and (ix) 2000 µg l^{-1}. Hg^{2+} = 10 mg l^{-1}, deposition time = 120 s, deposition potential = - 0.9 V, constant current = 1 µA.

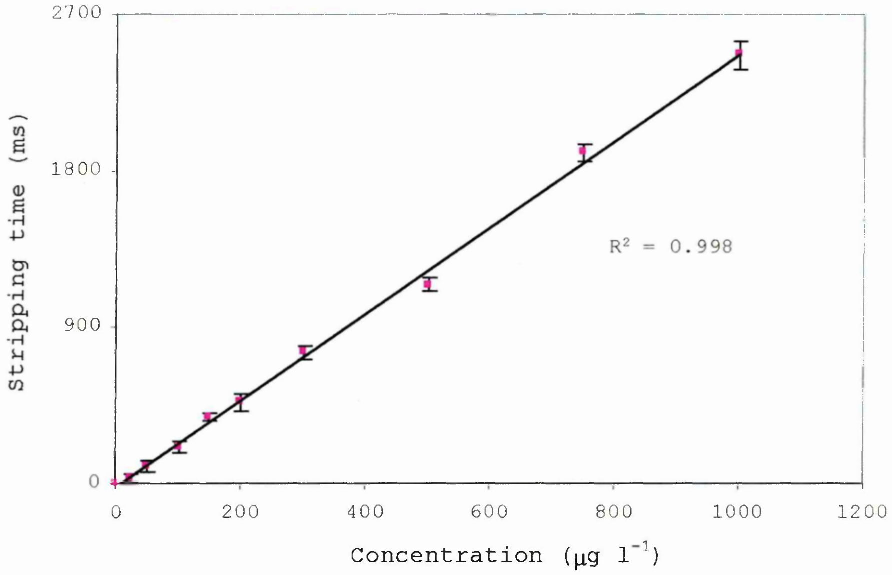


Figure 3.29: Calibration plot for increasing concentrations of copper (II) in 1 M ammonium acetate + 0.5 M HCl medium. Hg^{2+} = 10 mg l^{-1}, deposition time = 120s, deposition potential = - 0.9 V, constant current = 3 μA.

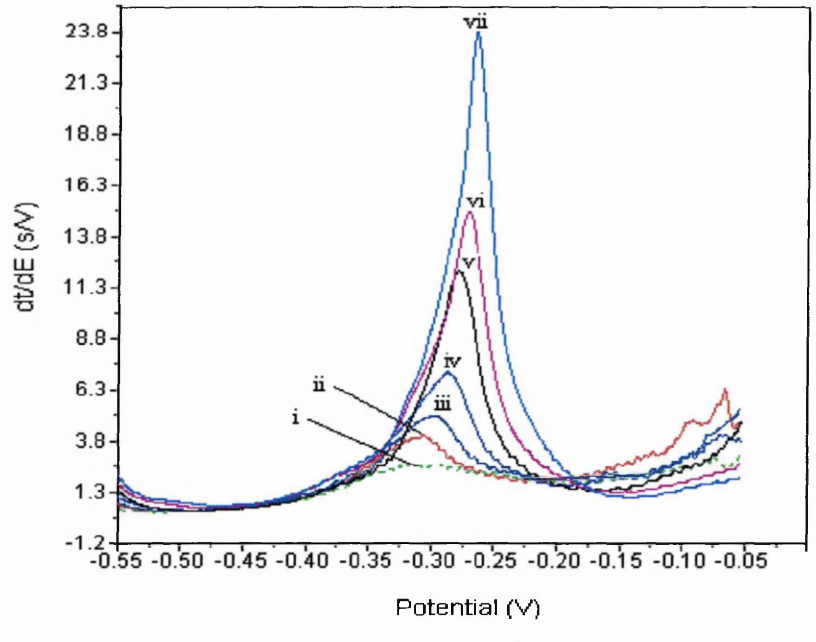


Figure 3.30: Stripping chronopotentiograms for increasing concentrations of copper (II) in 1 M ammonium acetate + 0.5 M HCl medium. (i) blank, (ii) 25, (iii) 50, (iv) 100, (v) 200, (vi) 300 and (vii) 500 μg l^{-1}.

3.3.4 Bismuth-film screen-printed electrodes as metal ion sensors

Some of the work presented here, have been accepted for publication in the Analytical and Bioanalytical Chemistry Journal (Kadara and Tothill, 2004).

In order to find an alternative electrode material to MFSPCE, the use of bismuth film SPCE (BFSPCE) was examined and the results of the studies carried out are presented below. The parameters, which most affect the CCSCP measurement process was optimised, i.e., concentration of bismuth (III) co-deposited with the target metals (lead and cadmium), deposition time (t_{dep}) and deposition potential (E_{dep}). Also the effect of coexisting metal ions was evaluated. Either 0.1 M HCl or 0.1 M HCl + 0.5 M ammonium acetate (pH 4.6) was used as supporting electrolyte.

3.3.4.1 *Effect of bismuth (III) concentration*

Figure 3.31 illustrates the effect of bismuth ion concentration upon the stripping chronopotentiometric responses of lead (II) and cadmium (II). The lead and cadmium peak responses increased upon raising the bismuth (III) concentration. There was a levelling off above 150 μg l^{-1} for lead and at 500 μg l^{-1} for cadmium. The stripping peak of 100 μg l^{-1} lead obtained on bismuth-coated electrode was much more defined compared to the stripping peak obtained on the bare SPCE (0 μg l^{-1} bismuth) as illustrated in Figure 3.32. On bare SPCE, no peak for cadmium was visible but a peak appeared when sufficient amounts of bismuth is co-deposited with the target metal ions on the SPCE surface. A bismuth concentration of 500 μg l^{-1} was used for subsequent lead and cadmium measurements.

3.3.4.2 Effect of deposition potential

Figure 3.33 shows the effect of deposition potential on the stripping chronopotentiometric response of lead (II) and cadmium (II). On the basis of sensitivity, resolution and reproducibility, E_{dep} of – 1.0 V was employed for simultaneous detection of lead and cadmium in mixed solution, while for separate measurements, E_{dep} of – 0.9 V and – 1.1 V were used for lead and cadmium detection, respectively. E_{dep} more negative than – 1.0 V could not be used for the simultaneous detection of lead and cadmium because the stripping response of lead was affected by interferences emanating from the electrode surface.

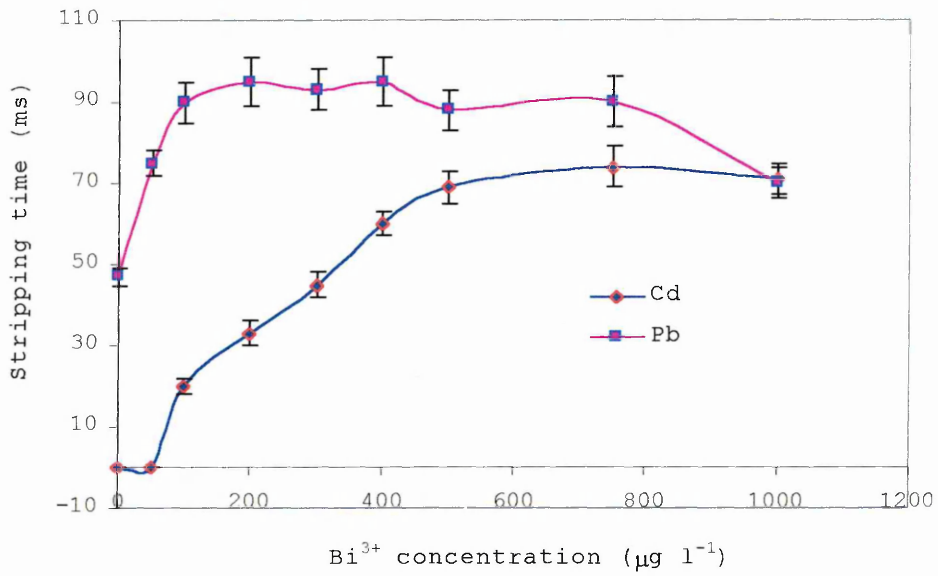


Figure 3.31: Effect of the bismuth concentration upon the stripping chronopotentiometric response of 100 μg l^{-1} lead (II) and cadmium (II), in 0.1 M HCl + 0.5 M ammonium acetate (pH 4.6). Deposition potential = - 1.0 V, deposition time = 120 s, constant current = 1 μA.

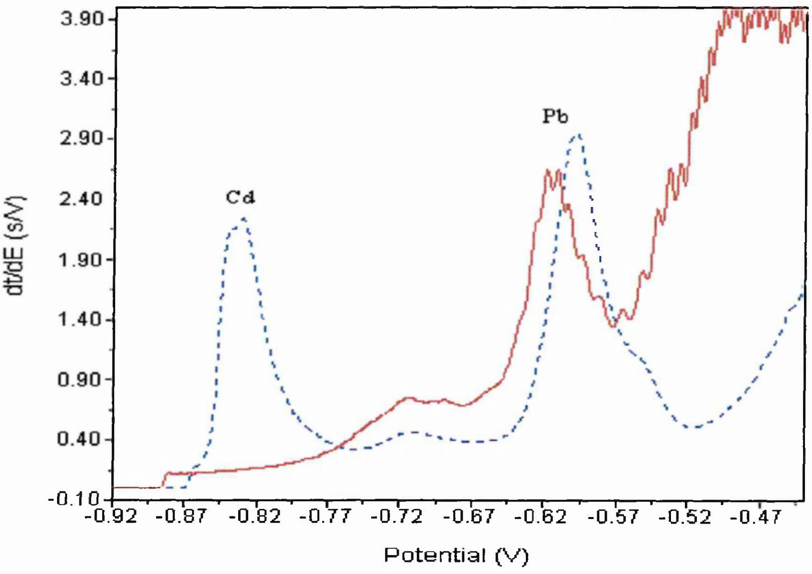


Figure 3.32: Stripping chronopotentiograms for 100 μg l^{-1} lead (II) and cadmium (II) in 0.1 M HCl + 0.5 M ammonium acetate on bare SPCE (solid line) and BFSPCE (broken line). Deposition potential = - 1.0 V, final potential = - 0.2 V, deposition time = 120 s, constant current = 1 μA. 500 μg l^{-1} Bi^{3+} is co-deposited with lead and cadmium for BFSPCE.

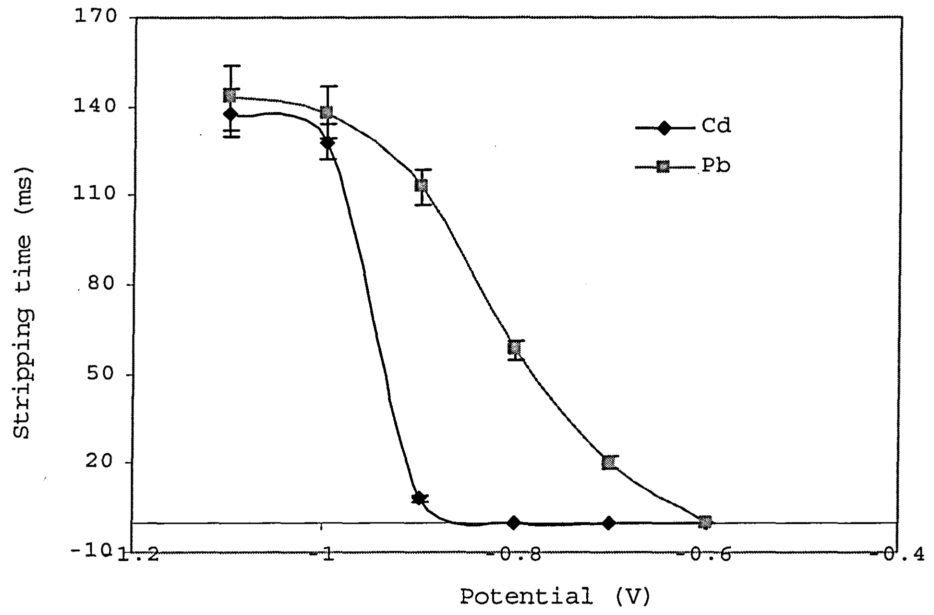


Figure 3.33: Effect of deposition potential on stripping responses of 200 µg l^{-1} lead (II) and cadmium (II) on BFSPCE. Deposition time = 60 s, Bi^{3+} = 500 µg l^{-1}, constant current = 1 µA.

3.3.4.3 Effect of deposition time

Figure 3.34 shows the effect of deposition time on the stripping chronopotentiometric response of lead (II). The stripping response increased linearly with the deposition time up to 300 s for 50 µg l^{-1} lead (II) and 240 s for 200 µg l^{-1} lead (II) and then levelled off afterwards for 200 µg l^{-1} lead due to the saturation of the bismuth film formed on the SPCE surface. The effect of deposition time on the stripping chronopotentiometric response of cadmium (II) is shown in Figure 3.35. A deposition time of 120 s was chosen for subsequent analytical work as a compromise between length of analysis and sensitivity.

3.3.4.4 Effect of interfering ions

The selectivity of the proposed method was investigated by examining the effect of co-existing ions on the responses for lead (II) and cadmium (II). The effect of different concentrations of copper (II) on the stripping peak responses of lead (II) was evaluated and the result is presented in Figure 3.36. Suppression of the lead stripping peak responses was observed in the presence of copper (II). The effect of other metal ions (cadmium (II) and chromium (III)) on lead (II), were not as significant as that of copper (II).

Possible interference by other ionic species in the stripping chronopotentiometric detection was investigated by the addition of chromium, copper, iron, lead, and sulphate ions to a solution containing 50 μg l^{-1} of cadmium (II) using the optimised conditions. The results of this study are summarised in Figure 3.37. There is an increase in the stripping response of cadmium in the presence of lead and chromium while in the presence of copper; there is a complete suppression of the stripping response.

To eliminate the detrimental effect of copper (II) on lead (II), ferricyanide was used to complex the copper (II). The concentration of ferricyanide added was determined by studying the effect of 2000 μg l^{-1} copper (II) on 60 and 200 μg l^{-1} lead (II) solutions (Figure 3.38). A concentration of 0.1 mM was found to be appropriate for suppressing the copper effect. As can observed from the stripping chronopotentiograms in Figure 3.39 and 3.40, the copper interference on the stripping response of lead was completely eliminated when 0.1 mM ferricyanide was added to the test solution.

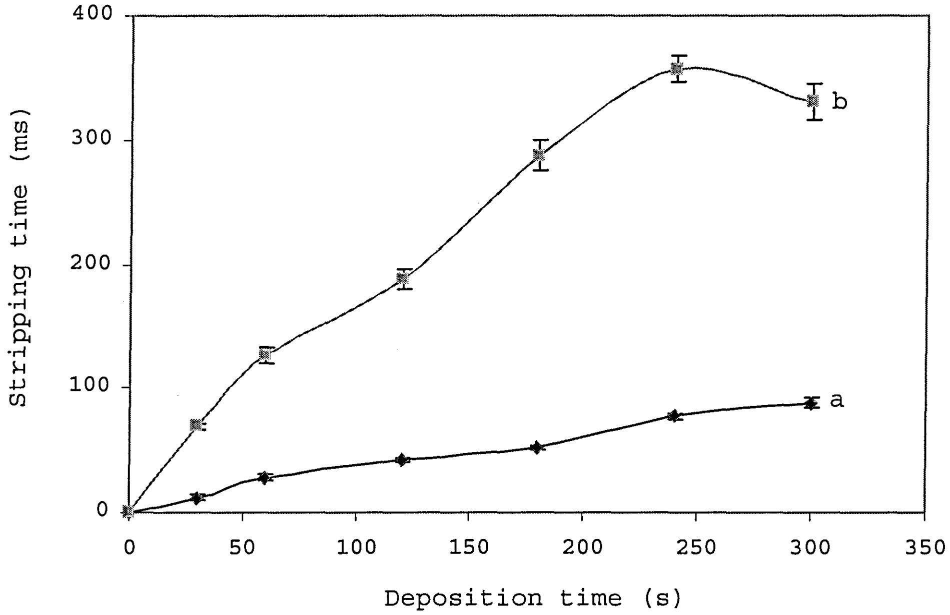


Figure 3.34: Effect of deposition time on stripping response of (a) 50, (b) 200 μg l^{-1} of lead (II). Deposition potential = - 0.9 V, Bi^{3+} = 500 μg l^{-1}, deposition time = 120 s, constant current = 1 μA.

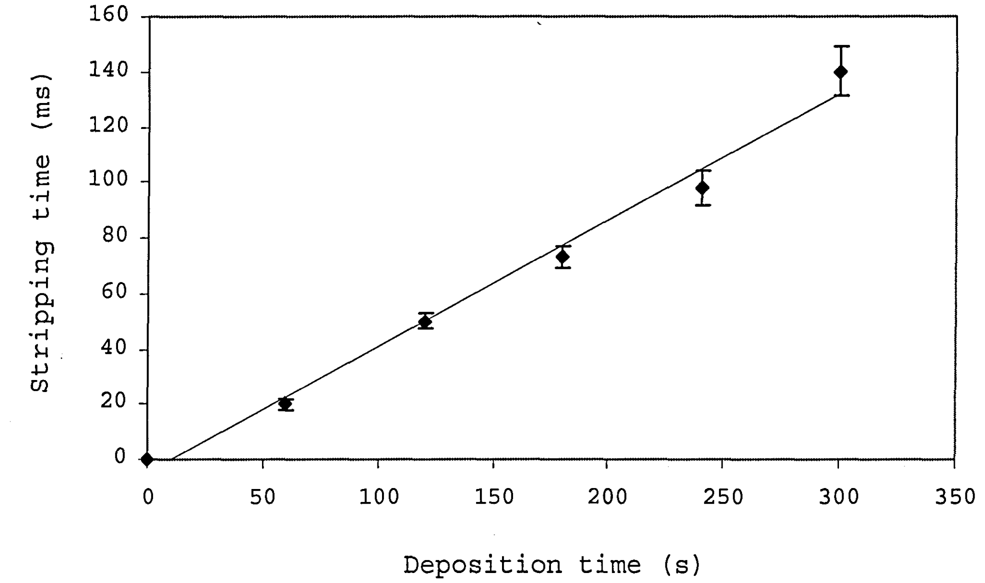


Figure 3.35: Effect of deposition time on stripping response of 75 μg l^{-1} of cadmium (II). Deposition potential = - 1.1 V, Bi^{3+} = 500 μg l^{-1}, deposition time = 120 s, constant current = 1 μA.

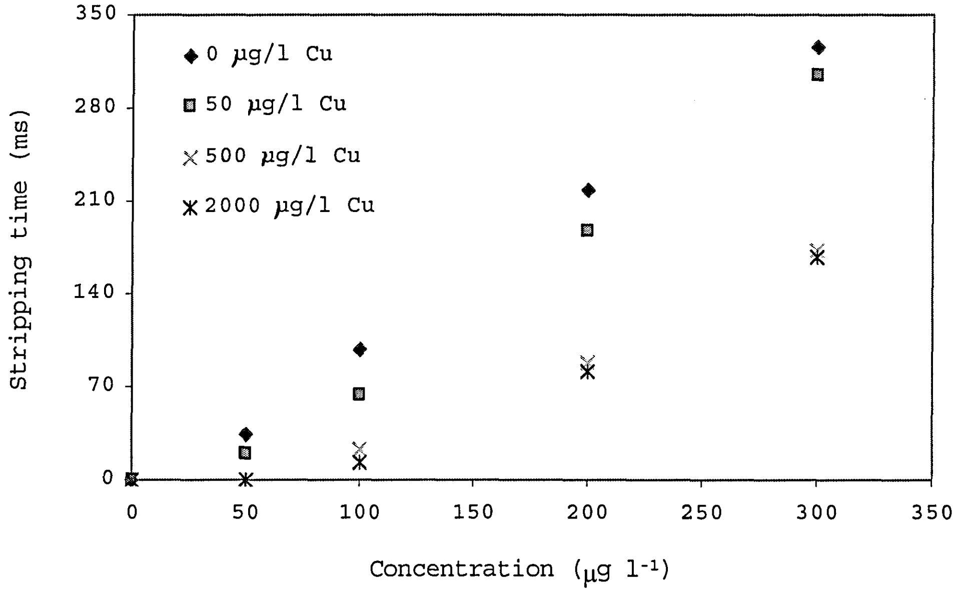


Figure 3.36: The effect of different concentrations of copper (II) on the stripping chronopotentiometric response of lead (II) on BFSPCE. Deposition potential = - 0.9 V, Bi^{3+} = 500 μg l^{-1}, deposition time = 120 s, constant current = 1 μA.

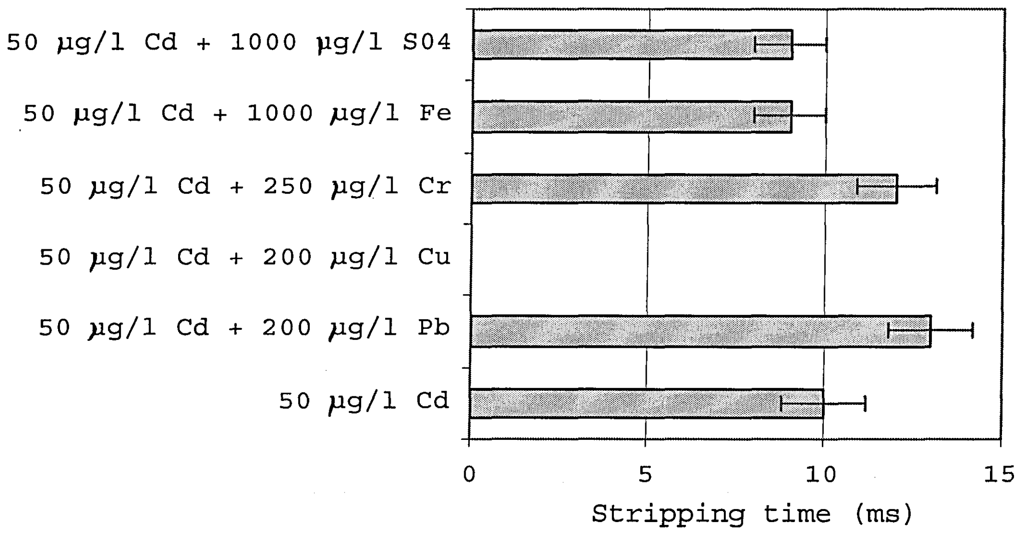


Figure 3.37: Graphical summation of the effect of different interfering ions on the stripping chronopotentiometric response of 50 µg l^{-1} cadmium (II) on BFSPCE. Deposition potential = - 1.0 V, Bi^{3+} = 500 µg l^{-1}, deposition time = 120 s, constant current = 1 µA.

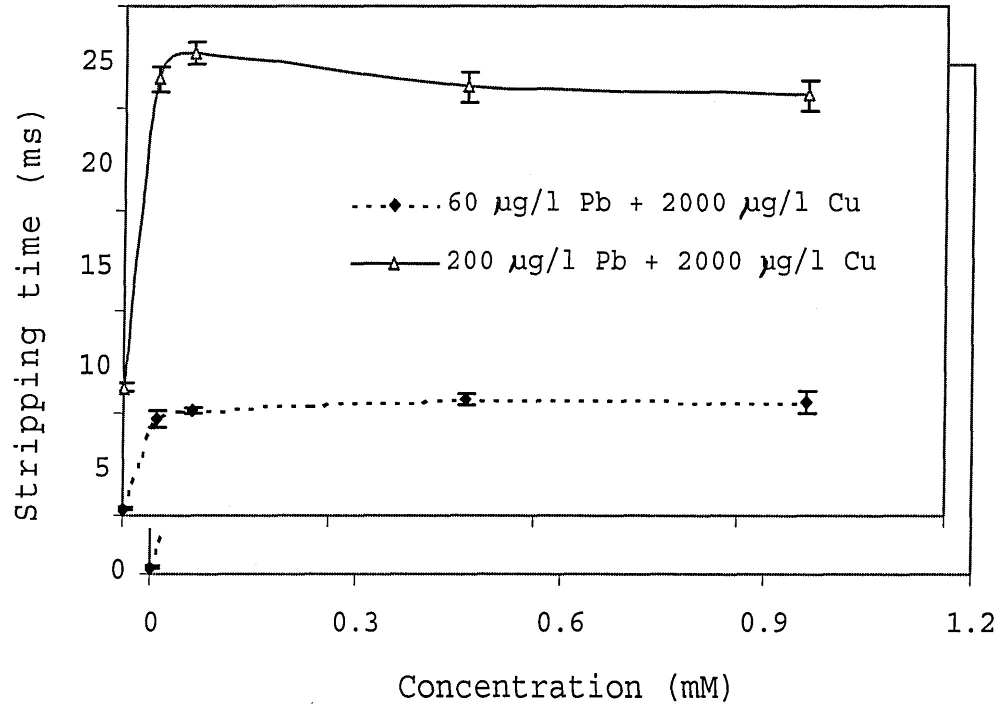


Figure 3.38: The effect of different amounts of ferricyanide in suppressing the copper (II) interference on lead stripping response. Deposition potential = - 0.9 V, Bi^{3+} = 500 µg l^{-1}, deposition time = 120 s, constant current = 1 µA.

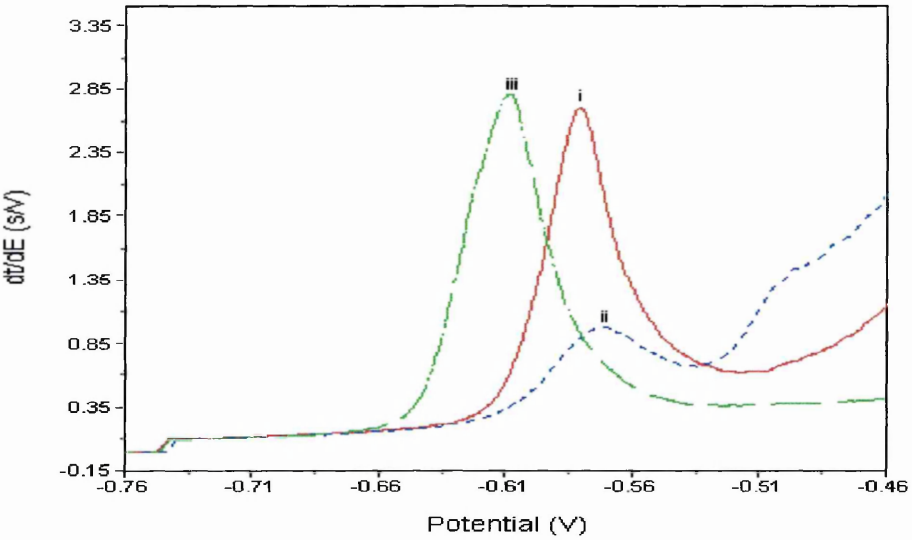


Figure 3.39: Elimination of the interference of copper on the stripping response of lead (II) on BFSPCE. (i) 100 μg l^{-1} lead (II) + 0 μg l^{-1} copper (II), (ii) 100 μg l^{-1} lead (II) + 500 μg l^{-1} copper (II) and (iii) 100 μg l^{-1} lead + 500 μg l^{-1} copper (II) + 0.1 mM ferricyanide. Deposition potential = - 0.9 V, Bi^{3+} = 500 μg l^{-1}, deposition time = 120 s, constant current = 1 μA.

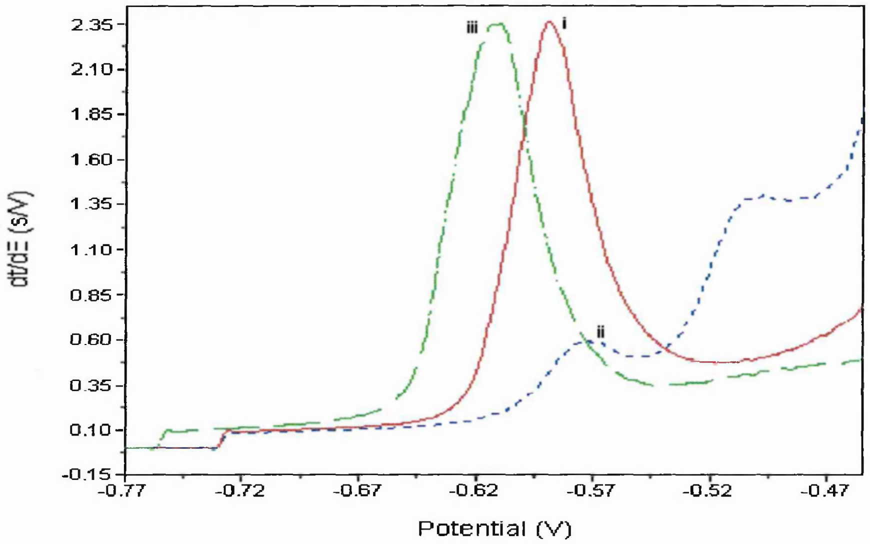


Figure 3.40: Elimination of the interference of copper on the stripping response of lead (II) on BFSPCE. (i) 100 μg l^{-1} lead (II) + 0 μg l^{-1} copper (II), (ii) 100 μg l^{-1} lead (II) + 2000 μg l^{-1} copper (II) and (iii) 100 μg l^{-1} lead + 2000 μg l^{-1} copper (II) + 0.1 mM ferricyanide. Deposition potential = - 0.9 V, Bi^{3+} = 500 μg l^{-1}, deposition time = 120 s, constant current = 1 μA.

3.3.4.5 Calibration curve

3.3.4.5a Lead (II)

CCSCP was carried out for lead (II) in 0.1 M HCl on BFSPCE. Deposition was carried out by holding the potential at – 0.9 V for 120 s. Figure 3.41 displays the calibration plot for stripping responses of lead (II) over the concentration range from 0 to 300 μg l^{-1}. The resulting plot was linear over the range studied (sensitivity = 1.06 ± 0.03 ms l μg^{-1}, $r^2 = 0.9938$). A detection limit of 12 μg l^{-1} was estimated following 120 s deposition. The stripping response was reproducible with fifteen repetitive measurements of 50 μg l^{-1} lead (120 s deposition) yielding a relative standard deviation (R.S.D) of 5.6 %.

3.3.4.5b Cadmium (II)

Figure 3.42 depicts the calibration plot of cadmium in 0.1 M HCl + 0.5 M ammonium acetate (pH 4.6) using a deposition potential of – 1.1 V and time of 120 s. The plot was linear over the range studied, 25 to 300 μg l^{-1}, with of sensitivity 0.97 ± 0.05 ms l μg^{-1} ($r^2 = 0.9851$). The stripping response was reproducible with fifteen repetitive measurements of 50 μg l^{-1} cadmium (120 s deposition) yielding a relative standard deviation (R.S.D) of 8.1 %.

3.3.4.5c Simultaneous measurement of lead (II) and cadmium (II)

The stripping chronopotentiograms for the simultaneous detection of lead and cadmium in 0.1 M HCl + 0.5 M ammonium acetate are shown in Figure 3.43. The two ions analysed simultaneously using a deposition potential of – 1.0 V, were linearly dependent on concentration in the same range (20 – 300 μg l^{-1}) as when they were detected separately. Sensitivities of 0.85 ± 0.04 ms l μg^{-1} and 0.84 ± 0.04 ms l μg^{-1} (correlation coefficient of $r^2 = 0.9966$ and 0.9977) were obtained for lead and cadmium, respectively. Detection limits of 8 and 10 μg l^{-1} were estimated for cadmium and lead in 0.1 M HCl + 0.5 M ammonium acetate (pH 4.6) following 120 s deposition. Six simultaneous measurements of 50 μg l^{-1} lead (II) and cadmium (II) yielded relative standard deviation (RSD) of 4.5 % and 5.1 % (following 2 minute deposition) respectively.

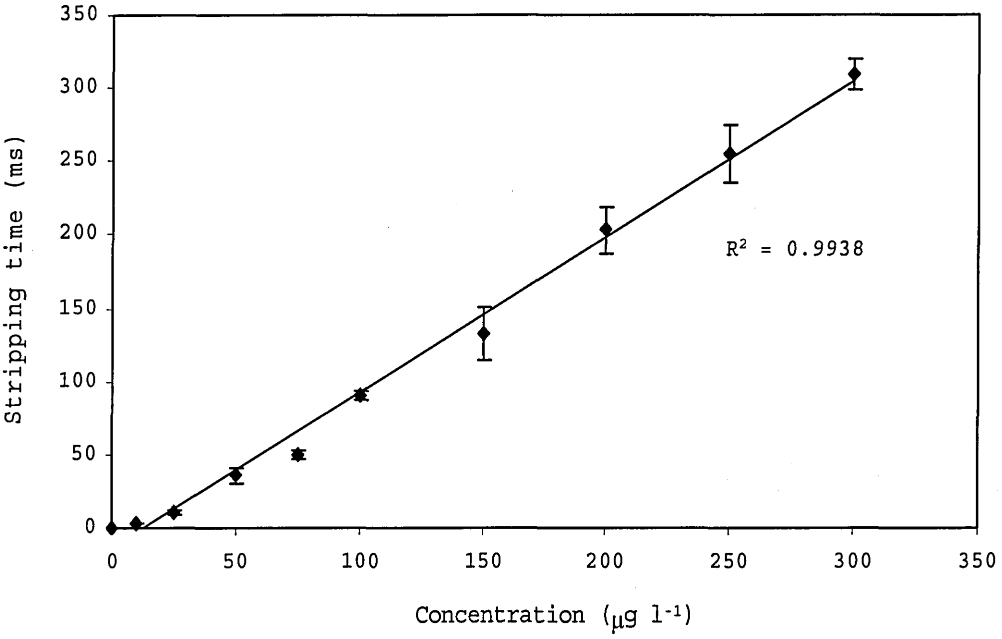


Figure 3.41: Calibration plot for increasing concentrations of lead (II) in 0.1 M HCl on BFSPCE. Deposition potential = - 0.9 V, Bi^{3+} = 500 μg l^{-1}, deposition time = 120 s, constant current = 1 μA.

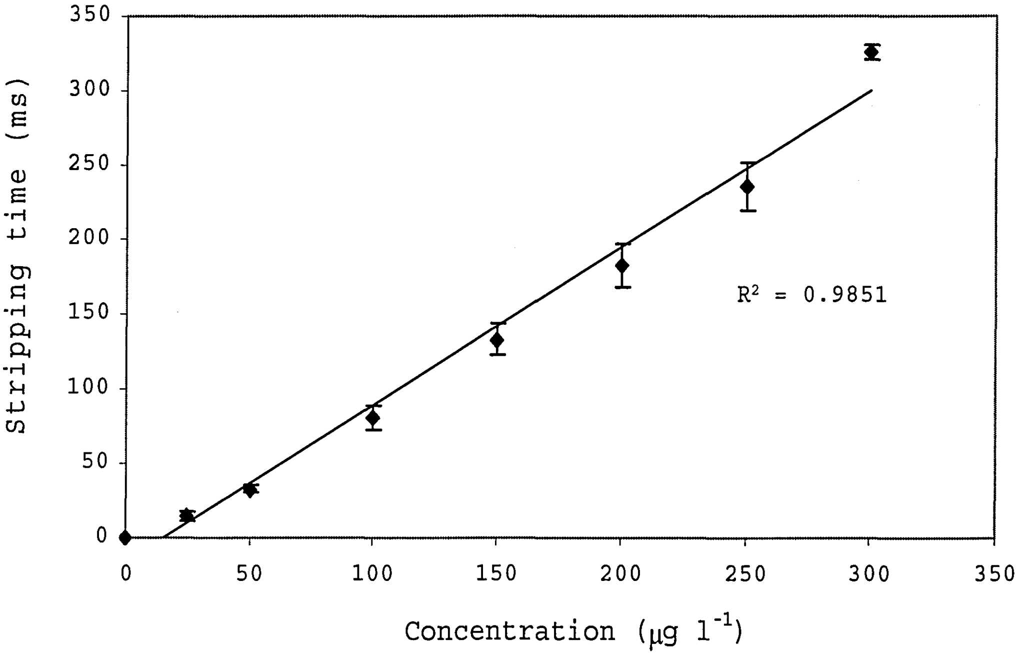


Figure 3.42: Calibration plot for increasing concentrations of cadmium (II) in 0.1 M HCl + 0.5 M ammonium acetate on BFSPCE. Deposition potential = - 1.0 V, Bi^{3+} = 500 μg l^{-1}, deposition time = 120 s, constant current = 1 μA.

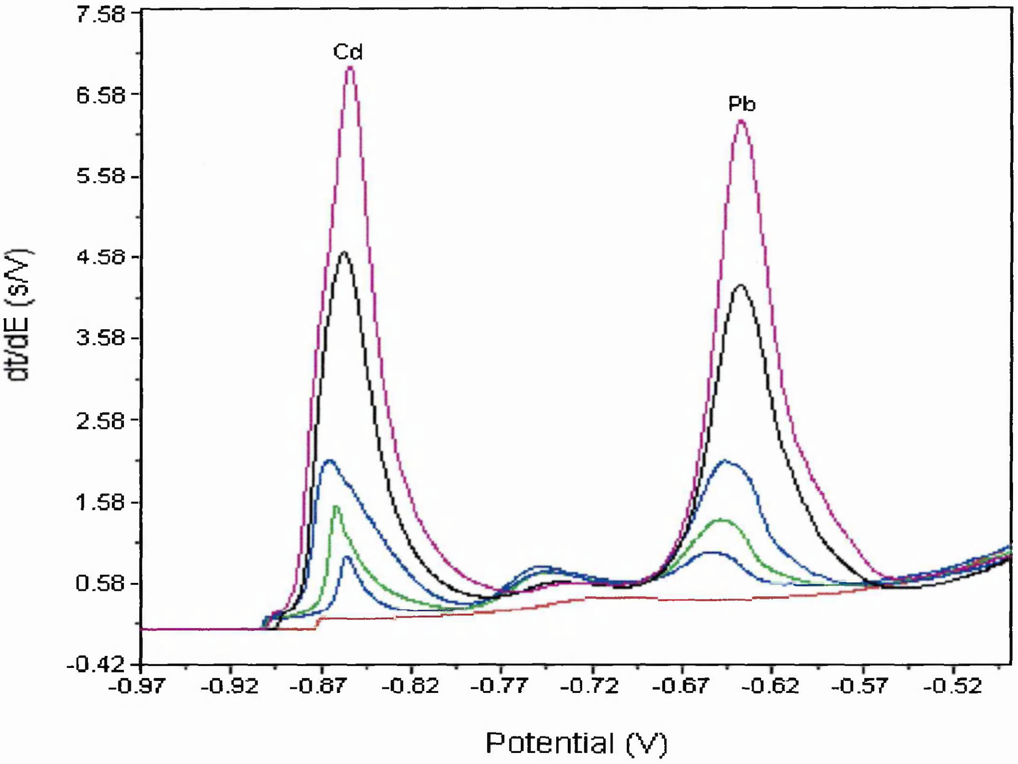


Figure 3.43: Overlaid stripping chronopotentiograms showing peaks obtained for 25, 50, 100, 200, 300 μg l^{-1} lead (II) and cadmium (II) on BFSPCE. Blank solution (base curve): 0.1 M HCl + 0.5 M ammonium acetate. Deposition potential = - 1.0 V, Bi^{3+} = 500 μg l^{-1}, deposition time = 120 s, constant current = 1 μA.

3.3.5 Bare screen-printed carbon electrode - Stripping chronopotentiometric detection of lead (II), cadmium (II) and copper (II)

Apart from studies carried out on MFSPCE and BFSPCE, the detection of lead, cadmium and copper was also investigated on bare SPCE. Deposition time of 120 s, as used in studies with mercury film SPCE and bismuth film SPCE was employed for analysis of lead (II), cadmium (II) and copper (II). On the basis of sensitivity, resolution and reproducibility, E_{dep} of - 0.9 V was employed for lead and copper measurements, while for cadmium, - 1.1 V was used. For simultaneous measurements of cadmium (II) and lead (II), – 1.0 V was used.

3.3.5.1 Lead (II)

CCSCP measurement of lead (II) was carried in 0.1 M HCl at bare SPCE by applying a deposition potential of – 0.9 V for 120 s. Figure 3.44 displays the calibration plot for stripping responses of lead (II) over the concentration range from 0 to 1000 μg l^{-1}. The resulting plot was linear over the range studied (sensitivity = 1.09 ± 0.03 ms l μg^{-1}, $r^2 = 0.993$). A detection limit of 35 μg l^{-1} was estimated following 120 s deposition. The stripping response was reproducible with ten repetitive measurements of 100 μg l^{-1} lead (120 s deposition) yielding a relative standard deviation (R.S.D) of 6.8 %.

The effect of copper on the stripping chronopotentiometric response of lead (II) was examined on bare SPCE. As the concentration ratio of copper to lead increased, the stripping chronopotentiometric response for lead (II) was suppressed. In order to reduce this effect, ferricyanide was added to the test solution. Table 3.3 shows the effect of copper (II) on the lead stripping response (200 μg l^{-1}) at different lead (II): copper (II) concentration ratios. Table 3.4 shows the effect of adding ferricyanide to mask the effect of copper. As can be seen from the results, the copper interference on the stripping response of lead is reduced significantly when 0.1 mM ferricyanide is added to the test solution.

3.3.5.2 Cadmium (II)

CCSCP measurement of cadmium (II) was carried in 0.1 M HCl + 0.5 M ammonium acetate at bare SPCE by applying a deposition potential of – 1.1 V for 120 s. Calibration plot of the stripping responses of cadmium (II) versus its concentration was established in the range 50 to 1000 μg l^{-1}. The resulting plot was linear (r^2 = 0.9918) with sensitivity of 0.76 ± 0.02 ms l μg^{-1}. A detection limit of 45 μg l^{-1} was estimated following 120 s deposition. The stripping response was reproducible with ten repetitive measurements of 200 μg l^{-1} cadmium (120 s deposition) yielding a relative standard deviation (R.S.D) of 10.1 %.

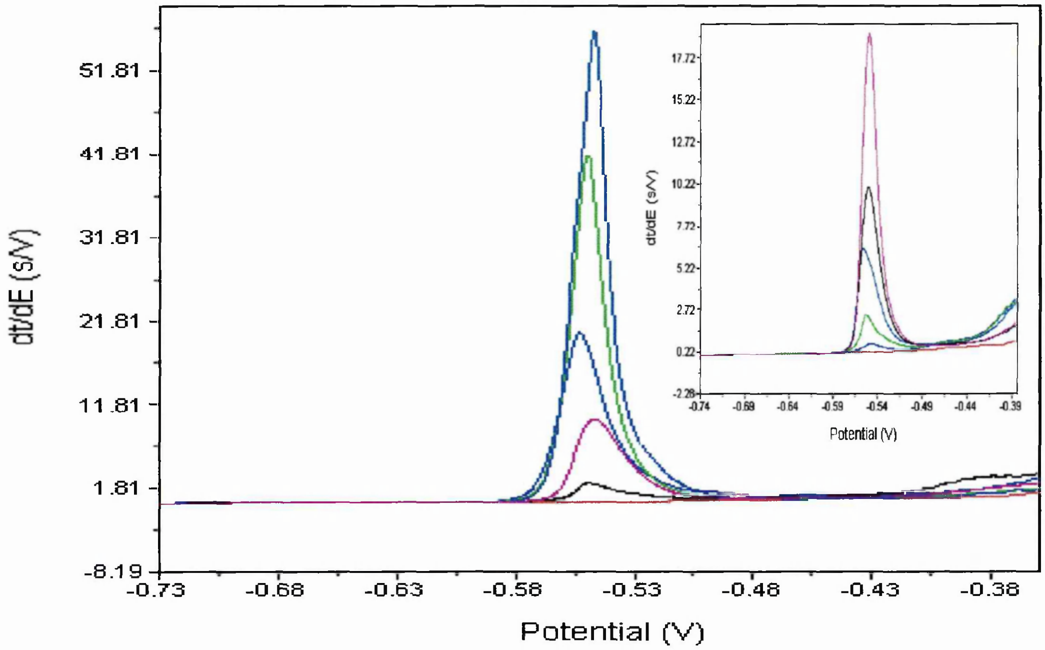


Figure 3.44: Overlaid stripping chronopotentiograms showing peaks obtained for 100, 300, 500, 750 and 1000 µg l^{-1} lead (II). Blank solution (base curve): 0.1 M HCl. (Inset: 50, 100, 200, 300, 400 µg l^{-1} lead (II)). Deposition potential = - 0.9 V, deposition time = 120 s, constant current = 1 µA.

Table 3.3: Effect of copper on lead response at different concentration ratios on bare SPCE.

Concentration ratio - lead: copper	% Signal change
1:1	- 26
1:5	- 95
1:10	- 98

Table 3.4: Effect of adding ferricyanide to the test solution containing lead (II) and copper (II) at different concentration ratio on bare SPCE.

Concentration ratio – lead: copper	% Signal change
1:1	- 8
1:5	- 17
1:10	- 29

3.3.5.3 Simultaneous measurement of lead (II) and cadmium (II)

The suitability of the bare SPCE for the simultaneous detection of lead and cadmium in mixed solutions containing both target metals was investigated at a deposition potential of – 1.0 V. Some stripping chronopotentiograms for the simultaneous detection of lead and cadmium in 0.1 M HCl + 0.5 M ammonium acetate are shown in Figure 3.45. The stripping responses were linearly dependent on concentration in the range from 0 to 600 μg l^{-1} (r^2 = 0.998) for lead (II) and from 200 to 600 μg l^{-1} (r^2 = 0.993) for cadmium (II). Sensitivities of 0.84 ± 0.01 ms l μg^{-1} and 0.53 ± 0.03 ms l μg^{-1} were obtained for lead and cadmium, respectively.

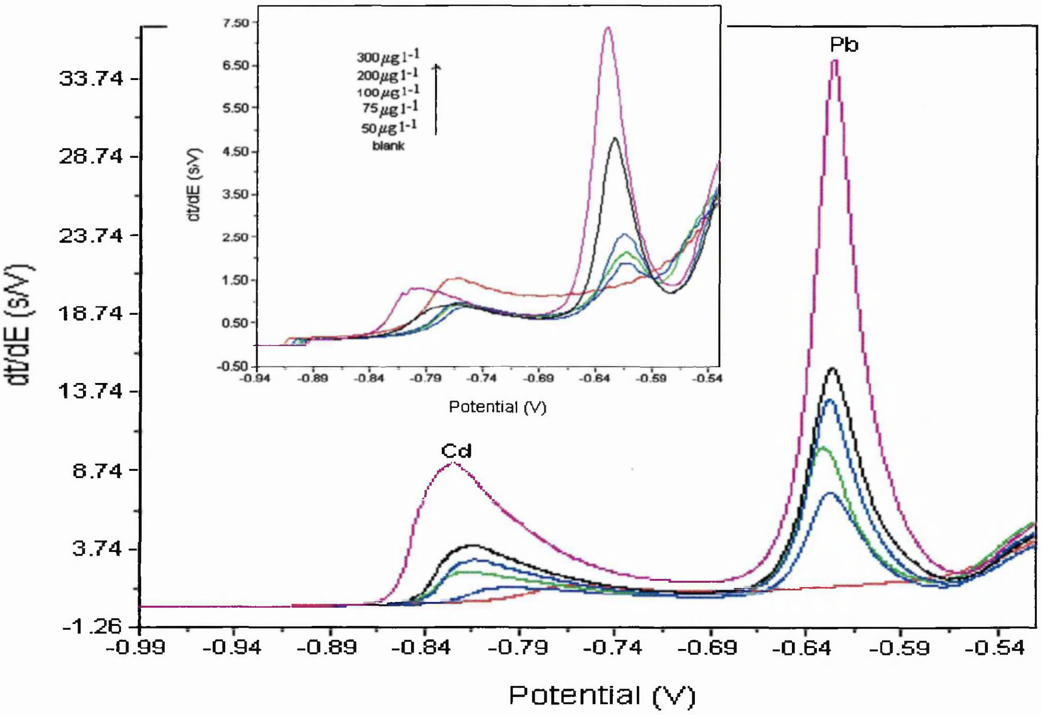


Figure 3.45: Overlaid stripping chronopotentiograms showing peaks obtained for 300, 400, 500, 600, 1000 μg l^{-1} lead (II) and cadmium (II). Base curve: Blank solution of 0.1 M HCl + 0.5 M ammonium acetate. (Inset: stripping responses at concentrations between 50 – 300 μg l^{-1}). Deposition potential = - 1.0 V, deposition time = 120 s, constant current = 1 μA.

3.3.5.4 Copper

The detection of copper (II) on bare-SPE was investigated using 0.1 M HCl as supporting electrolyte. Similar to result obtained on mercury film SPCE for copper using 0.1 M HCl as supporting electrolyte (see Section 3.3.3.1), the detection of copper using the same electrolyte was not possible with CCSCP coupled to the untreated bare SPCE. Hence the bare SPCE was pretreated in order to reduce the interfering peaks emanating from the electrode surface in the potential range (- 0.4 V to 0.1 V) in which copper is detected (Figure 3.46). By pretreating the bare SPCE at – 0.9 V for 120 s using 0.1 M HCl and then drying the electrode, stripping chronopotentiometric measurements of copper were able to be carried out (Figure 3.47).

To ascertain the effect interfering ions on the copper response, a fixed concentration of copper (200 μg l^{-1}) was spiked with various concentrations (200, 500 and 1000 μg l^{-1}) of bismuth, cadmium, lead and zinc. The copper peak was affected in the presence of bismuth, as its peak was not well resolved from the bismuth peak (copper and bismuth peaks were overlapping). The copper response was also reduced in the presence of lead probably due to the formation of intermetallic or solid solution between copper and lead on the bare SPCE surface. Cadmium (II) and zinc (II) had no effect on the copper (II) response due to copper being deposited at a potential (- 0.9 V) where not enough cadmium and zinc is deposited.

A calibration curve was obtained for copper (II) standards in 0.1 M HCl solution on bare SPE surface using CCSCP. The plot in Figure 3.48 shows this, with a linear copper (II) response between 0 – 1000 μg l^{-1} (sensitivity = 1.07 ± 0.04 ms l μg^{-1}, r^2 = 0.9886). A detection limit of 59 μg l^{-1} was estimated for copper in 0.1 M HCl following 120 s deposition. The stripping response was reproducible with ten repetitive measurements of 200 μg l^{-1} copper (120 s deposition) yielding a relative standard deviation (R.S.D) of 10.8 %.

3.3.5.5 Simultaneous measurement of lead (II) and copper (II)

The simultaneous detection of copper (II) and lead (II) was possible on bare-SPCE pretreated with 0.1 M HCl. Although the stripping peaks for copper (II) and lead (II) were well separated, there seems to be a peak forming between the two metal ions

(Figure 3.49, pointed arrow). This could be due to an inter-metallic compound forming between both metal ions. Both metal ions gave linear responses between 0 – 1000 μg l^{-1}.

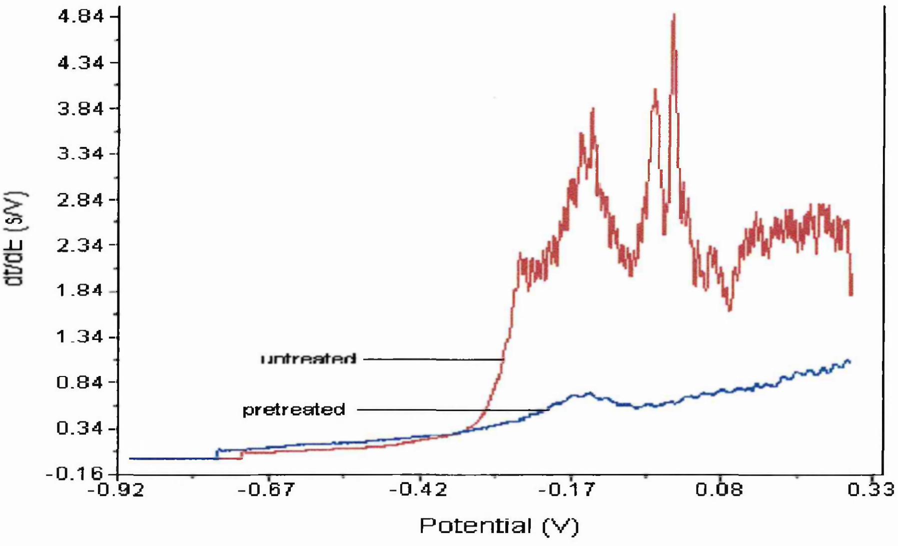


Figure 3.46: Stripping chronopotentiograms showing responses obtained for blank solution of 0.1 M HCl on bare SPCE. Deposition potential = - 0.9 V, deposition time = 120 s, constant current = 3 μA.

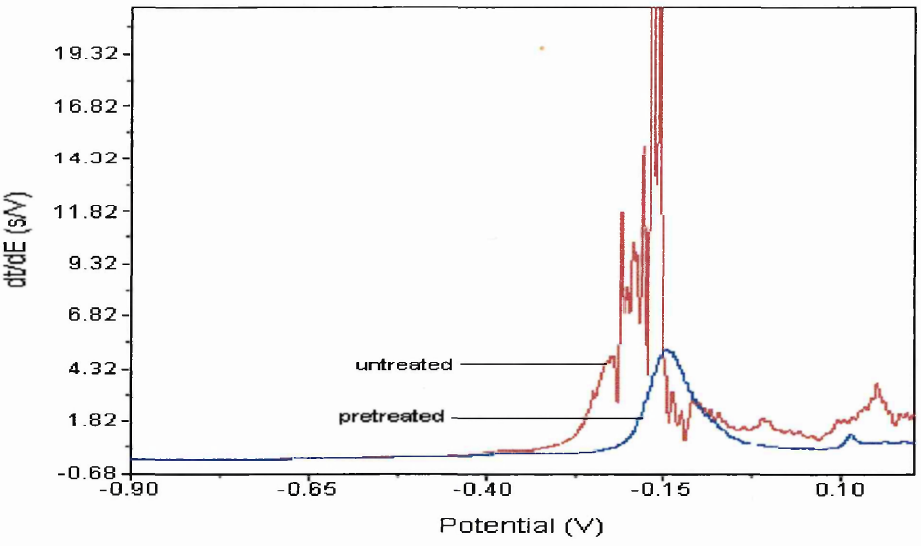


Figure 3.47: Stripping chronopotentiograms showing responses obtained for 250 μg l^{-1} copper (II) in 0.1 M HCl on bare SPCE. Deposition potential = - 0.9 V, deposition time = 120 s, constant current = 3 μA.

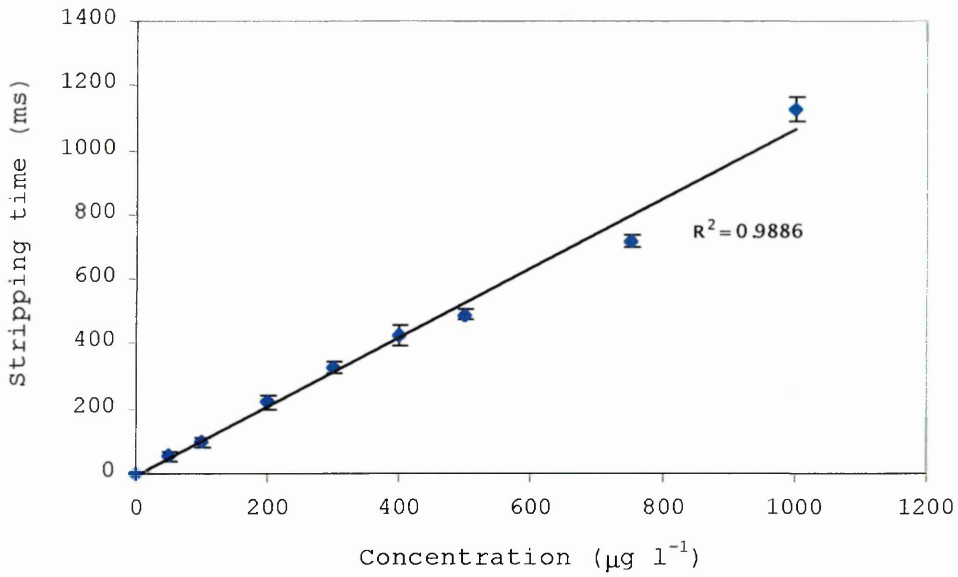


Figure 3.48: Calibration plot for increasing concentrations of copper (II) in 0.1 M HCl. Deposition potential = - 0.9 V, deposition time = 120 s, constant current = 3 μA.

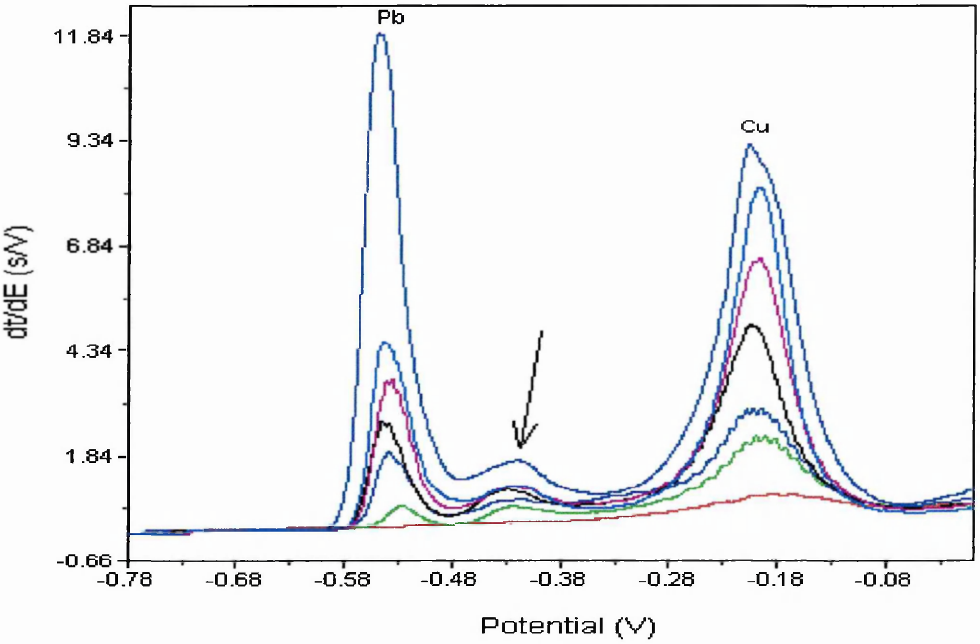


Figure 3.49: Overlaid stripping chronopotentiograms showing peaks obtained for 100, 200, 300, 400, 500, 1000 μg l^{-1} of lead (II) and copper (II). Blank solution (base curve): 0.1 M HCl. Deposition potential = - 0.9 V, deposition time = 120 s, constant current = 3 μA.

3.4 CONCLUSION

In this Chapter, results obtained for electrochemical stripping analysis of lead, cadmium and copper using the in-house fabricated electrodes has been presented. The fabricated three-electrode configuration system coupled with SWASV and CCSCP has provided a means of a relatively inexpensive on-site detector for trace levels of toxic metals. Detection of these metals was carried out on MFSPCE, BFSPCE and bare SPCE using the optimised procedures developed for measurements. The bare SPCE acted as supports for the *in situ* plating of bismuth and mercury films. With the optimised working conditions, the results obtained indicate that the screen-printed electrochemical sensors are sensitive and reproducible enough for the CCSCP and SWASV determination of lead, copper and cadmium in the parts per billion range.

Because the linear concentration range obtainable with SWASV is narrow, CCSCP was the preferred technique used due to the fact that it's reasonably broad linear range provided an opportunity to develop a screening device, which can be used over a broad concentration range for determining lead, copper and cadmium in samples. Another reason for CCSCP being the preferred technique was that the use of electrodes from different batches did not really affect the sensitivity of measurements carried out with CCSCP but did with SWASV measurements. Results and discussion on the batch-to-batch variation study for SPEs are documented in Appendix 1.1.

The preconcentration of lead, copper and cadmium metallic phase from aqueous solutions was favoured when co-depositing with either mercury or bismuth film onto the bare electrode surface. These approaches allowed the trace metal determination of lead, cadmium and copper (cannot detect copper with the bismuth film electrode), with limits of detection below 20 $\mu g\ l^{-1}$. The reproducibility of the measurements, which also contributed to interest in electrochemical system, was below 15 % for both BFSPCE and MFSPCE. Although the performance of the bismuth film SPCE used for CCSCP measurements measured up to those at MFSPCE, the only downside is the undesirable effect of copper. To eliminate the detrimental effect of copper (II), excess amount of ferricyanide was used to complex the copper (II).

The sensitivity of measurement on bare SPCE was lower compared to that on MFSPCE and BFSPCE, but that did not hinder the detection of lead (II), copper (II) and cadmium in test solutions with high concentrations. Detection limits were 35, 45 and 59 $\mu g\ l^{-1}$ for lead, cadmium and copper, respectively.

The difficulty in obtaining a stable and well-defined stripping chronopotentiometric peak for copper on the bare SPCE and MFSPCE was resolved by pretreating the electrode or using 2 M HCl as the supporting medium to suppress the background signal obscuring the copper stripping response.

The application of the BFSPCE, MFSPCE and bare SPCE to real samples is presented in Chapter 5. Also detailed discussion of the results generated in this work can be found in Chapter 6.

CHAPTER 4

• 4 DETECTION WITH MODIFIED SCREEN-PRINTED ELECTRODES

4.1 INTRODUCTION

Chemically modified electrodes (CMEs) are electrodes, which have been coated with reagents having desirable properties, so as to take on the properties of the used reagent (Arrigan, 1994). Basically, the modification of the surface or bulk matrix material of the electrode involves immobilisation or inclusion of reagents that change the electrochemical characteristics of the electrode. Such manipulation of the molecular composition of the electrode thus allows one to tailor the electrode response to meet specific sensing needs (Kutner *et al.*, 1998). Also these "mercury-free" surfaces address growing concerns associated with field applications of mercury-based electrodes.

Sensors based on modified electrodes hold great promise for the task of environmental monitoring. This is due to the benefit that can be attained through enhanced electron-transfer kinetics, permselective transport or preferential accumulation uptake (Wang and Golden, 1989; Wang 2000).

With the concern over the use of mercury-based electrodes for metal ion analysis, substantial research efforts are being devoted to the development of electrochemical sensors based on the judicious modification of the working electrode surfaces with chelating reagents. One such working electrode surface is that of SPCE. Modification of the SPCE surfaces is a very promising approach. The modification of the electrode surface can be exploited by incorporating the modifying reagents into the carbon ink prior to the printing step or drop coating the concentrated solution onto the transducer surface (Honeychurch and Hart, 2003).

Various interactions (covalent, ion exchange, etc.) between the surface modifier and metal ions have been exploited recently to obtain the better selectivity and sensitivity. Various compounds with chelation or ion-exchange properties have been attached to electrode surfaces and used to concentrate metal ions from diluted solutions.

Somasundrum and Bannister (1993) described an amperometric detection method for the determination of copper using disposable SPCEs modified with the ligand, bis-cyclohexane oxaldihydrazone. The bis-cyclohexane oxaldihydrazone was incorporated into the electrode matrix by mixing with the carbon paste, hydroxyethyl cellulose and ethylene glycol printing solution. The electrodes operated at + 0.25 V versus SCE (saturated calomel electrode) and gave a linear response for copper in the range 0.03 to 0.3 mM (1.9 – 19 mg l^{-1}).

Similarly, a strip-type, preconcentrating voltammetric sensors, prepared by incorporating a cation-exhange resin (Dowex 50W-X8) within screen-printed carbon inks was described by Neuhold *et al.* (1995). The efficient electrostatic preconcentration of copper from dilute solutions at open circuit was followed by voltammetric measurements in a separate blank solution. Lead (II) at the same concentration level (as copper) and at tenfold excess interfered by decreasing the magnitude of the stripping response. Silver (I) and mercury (II) at tenfold excess also interfered. A detection limit of 0.5 μg l^{-1} was estimated for a 10 minute preconcentration at open circuit. The Dowex 50W-X8 modified SPCEs was applied to determination of copper (II) in drinking water.

Wang *et al.* (1996a) doped screen-printing carbon ink with dimethylglyoxime (DMG) and used the resulting disposable complexing (preconcentrating) sensor strip for detecting nickel. The response was characterised with respect to the ink composition, preconcentration time and potential, solution conditions and other variables. For a deposition time of 30 s, a detection limit of 5 μg l^{-1} nickel was obtained.

SPCEs modified with Sumichelate Q10R, a chelating resin containing dithiocarbamate groups, were successfully applied to the determination of mercury (II) by Ugo *et al.* (1998). The chelating resin was incorporated into the electrode matrix by mixing with the screen-printing carbon ink. Trace determination of mercury (II) was obtained by adopting a complexation-anodic stripping procedure with detection via DPV. A detection limit of 0.002 μg l^{-1} was estimated for a 30-minute preconcentration at open circuit and a 5- minute preconcentration at – 0.5 V (close circuit). The modified SPCEs were applied to analysis of water samples from the channels of Venice (Italy).

Dithizone (diphenylthiocarbazone) and Nafion were used by Palchetti *et al.* (2000) to obtain modified screen-printed strips for lead determination. Dithizone was mixed with graphite ink and screen-printed on a plastic substrate. The detection limit for lead (II) using this dithizone modified electrode was 12 μg l^{-1} with SWASV. The Nafion modified electrodes were made by casting 2 μl Nafion® solution onto the working electrode surface of screen-printed electrodes and allowing the evaporation of solvent. Lead was detected with a detection limit of 15 μg l^{-1} using PSA.

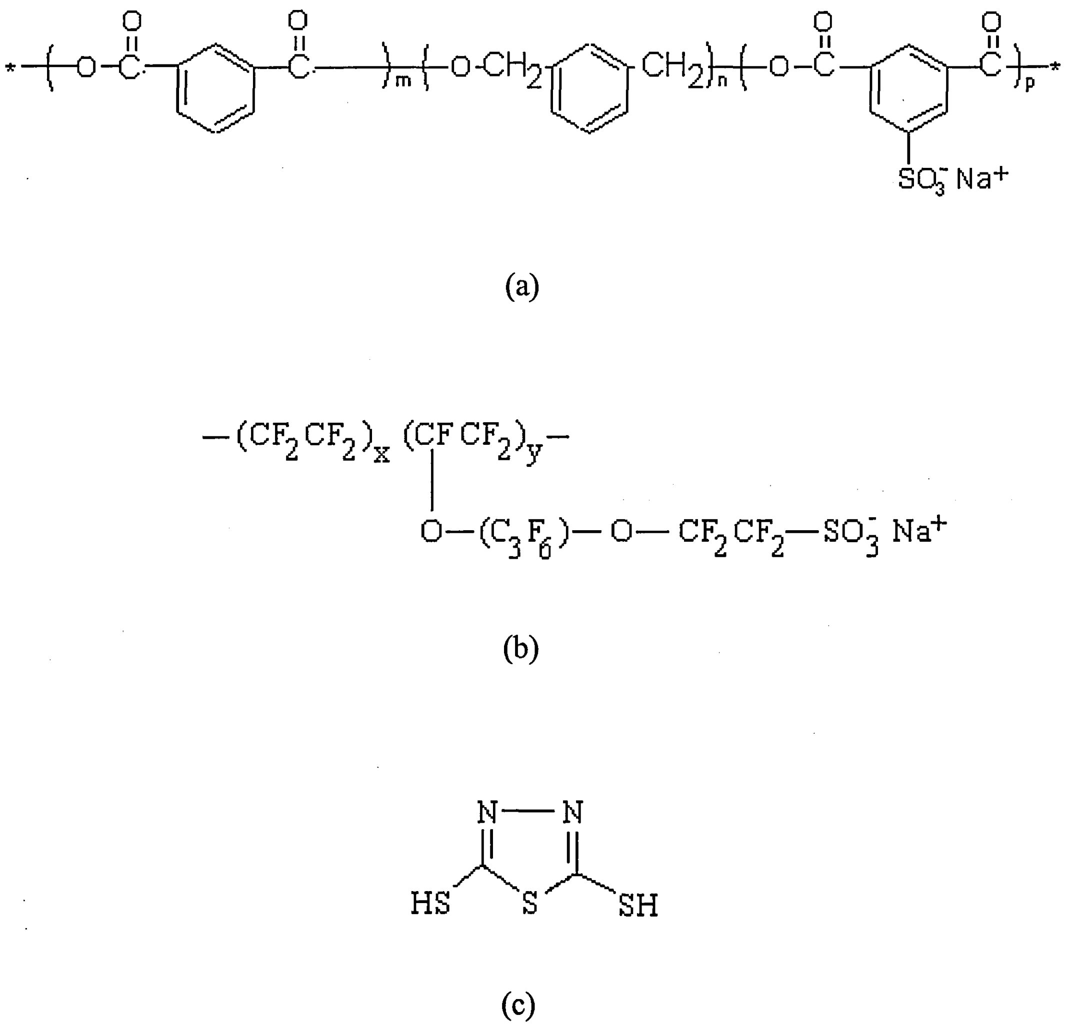
Honeychurch *et al.*, (2001a; 2001b; 2002b) have described a number of modified SPCEs with chelating agents for the trace determination of lead (II) and cadmium (II). A disposable 1-(2-pyridylazo)-2-naphthol (PAN) modified SPCE (PAN-SPCE) prepared by drop coating technique was used for lead (II) detection (Honeychurch *et al.*, 2001a). Also SPCEs modified by drop coating with two different calixarenes; a novel thiocalix[4]arene (5,11,17,23, tetra-tert-butyl-25,26,27,28-tetrakis(2-mercaptoethoxy)-calix[4]arene) and a commercially available 25,26,27,28-tetrahydroxy-calix[4]arene were used for lead (II) determination (Honeychurch *et al.*, 2001b). Lead ions were preconcentrated from ammonia buffer on the modified SPCE surface by the formation of a chemical complex at open circuit. The resulting SPCE with the complexed lead was then transferred to 0.1 M HCl and subjected to DPASV measurements at close circuit. A detection limit of 15 μg l^{-1} was obtained for lead (II) on PAN-SPCE for 5 minute preconcentration time. The principal metal interference was copper, but this problem was successfully overcome by the addition of iodide to the ammonia buffer prior to the accumulation step. For the SPCE modified with thiolated calixarene (which was found superior in terms of sensitivity compared to the 25,26,27,28-tetrahydroxy-calix[4]arene modified SPCE), a detection limit of 5 μg l^{-1} was obtained following a preconcentration time of 10 minutes for lead (II). The methods were evaluated by carrying out lead determinations on spiked potable and river water samples.

The same calixarene modified SPCEs were also applied to the determination of cadmium (II) using a 15 minute preconcentation time at open circuit, followed by DPASV measurement (Honeychurch *et al.*, 2002b). A detection limit of 2.8 μg l^{-1}

was obtained. The method was evaluated by carrying out cadmium determinations on spiked river water samples.

In this work, modified electrodes obtained by attachment of a modifying agent to the surface of the bare SPCE and by introduction of modifying agent into the graphite ink matrix and then screen-printing are studied for metal ions detection. The investigated modifying reagents include (i) Polyester sulphonic acid (PESA), (ii) Nafion®, (iii) 2,5- Dimercapto-1,3,4- thiadiazole (DMTD), (iv) Bismuth oxide (Bi_2O_3), and (v) Polyethylenimine (PEI). The structures of PESA, Nafion, DMTD and PEI are given in Figure 4.1.

PESA and Nafion® are ion-exchange polymers which allow the quick preconcentration and simultaneous electrochemical detection of ion redox analytes (Ugo and Moretto, 1995) as well as discrimination against potential interferents (Brett and Fungaro, 2000a). DMTD is a chelating agent containing sulphur donor atoms, which permits good adsorption on solid phase (electrode) as well as strong metal complexation (Li *et al.*, 1988). PEI is a water-soluble complexing polymeric agent that can efficiently concentrate metal ions. It is also known to reduce the intermetallic interactions of metal ion components at the stage of preconcentration at the working electrode surface (Osipova *et al.*, 1997; 1998; 2000; Sladkov and Osipova, 2001).



(a)

$-(CF_2CF_2)_x(CFCF_2)_y-$ with $O-(C_3F_6)-O-CF_2CF_2-SO_3^- Na^+$

(b)

(c)

$(\text{-NHCH}_2\text{CH}_2)_x\ [\text{-N(CH}_2\text{CH}_2\text{CH}_2\text{)CH}_2\text{CH}_2\text{-}]_y$

(d)

Figure 4.1: Structure of (a) Polyester sulphonic acid (PESA), (b) Nafion, (c) 2,5-Dimercapto-1,3,4- thiadiazole (DMTD) and (d) Polyethylenimine (PEI).

4.2 EXPERIMENTAL

4.2.1 Apparatus and reagents

A computer-controlled Autolab PGSTAT10 Electrochemical Analyser with general-purpose electrochemical software operating system GPES version 4.8 (Eco Chemie B.V., Utrecht, The Netherlands) was used for cyclic voltammetric and constant current stripping chronopotentiometric measurements. The in-house fabricated screen-printed electrodes (see Section 2.2.4 in Chapter 2) were used for the electrochemical measurements with connection made to the electrochemical analyser using a specially adapted electrical edge connector from Maplin (Milton Keynes, U.K).

All chemicals were of analytical reagent grade and used as received. Hydrochloric acid (HCl), potassium chloride (KCl), acetic acid (glacial, 99 %), Tris-HCl, ammonia and ammonium chloride (NH_4Cl) were obtained from BDH Ltd (Poole, England) while ammonium acetate was obtained from Merck (Darmstadt, Germany). Bismuth (III) oxide (Bi_2O_3, 99.9 %) was obtained from Acros Organics (Geel, Belgium). The Eastman AQ 29D polymer (polyester sulphonic acid, 30 % dispersion) was supplied by Eastman Company Ltd (U.K). Potassium hexacyanoferrate (III) (also known as potassium ferricyanide), dimercapto-1,3,4-thiadiazole (DMTD), Nafion® (perfluorinated ion-exchange 5 wt. % in lower aliphatic alcohols + water), Polyethylenimine (PEI, 50 % (w/v) aqueous solution) and standard stock solutions (1000 mg l^{-1} in 1 wt. % HNO_3) of cadmium (II), lead (II) and copper (II) were obtained from Aldrich (Gillingham, Dorset, U.K).

4.2.2 Risk assessments and codes of practice

Experiments were performed in compliance with Departmental codes of practice and C.O.S.H.H regulations; waste was disposed of by approved routes.

4.2.3 Electrode preparation

4.2.3.1 Polyester sulphonic acid (PESA) and Nafion modified electrodes

The PESA and Nafion modified electrodes were prepared by drop-coating 2 µl of the polymer (Nafion and PESA) solutions onto the SPCE electrode surface with a microliter pipette. These polymer solutions used were prepared by diluting the stock Nafion® solution (5 wt. % in ethanol + water) and PESA AQ 29D (30 %) dispersion solution with ethanol. The solvent was allowed to evaporate at room temperature.

4.2.3.2 Preparation of the Dimercapto-1,3,4-thiadiazole (DMTD) modified electrodes

DMTD modified electrodes were prepared by dip-coating bare SPCEs (fabrication procedure described in Chapter 2, Section 2.2.4) in a solution of DMTD (1 x 10^{-3} M) for 10 minutes and then drying in air.

Another type of DMTD modified electrode (screen-printed DMTD modified electrode) was fabricated by mixing 9.5 g of graphite carbon ink (E423-SS graphite-based ink from Acheson Colloids, U.K) with 0.5 g of DMTD in a mortar and the mixture, homogenized. The DMTD and graphite carbon ink mixture was then screen-printed onto the working electrode surface using the same fabrication procedure used in Chapter 2 (Section 2.2.4).

4.2.3.3 Preparation of the Nafion-DMTD modified electrodes

SPCEs modified with Nafion and DMTD were prepared by drop-coating 2 µl of the mixed ethanol solutions of 0.5 % Nafion (diluted from a 5 wt. % Nafion® solution in alcohols and water) and 5 x 10^{-4} M DMTD on the SPCE electrode surface with a microliter pipette. The solvent was allowed to evaporate at room temperature.

4.2.3.4 Preparation of the Screen-printed Bi_2O_3 modified electrodes

SPCEs modified with Bi_2O_3 were fabricated by direct mixing of Bi_2O_3 and the graphite carbon ink in a mortar followed by intimate homogenization. The mixture of the Bi_2O_3 and graphite carbon ink was then screen-printed. Screen-printed Bi_2O_3 modified electrodes with Bi_2O_3 contents of 0.5, 2 and 7 % were printed on the working electrode surface following the fabrication procedure described in Chapter 2 (Section 2.2.4).

4.2.3.5 Preparation of the Screen-printed Polyethylenimine (PEI) modified electrodes

SPCEs modified with 0.5 % PEI were fabricated by mixing 0.05 g of PEI into 9.95 g of graphite carbon ink and then screen-printing the mixture onto the working electrode surface using the same fabrication procedure described in Chapter 2 (Section 2.2.4).

4.2.4 Procedure

Electrochemical studies were carried out by placing a 100 µl sample drop on the three-electrode strip. Each measurement was carried out in triplicates with a new modified SPE and unmodified SPE strip in non-dearated and unstirred solution. Metal standard solutions were prepared by diluting the standard stock solution (1000 $mg \cdot l^{-1}$) with deionised water and supporting electrolyte. All experimental runs carried out in this work, was repeated three to five times with different electrodes. Each response data point represents the mean value of three measurements and the error bar represents the standard deviation. The mean, standard deviation and relative standard deviation (R.S.D) were calculated for the measurements using Microsoft Excel 2000 and SigmaStat Statistical Software Version 2.03.

4.2.4.1 Cyclic voltammetry (CV) measurements

Cyclic voltammograms were recorded over the range of potentials between – 1.0 V and + 1.0 V (unless otherwise stated) at a scan rate of 50 mV s^{-1}.

4.2.4.2 Constant current stripping chronopotentiometric (CCSCP) measurements

The modified SPCEs were used without further pre-treatment apart from the PEI modified SPCEs (and bare SPCEs used for measurement of copper) which were electrochemically cleaned in 0.1 M HCl by holding at – 0.9 V (vs. screen-printed Ag/AgCl reference electrode) for 120 s and then a positive going voltage scan applied from – 0.9 to 0.3 V. The 0.1 M HCl solution was removed from the electrode surface and the electrode allowed to dry.

CCSCP measurements with the modified SPCEs and bare SPCEs (unmodified SPCE) were carried out at closed circuit by applying a sufficiently negative potential (vs. screen-printed Ag/AgCl reference electrode) to preconcentrate for a time period.

After the accumulation period, a constant current was applied to strip the accumulated analytes until a limit of 0 V (unless otherwise stated). The background signal for the supporting electrolyte was also measured in the same manner.

4.3 RESULTS

4.3.1 Characterisation of lead (II) on Nafion and PESA modified electrodes

Initially, Nafion and PESA were investigated as electrode modifiers for enhancing the preconcentration of lead by means of ion exchange from aqueous solutions onto the electrode surface. Figure 4.2 compares the stripping chronopotentiograms of 300 μg l^{-1} lead (II) obtained at the modified SPCEs and the bare SPCE. The stripping chronopotentiometric response obtained with the Nafion modified SPCE clearly shows the ability of the Nafion in accumulating lead (II) from the bulk solution compared to the bare SPCE (unmodified SPCE). Improvement on the Nafion modified electrode reflects the ion-exchange property of Nafion. The sensitivity of lead (II) response at the PESA modified electrode was lower than those for the bare SPCE and Nafion modified SPCE. The thickness of the film might have caused this, creating a diffusion barrier, which affects the uptake of lead (II) from the test solution. Further reduction of the PESA content in the PESA modified SPCE was examined but there was no improvement on the lead stripping response that can be obtained with unmodified bare SPCE.

With the magnitude of lead response obtained on the PESA modified electrode not showing any improvement on the one generated at the bare SPCE (unmodified SPCE), further work with the electrode was discontinued. Further investigation was carried out with Nafion modified SPCE. Figure 4.3 shows the effect of different amounts of Nafion solution drop-coated on the SPCE for lead (II) detection. From the stripping chronopotentiograms obtained, a Nafion solution coating of 0.5 % was found to be the appropriate amount to drop-coat on the SPCE.

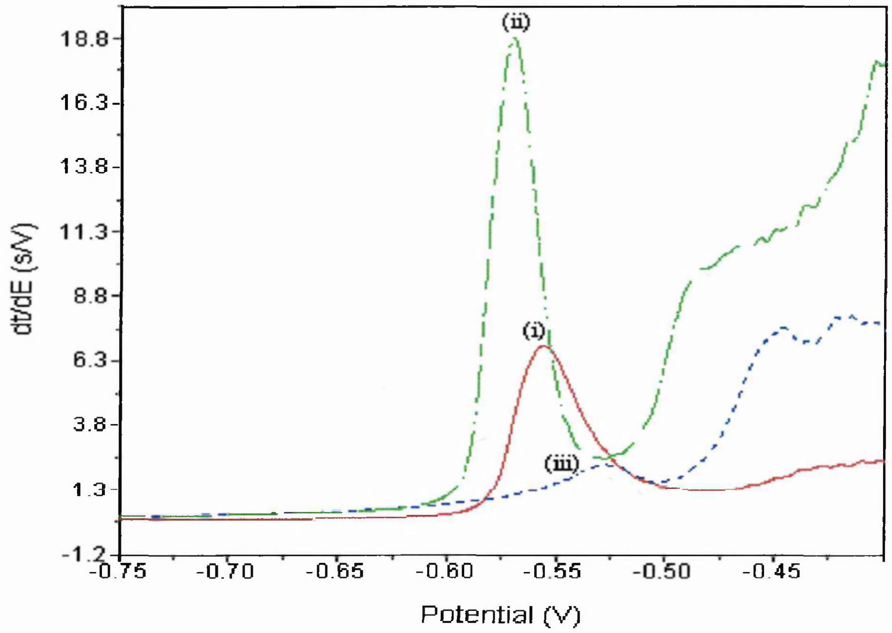


Figure 4.2: Stripping chronopotentiograms for 300 μg l^{-1} lead in 0.1 M HCl at (i) bare SPCE, (ii) 1 % Nafion modified SPCE and (iii) 0.05 % PESA modified SPCE. Deposition potential = - 0.9 V, deposition time = 120 s, constant current = 1 μA.

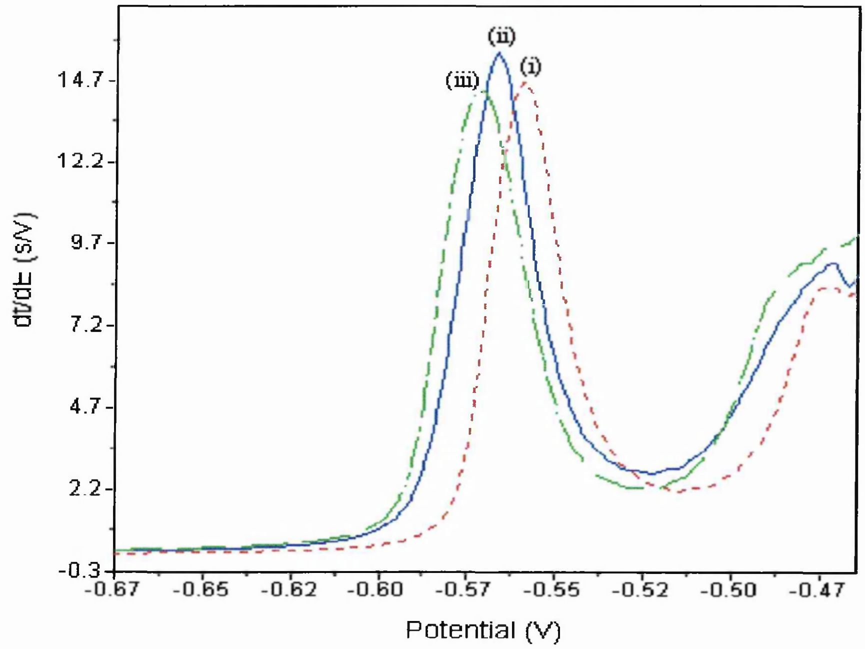


Figure 4.3: Stripping chronopotentiograms for 300 μg l^{-1} lead (II) at SPCE modified with different amounts of Nafion solution: (i) 0.1 (ii) 0.5 and (iii) 1 %. Deposition potential = - 0.9 V, deposition time = 120 s, constant current = 1 μA.

In order to increase the selectivity and sensitivity towards lead (II), a complexing agent was incorporated within the Nafion matrix. Mixing Nafion with a complexing agent brings about a specific interaction with solution species, thus achieving preconcentration by ion exchange and complexation. A 0.5 % Nafion mixed with 5 x 10^{-4} M DMDT was investigated. Contrary to expectations, there was not much of a difference (peak area or stripping time) between the stripping responses of lead (II) on the Nafion modified SPCE and the Nafion-DMDT modified SPCE (Figure 4.4). It could be that the DMDT incorporated into the Nafion film dissolves rapidly from the electrode surface in spite of the retaining Nafion film. Further studies were carried out with the Nafion modified SPCE.

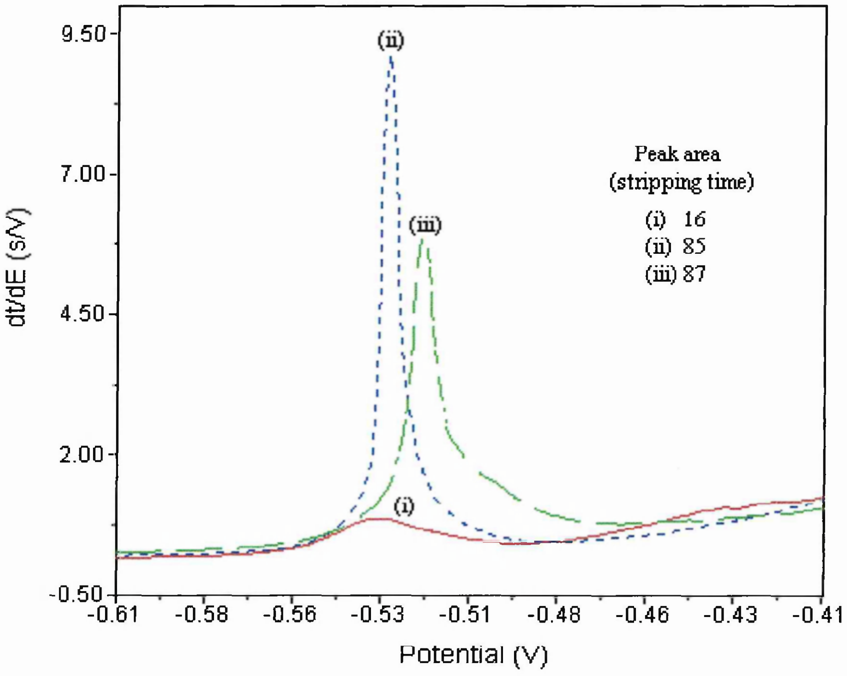


Figure 4.4: Stripping chronopotentiograms for 50 μg l^{-1} lead (II) at (i) bare SPCE, (ii) Nafion modified SPCE and (iii) Nafion-DMTD modified SPCE. Deposition potential = - 0.9 V, deposition time = 180 s, constant current = 1 μA.

4.3.1.1 Effect of deposition time

As common for stripping analysis, the time afforded to the accumulation to take place has a significant influence on the stripping response. Figure 4.5 exhibits the dependence of the stripping response on the deposition time for 100 μg l^{-1} lead (II) on

Nafion modified SPCE. As can be observed, the stripping response increased rapidly with deposition time up to 180 s before it started to level out. Hence a deposition time of 120 or 180 s was used for subsequent experiments.

4.3.1.2 Calibration curve

A linear calibration curve was obtained for lead (II) on the Nafion modified SPCE (Figure 4.6). The plot obtained using a deposition potential of – 0.9 V and a deposition time of 180 s was linear in the range examined (0 – 1000 μg l^{-1}, r^2 = 0.9984) with sensitivity of 1.40 ± 0.02 ms l μg^{-1}. A detection limit of 18 μg l^{-1} was estimated following 180 s deposition. For a deposition time of 120 s, a detection limit of 22 μg l^{-1} was obtained. The stripping chronopotentiometric response was reproducible with ten repetitive measurements of 50 μg l^{-1} lead (120 s deposition) yielding a relative standard deviation (R.S.D) of 5.6 %.

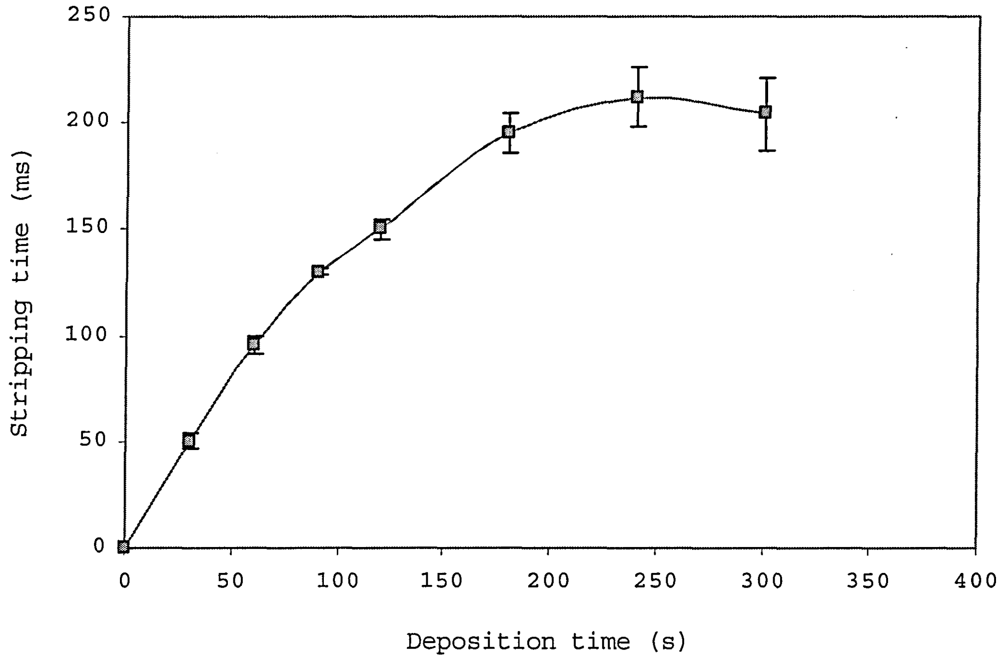


Figure 4.5: Effect of deposition time on the stripping response of 100 μg l^{-1} lead (II) in 0.1 M HCl obtained at Nafion modified SPCE. Deposition potential = - 0.9 V, constant current = 1 μA.

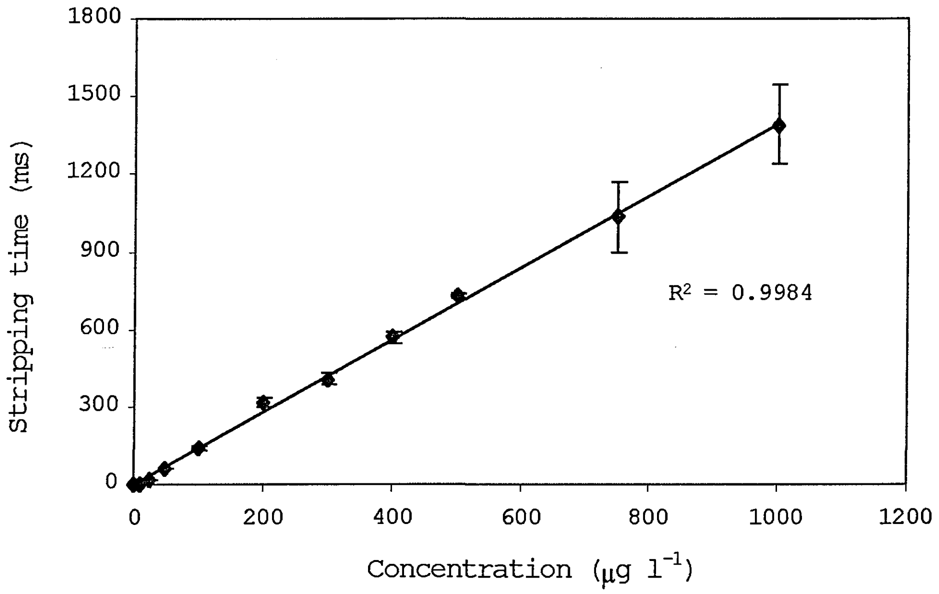


Figure 4.6: Calibration curve for increasing concentrations of lead (II) in 0.1 M HCl at Nafion modified SPCE. Deposition potential = - 0.9 V, deposition time = 180 s, constant current = 1 μA.

4.3.2 Characterisation of lead (II) on DMTD modified electrodes

Initially, DMTD was examined as a complexing agent for accumulating lead (II) onto the electrode surface by dip-coating SPCEs with 1 x 10^{-3} M DMTD. Figure 4.7 shows the stripping chronopotentiogram obtained for 100 μg l^{-1} lead (II). The lead oxidation peak shifts from – 0.54 V on the unmodified bare SPCE to ~ – 0.50 V on the screen-printed DMTD modified electrode. The inclusion of DMTD improved the sensitivity of lead detection at SPCE on the basis of lead interaction with the functional group on DMTD.

After the initial investigations, further studies moved on to modifying the SPCE surface by incorporating DMTD (5 %) into the graphite ink matrix and then screen-printing. Cyclic voltammetry (CV) and stripping chronopotentiometry were used for characterising the screen-printed DMTD modified electrodes.

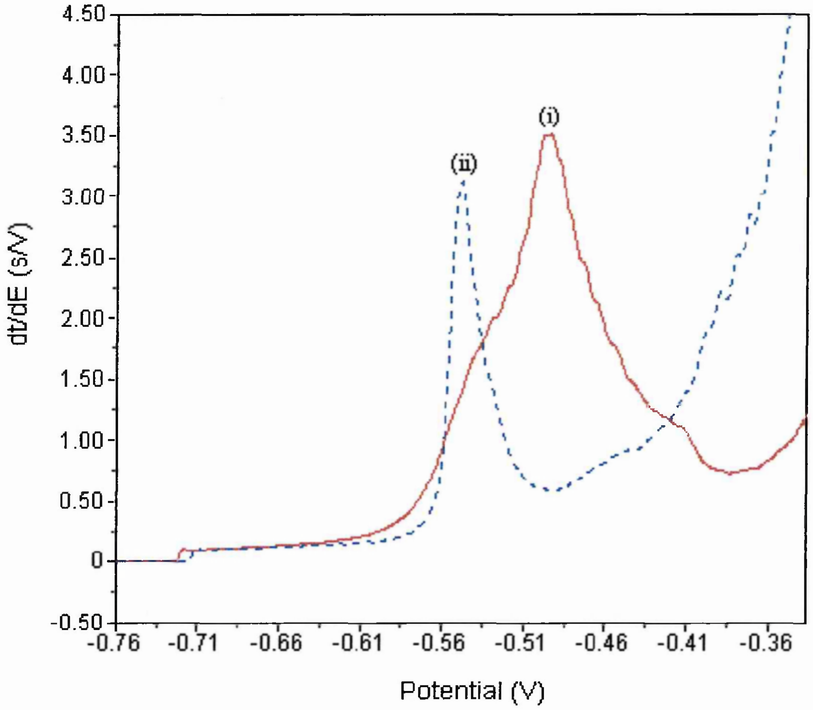


Figure 4.7: Stripping chronopotentiograms for 100 μg l^{-1} lead (II) at (i) unmodified bare SPCE and (ii) DMTD modified SPCE (SPCE dip-coated in 1 x 10^{-3} M DMTD solution for 10 min). Deposition potential = - 0.9 V, deposition time = 120 s, constant current = 1 μA.

4.3.2.1 CV studies

Cyclic voltammetry was applied to determine the electrochemical behaviour of lead (II) at screen-printed DMTD modified electrode. Cyclic voltammograms were recorded over the range of potentials between – 1.0 V and + 1.0 V at a scan rate of 50 mV s^{-1}. Figure 4.8 presents cyclic voltammograms obtained with screen-printed DMTD modified electrode and unmodified bare SPCE in 0.1 M HCl. The response obtained with the screen-printed DMTD modified electrode shows that the DMTD compound incorporated into the graphite-carbon ink (used for screen-printing the electrodes) is an electro-active reagent (pointed arrows) but there was no peak observed in the potential range in which the oxidation peak of lead appears.

When the accumulation process was carried out for 30 s at – 1.0 V in a solution containing 4 mg l^{-1} lead (II), at the unmodified bare SPCE, the lead (II) oxidation peak appeared at ~ – 0.56 V while the screen-printed DMTD modified electrode exhibited an anodic peak at ~ – 0.52 V (Figure 4.9). A broad lead peak was obtained for the screen-printed DMTD modified electrode in comparison to the narrow peak obtained with the unmodified bare SPCE. Using the peak area (Coulomb) under the voltammetric peak for quantification instead of the peak height (Ampere), the peak area response for 4 mg l^{-1} lead (II) was found to be higher using the screen-printed DMTD modified electrode (peak area = 1.15 ± 0.07 x 10^{-5} C) compared to the bare electrode (peak area = 0.5 ± 0.03 x 10^{-5} C).

The effects of other supporting electrolyte such as 0.1 M ammonium chloride (NH_4Cl), 0.5 M ammonium acetate + 0.1 M HCl, 0.1 M acetic acid + 0.1 M KCl and 0.1 M Tris-HCl + 0.1 M KCl, on the voltammetric response (peak area) were studied. Table 4.1 presents the results obtained for the cyclic voltammetric measurement. The results show that lead (II) has its best voltammetric response using 0.1 M HCl.

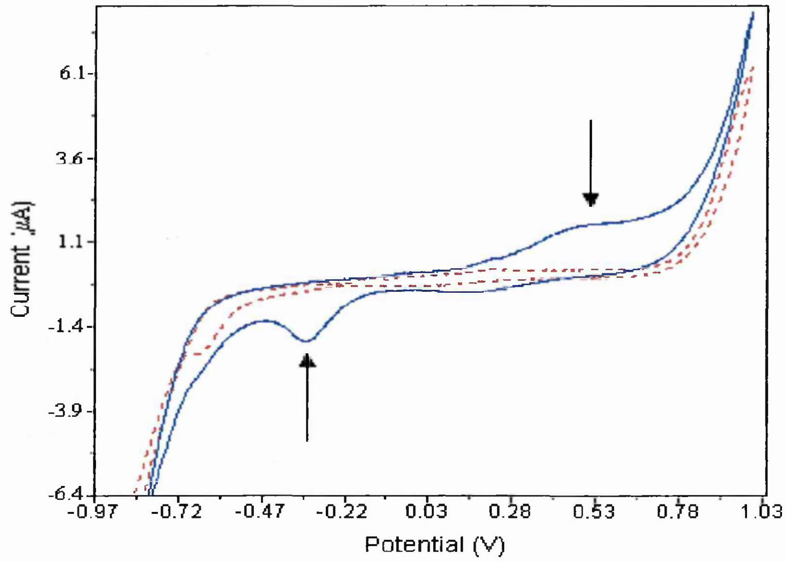


Figure 4.8: Cyclic voltammograms for 0.1 M HCl at bare SPCE (broken line) and screen-printed DMTD modified electrode (solid line). Potential interval = - 1.0 V to 1.0 V (vs. screen-printed Ag/AgCl reference Electrode), deposition time = 30 s, scan rate = 50 mV s^{-1}.

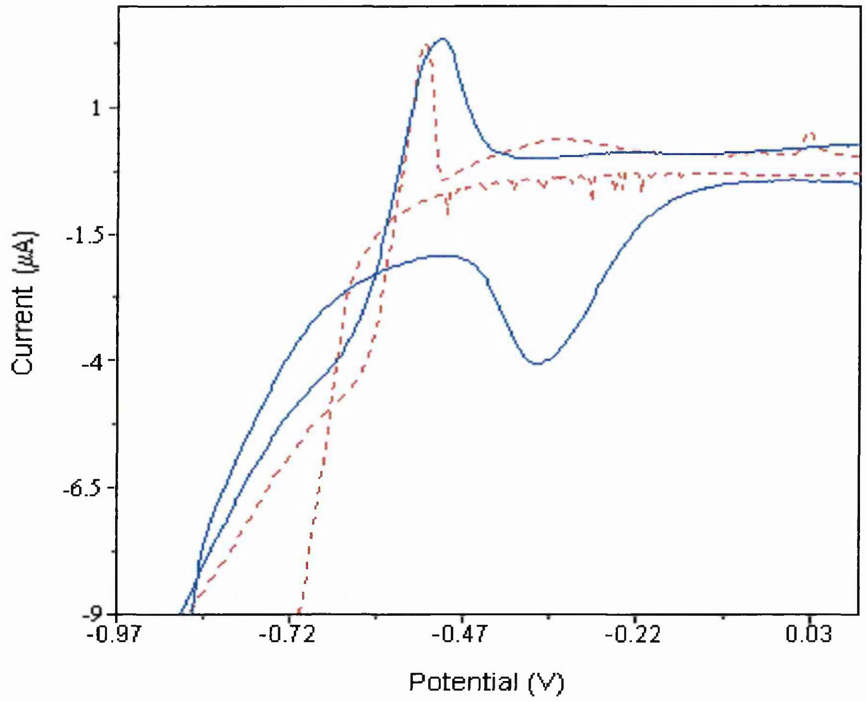


Figure 4.9: Cyclic voltammograms for 4 mg l^{-1} lead in 0.1 M HCl at bare SPCE (broken line) and screen-printed DMTD modified electrode (solid line). Potential interval = - 1.0 V to 0.1 V (vs. screen-printed Ag/AgCl reference electrode), deposition time = 30 s, scan rate = 50 mV s^{-1}.

Table 4.1: Effect of supporting electrolyte on the cyclic voltammetric response of 4 mg l^{-1} lead (II) at screen-printed DMTD modified electrode using conditions shown in Figure 4.9.

Supporting electrolyte	Peak area (C)
0.1 M HCl (pH 1.3)	$1.15 \pm 0.07 \times 10^{-5}$
0.1 M acetic acid + 0.1 M KCl (pH 2.9)	$1.10 \pm 0.06 \times 10^{-5}$
0.1 M NH_4Cl (pH 5.0)	$0.98 \pm 0.06 \times 10^{-5}$
0.5 M ammonium acetate + 0.1 M HCl (pH 5.0)	$0.65 \pm 0.04 \times 10^{-5}$
0.1 M Tris-HCl + 0.1 M KCl (pH 7.5)	No response

mean for 3 measurements

4.3.2.2 CCSCP studies

After the CV studies, SCP was applied to lead (II) detection on the DMTD modified electrode. Factors affecting the preconcentration of lead (II) ions onto the electrode surface, which in turn affect the stripping chronpotentiometric responses in the detection step, i.e. deposition time, presence of co-existing metal ions and metal ion concentration, were studied. The spontaneous accumulation of lead onto the screen-printed DMTD modified electrode is indicated from the plot in Figure 4.10, following different deposition times, 0 to 360 s. The stripping chronopotentiometric response increased rapidly with the metal concentration up to 180 s for 500 μg l^{-1} lead (II) and 240 s for 200 μg l^{-1} lead (II) and then levelled off. These profiles, which reflect the kinetics of the lead uptake, indicate a very rapid accumulation process. Thus for practical reasons, a short accumulation time of 120 s was chosen, as it is sufficient for realising the preconcentration advantage.

The influence of copper (II) ions, commonly found in analytical samples on the accumulation and anodic stripping of lead (II) was studied. Copper was found to have adverse effect on lead response obtained on the screen-printed DMTD modified electrode. Table 4.2 shows the effect of copper (II) on the 200 μg l^{-1} lead (II) stripping response at different lead (II): copper (II) concentration ratios. To reduce the effect of copper, ferricyanide was added to the test solution. Figure 4.11 shows the outcome of adding ferricyanide to mask the effect of copper. As can be seen from the results, the copper interference on the stripping chronopotentiometric response of lead is suppressed when 0.1 mM ferricyanide is added to the test solution.

Figures 4.12 and 4.13 depict the stripping chronopotentiograms and calibration plot for increasing concentration of lead (II) in 0.1 M HCl. The calibration plot of stripping time versus the concentration, gave a linear response in the range examined (0 – 1000 μg l^{-1}, $r^2 = 0.9945$) with sensitivity of 1.84 ± 0.05 ms 1 μg^{-1}. A detection limits of 20 μg l^{-1} was estimated following 120 deposition. The stripping response was reproducible with ten repetitive measurements of 200 μg l^{-1} lead (II) (120 s deposition) yielding a relative standard deviation (R.S.D) of 10.6 %.

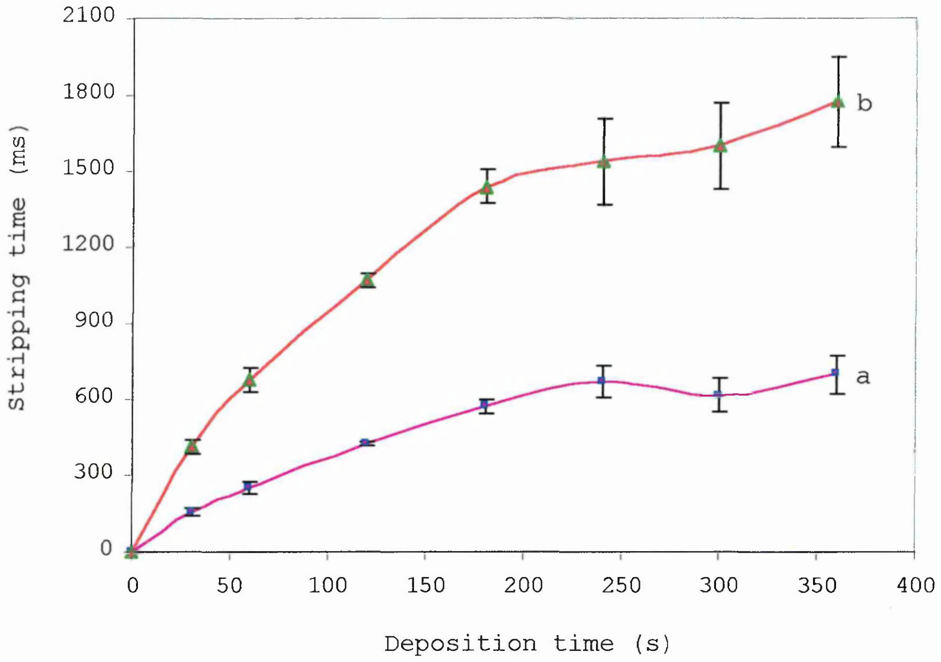


Figure 4.10: Dependence of the stripping chronopotentiometric response of lead accumulated at 5 % (w/w) DMTD screen-printed electrode on deposition time (a = 200 μg l^{-1}, b = 500 μg l^{-1}). Deposition potential = - 0.9 V, constant current = 1 μA.

Table 4.2: Effect of copper on lead response at different concentration ratios on screen-printed DMTD modified electrode.

Concentration ratio – lead : copper	% Signal change
1:1	- 55
1:2.5	- 64
1:10	- 83

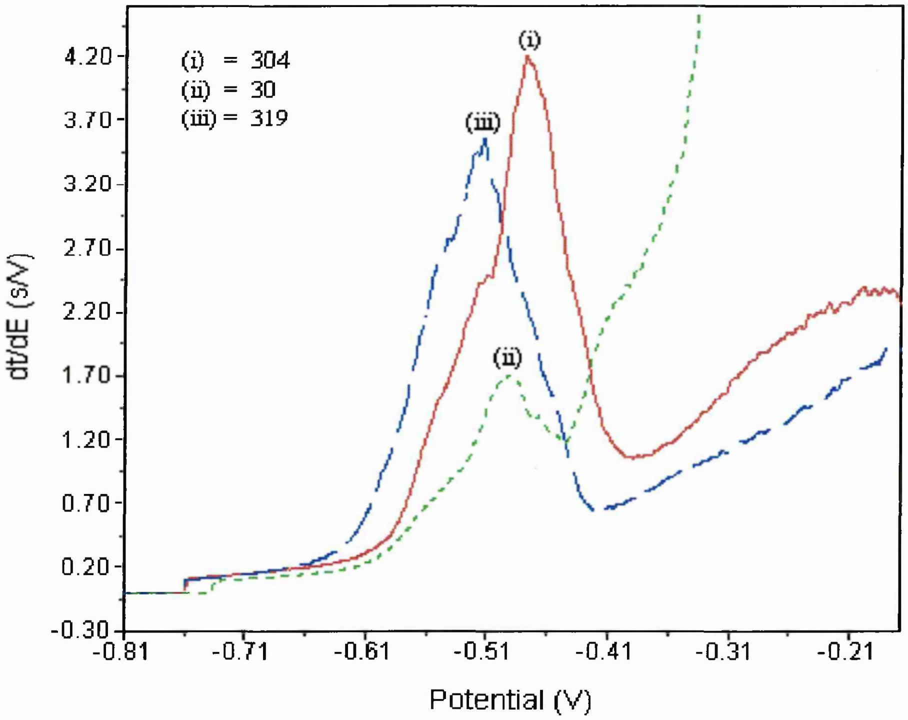


Figure 4.11: Masking of the copper interference on the stripping response of lead (II) at the screen-printed DMTD modified electrode. (i) 200 μg l^{-1} lead (II) + 0 μg l^{-1} copper (II), (ii) 200 μg l^{-1} lead (II) + 2000 μg l^{-1} copper (II) and (iii) 200 μg l^{-1} lead + 2000 μg l^{-1} copper (II) + 0.1 mM ferricyanide. Deposition potential = - 0.9 V, deposition time = 120 s, constant current = 1 μA.

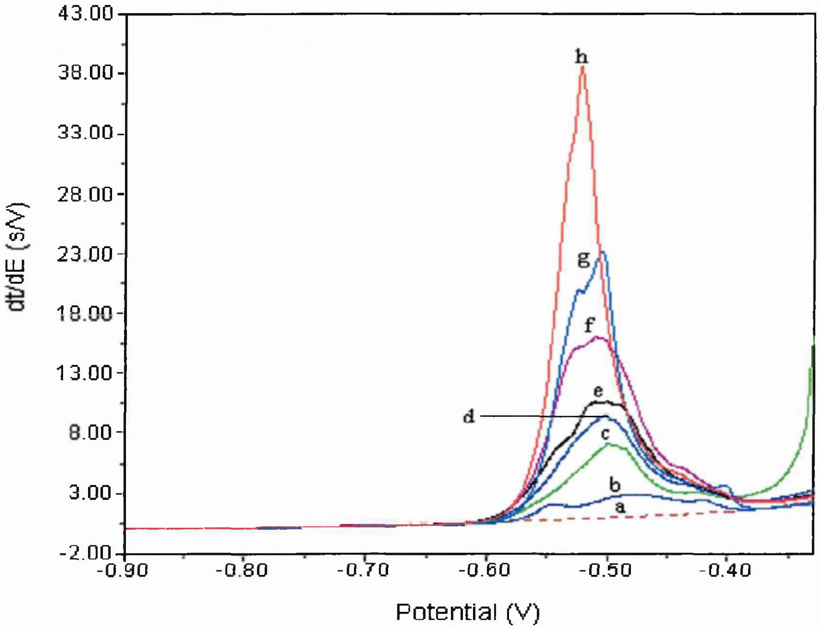


Figure 4.12: Stripping chronopotentiograms for increasing concentrations (a: blank, b: 100, c: 200, d: 300, e: 400, f: 500, g: 750 and h: 1000 μg l^{-1}) of lead (II) in 0.1 M HCl at screen-printed DMTD modified electrode. Deposition potential = - 0.9 V, deposition time = 120 s, constant current = 1 μA.

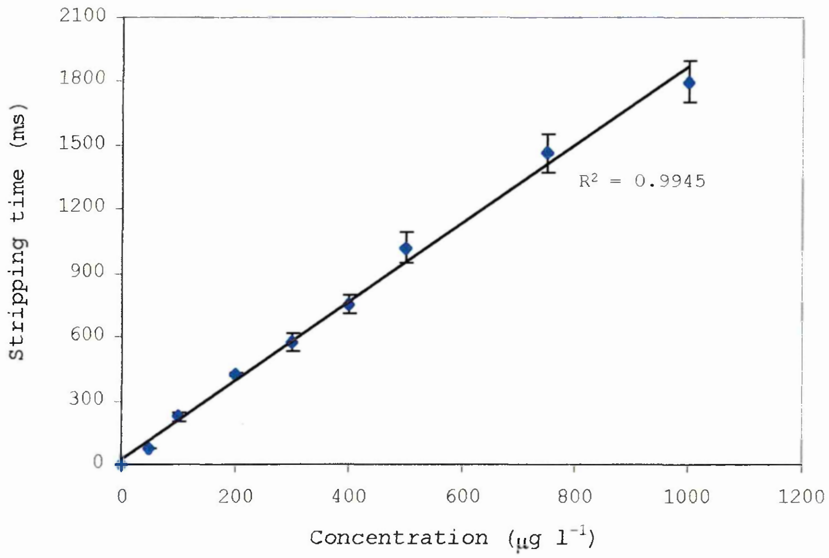


Figure 4.13: Calibration plot for increasing concentrations of lead (II) in 0.1 M HCl at screen-printed DMTD modified electrode. Deposition potential = - 0.9 V, deposition time = 120 s, constant current = 1 μA.

4.3.3 Screen-printed Bi_2O_3 modified electrode

The intention of this investigation is to examine if screen-printed bismuth oxide modified electrodes can replicate the excellent results obtained in Section 3.3.4 with the BFSPCE (*in situ* plated). Measurements were carried out using the optimised parameters obtained for CCSCP detection of lead (II) and cadmium on BFSPCE and bare SPCE in Chapter 3.

4.3.3.1 Available potential window

First, the accessible potential range of the screen-printed bismuth oxide modified electrode was examined employing CV measurements in both solutions of 0.1 M HCl and 0.5 M ammonium acetate + 0.1 M HCl (Figure 4.14). The positive (least negative) limit of the bulk-modified electrode represents the reoxidation of the reduced bismuth (incorporated within the working electrode surface), which occurs at potentials less than – 0.15 V for 0.1 M HCl solution (pH 1.3) and – 0.33 V for 0.1 M HCl + 0.5 M ammonium acetate (pH 4.6). The negative potential limit of the electrode is – 0.78 V for 0.1 M HCl and – 1.20 V for 0.1 M HCl + 0.5 M ammonium acetate.

The potential range obtained for 0.1 M HCl + 0.5 M ammonium acetate is sufficient for detecting both lead (II) and cadmium (II) because their oxidation peak potentials appear in this range. Within the potential range obtained for 0.1 M HCl, lead can be detected but not cadmium. This is due to the early commencement of the hydrogen evolution on the electrode, which obscures the cadmium stripping peak.

4.3.3.2 Effect of the amount of Bi_2O_3

To optimise the amount of Bi_2O_3 included in the graphite-carbon ink, the amount of Bi_2O_3 in the ink screen-printed onto the working electrode surface was varied between 2 and 7 % (w/w). Figure 4.15 shows the effect of various amounts on the stripping chronopotentiometric response of lead (II). Based on the stripping chronopotentiometric responses of 50 and 200 $\mu g\ l^{-1}$ lead, a screen-printed Bi_2O_3 modified electrode containing 2 % Bi_2O_3 in weight was used for subsequent measurements. At the highest percentage of Bi_2O_3 (7 %), the lead stripping response was reduced probably due to a decrease in conductivity.

4.3.3.3 Comparison between screen-printed Bi_2O_3 modified electrode, BFSPCE and bare SPCE

Figure 4.16 shows the stripping chronopotentiometric response for 50 μg l^{-1} lead (II) obtained with the screen-printed Bi_2O_3 modified electrode in comparison to that obtained on BFSPCE and bare SPCE. The stripping chronopotentiometric response for lead (II) was enhanced at the 2 % Bi_2O_3 modified electrode (although not as much as that of BFSPCE) compared to the bare SPCE for this low lead concentration. The magnitude of the stripping response obtained for cadmium was also slightly enhanced on the Bi_2O_3 modified electrode compared to that on the bare SPCE (Figure 4.17).

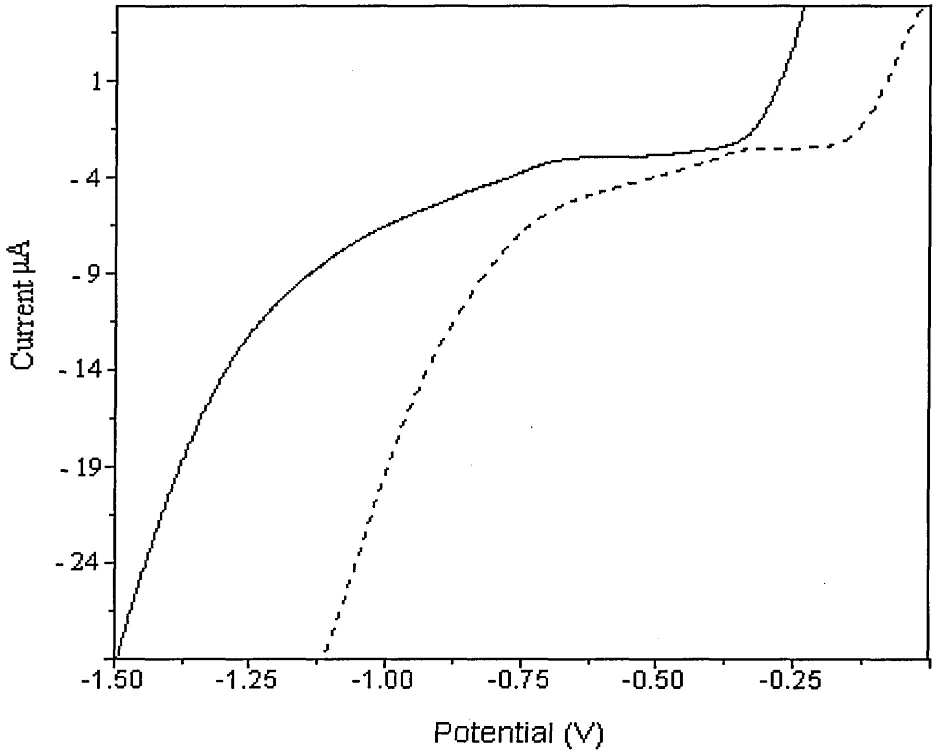


Figure 4.14: Cyclic voltammetric measurements of blank solutions of 0.1 M HCl + 0.5 M ammonium acetate solution (solid line) and 0.1 M HCl solution (broken line) on screen-printed Bi_2O_3 modified electrode. Scan range: – 1.5 V to + 0.3 V, scan rate: 50 mV s^{-1}.

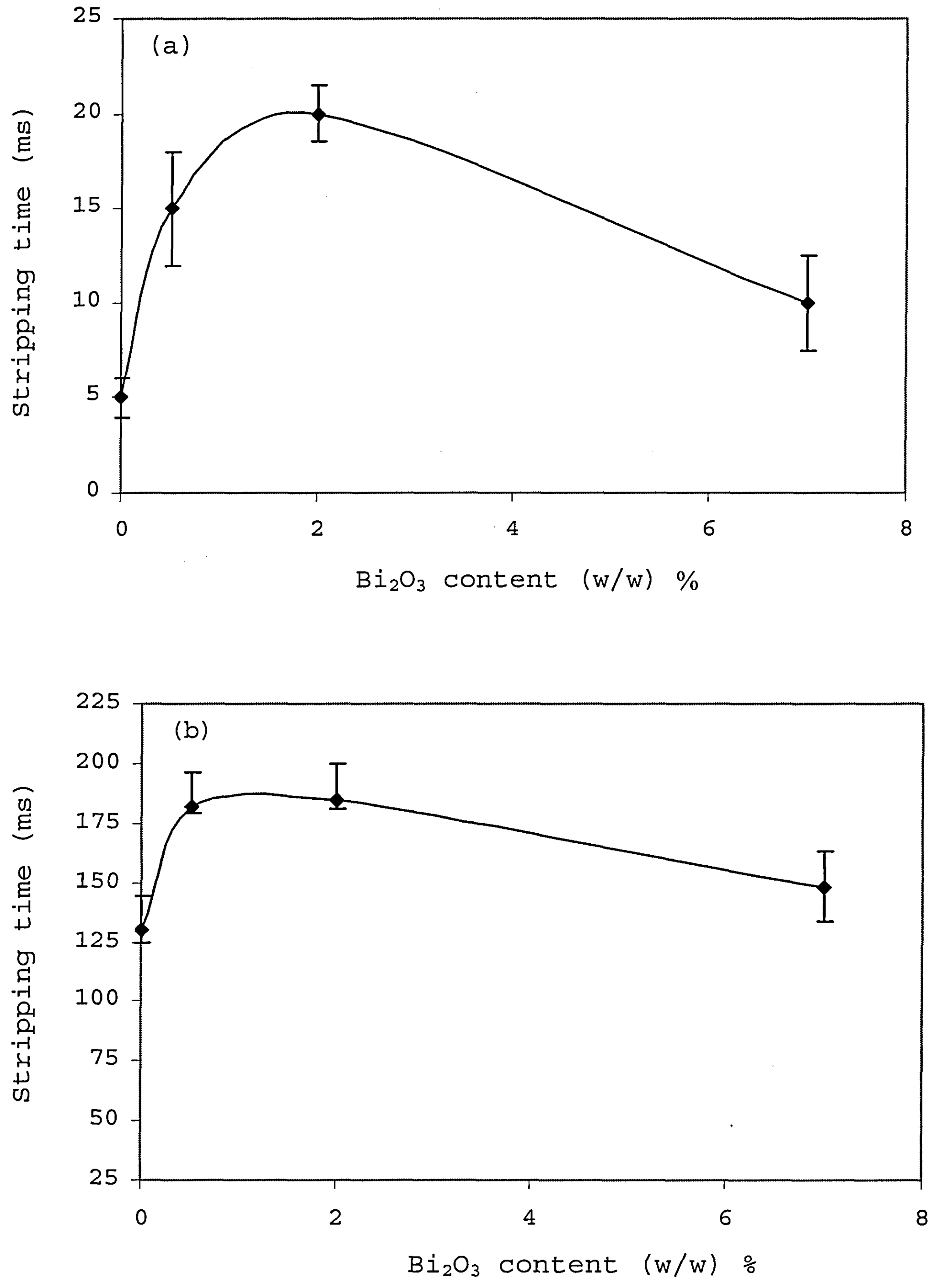


Figure 4.15: Effect of Bi_2O_3 content on the stripping chronopotentiometric responses of (a) 50 and (b) 200 μg l^{-1} lead in 0.1 M HCl. Deposition potential = - 0.9 V, deposition time = 120 s, constant current = 1 μA.

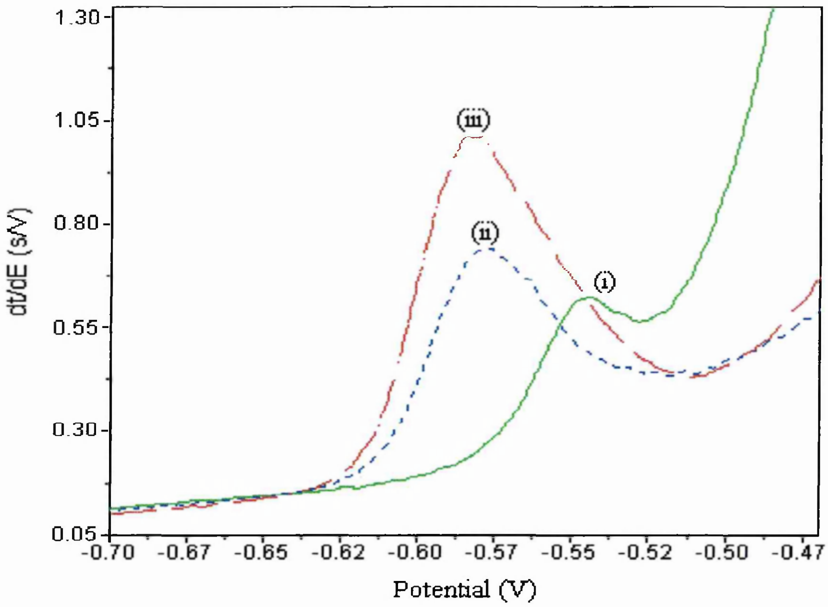


Figure 4.16: Stripping chronopotentiograms for 50 μg l^{-1} lead (II) at (i) unmodified bare SPCE, (ii) 2 % Bi_2O_3 modified electrode and (iii) BFSPCE. Deposition potential = - 0.9 V, deposition time = 120 s, constant current = 1 μA.

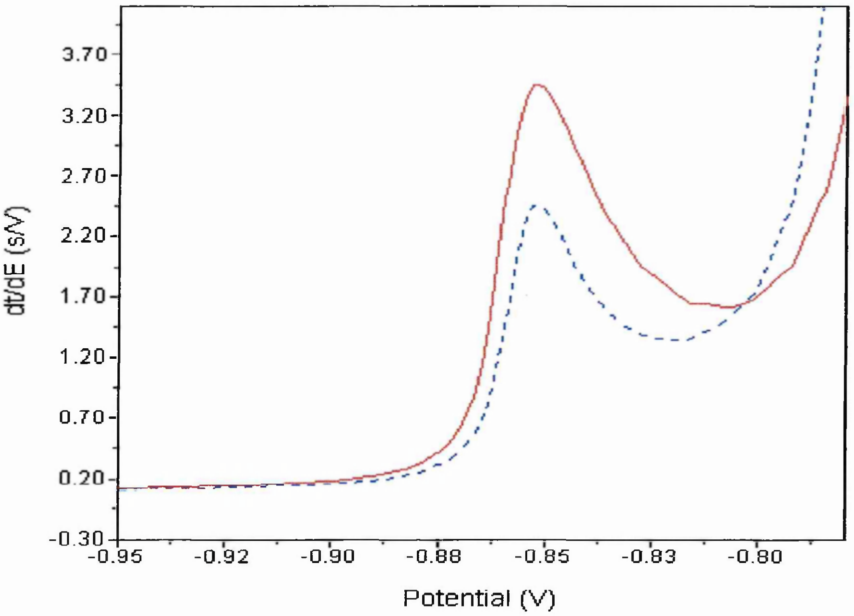


Figure 4.17: Stripping chronopotentiograms for 100 μg l^{-1} cadmium (II) at unmodified bare SPCE (broken line) and 2 % Bi_2O_3 modified electrode (solid line). Deposition potential = - 1.1 V, deposition time = 120 s, constant current = 1 μA.

4.3.3.4 Calibration plot, detection limit and precision

Figure 4.18 depicts the linear calibration curve obtained for lead (II) on the screen-printed Bi_2O_3 modified electrode. The plot obtained using a deposition potential of – 0.9 V and a deposition time of 120 s was linear in the range examined (0 – 500 μg l^{-1}, $r^2 = 0.9975$) with sensitivity of 1.05 ± 0.02 ms l μg^{-1}. A detection limit of 16 μg l^{-1} was estimated following 120 s deposition. The stripping response was reproducible with ten repetitive measurements of 50 μg l^{-1} lead (120 s deposition) yielding a relative standard deviation (R.S.D) of 5.6 %.

For the single detection of cadmium, a deposition potential of – 1.1 V was used. The plot of stripping response versus concentration, constructed for cadmium (II) was linear in the range examined (0 – 300 μg l^{-1}, $r^2 = 0.9940$), with sensitivity of 0.84 ± 0.03 ms l μg^{-1}. A detection limit of 16 μg l^{-1} was estimated following 120 s deposition. The stripping response was reproducible with ten repetitive measurements of 100 μg l^{-1} cadmium (120 s deposition) yielding a relative standard deviation (R.S.D) of 9.1 %.

The simultaneous detection of cadmium (II) and lead (II) was attempted but this was not possible. This is due to the resolution and stability of the stripping peak of lead being affected at deposition potential more negative than – 1.0 V.

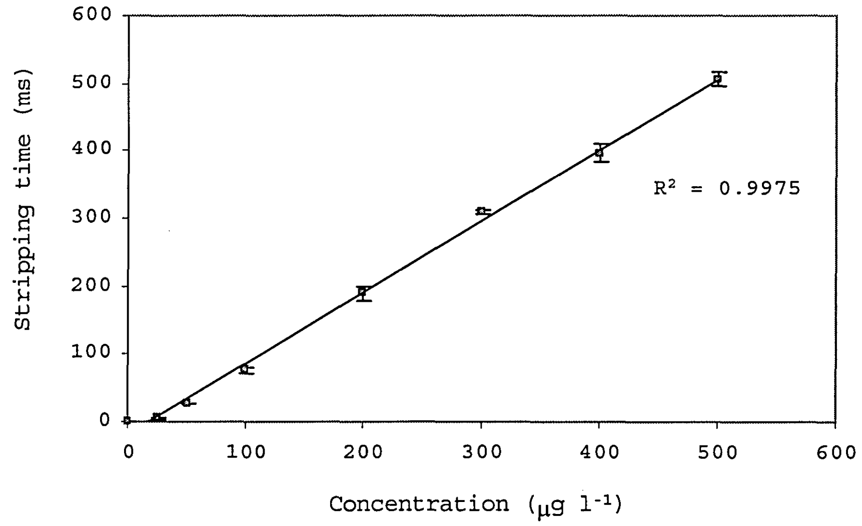


Figure 4.18: Calibration plot for increasing concentration of lead (II) in 0.1 M HCl at screen-printed Bi_2O_3 modified electrode. Deposition potential = - 0.9 V, deposition time = 120 s, constant current = 1 μA.

4.3.4 Screen-printed PEI modified electrode

4.3.4.1 Cyclic voltammetric behaviour of copper (II) and lead (II) on the modified electrode

Cyclic voltammetry was applied to examine the electrochemical behaviour of copper and lead (II) at the 0.5 % PEI modified electrode. Cyclic voltammograms were recorded over the range of potentials between – 0.9 V and + 0.1 V at a scan rate of 50 mV s^{-1}. Figure 4.19 presents cyclic voltammograms obtained for separate analysis of 5 mg l^{-1} copper (II) and lead (II) with screen-printed PEI modified electrode and unmodified bare SPCE in 0.1 M HCl. The copper oxidation peak obtained at ~ – 0.12 V was slightly enhanced on the modified electrode while for lead, the oxidation peak at ~ – 0.52 V was similar that obtained on the unmodified bare SPCE.

When copper (II) and lead (II) were simultaneously measured, the copper oxidation peak area obtained with the modified electrode was more than twice that obtained with the unmodified bare SPCE (Figure 4.20). However, the lead response in the presence of copper on the modified electrode was slightly reduced compared to that obtained at the unmodified bare SPCE. There was also an increase in the response for the single analysis of copper carried out on the PEI modified electrode compared to the unmodified bare SPCE. For the single analysis of lead, the responses on both the modified and unmodified electrodes were the same. Table 4.3 presents the peak areas obtained for 5 mg l^{-1} concentration of copper and lead by single analysis and by simultaneous analysis.

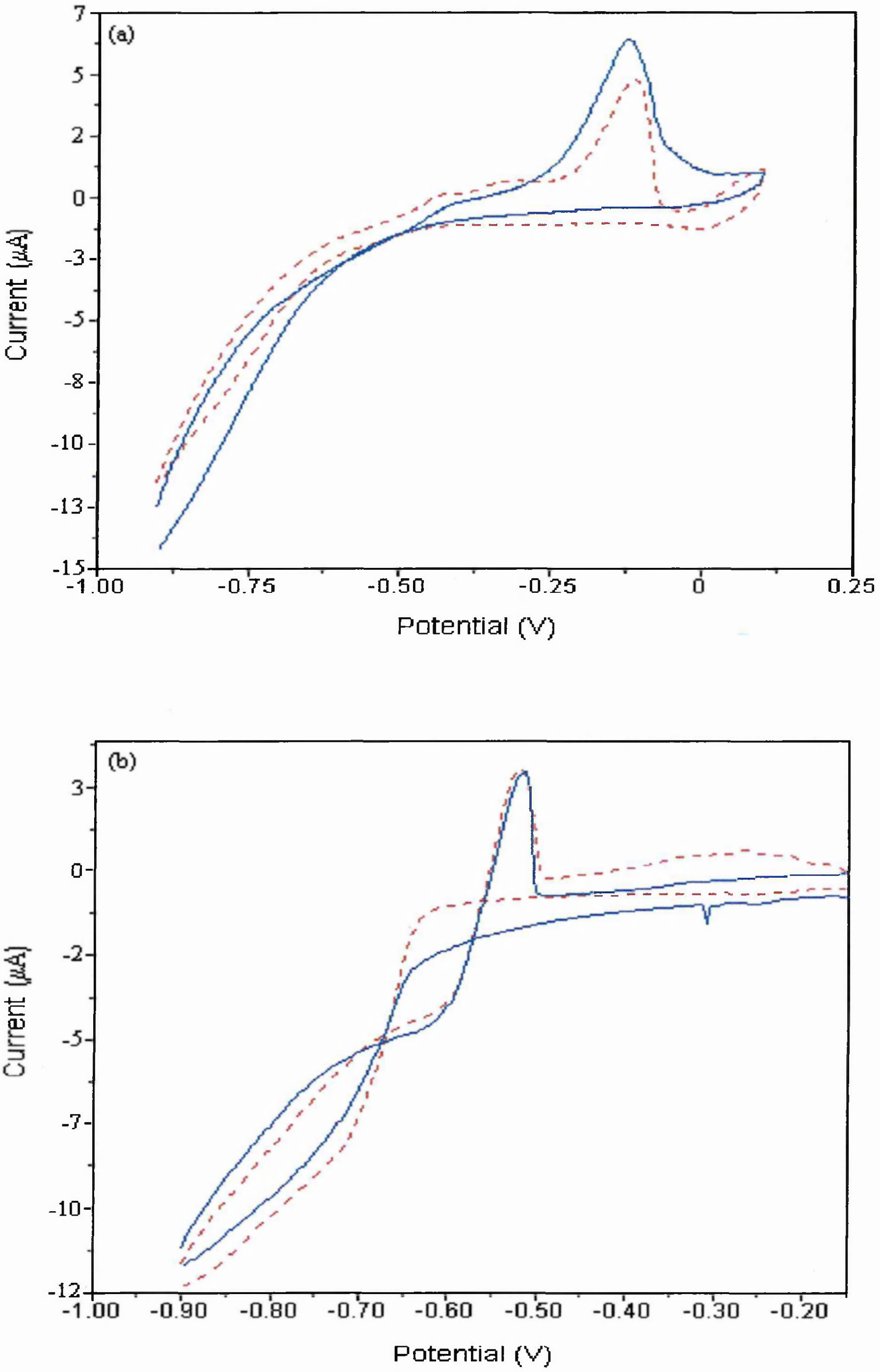


Figure 4.19: Cyclic voltammograms for (a) 5 mg l^{-1} copper and (b) 5 mg l^{-1} lead in 0.1 M HCl at unmodified bare SPCE (broken line) and screen-printed PEI modified electrode (solid line). Potential interval = - 0.9 V to 0.1 V (vs. screen-printed Ag/AgCl reference electrode), deposition time = 30 s, scan rate = 50 mV s^{-1}.

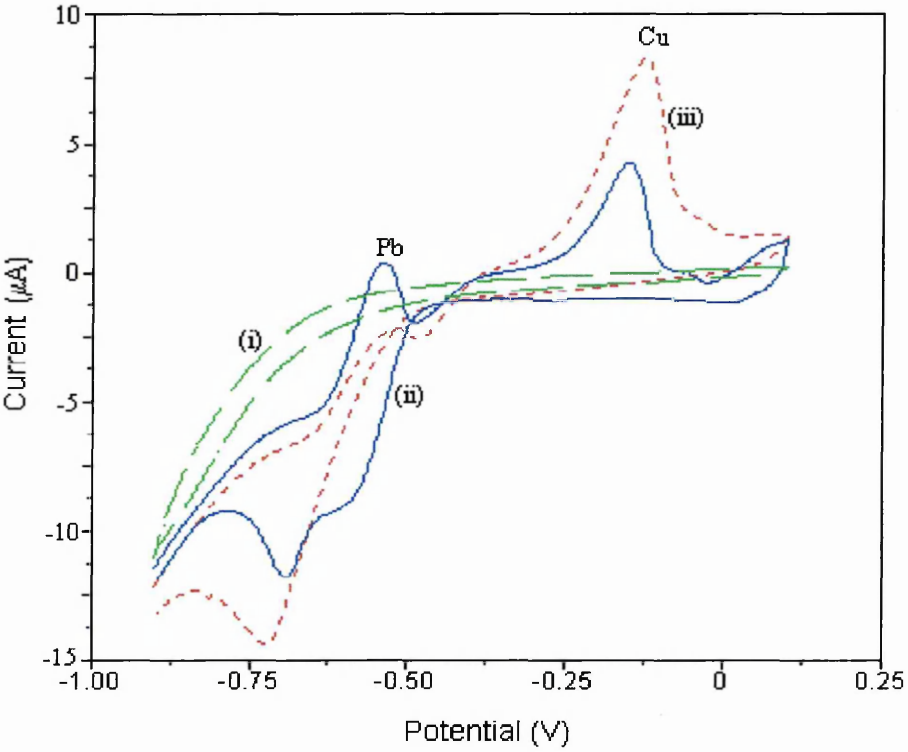


Figure 4.20: Cyclic voltammograms obtained for (i) modified electrode in the presence only of 0.1 M HCl, (ii) unmodified in the presence of 5 mg l^{-1} copper (II) and lead (II) in 0.1 M HCl and (iii) modified electrode in the presence of 5 mg l^{-1} copper (II) and lead (II) in 0.1 M HCl. Potential interval = - 1.0 V to 0 V (vs. screen-printed Ag/AgCl reference electrode), deposition time = 30 s, scan rate = 50 mV s^{-1}.

Table 4.3: The peak areas obtained for 5 mg l^{-1} copper (II) and lead (II) by single and simultaneous analysis using conditions shown in Figure 4.20.

	PEI modified electrode Single analysis	**PEI modified electrode** Simultaneous analysis	**Unmodified bare electrode** Single analysis	**Unmodified bare electrode** Simultaneous analysis
Analyte	Peak area (C)	Peak area (C)	Peak area (C)	Peak area (C)
Copper (II)	14.6 ± 0.3 x 10^{-6}	17.5 ± 1.2 x 10^{-6}	8.3 ± 0.5 x 10^{-6}	7.3 ± 0.2 x 10^{-6}
Lead (II)	4.7 ± 0.5 x 10^{-6}	3.1 ± 0.3 x 10^{-6}	4.7 ± 0.4 x 10^{-6}	4.7± 0.5 x 10^{-6}

mean for 4 measurements

4.3.4.2 CCSCP studies

After the CV studies, SCP was applied to copper (II) and lead (II) determination on the PEI modified electrode. The stripping chronopotentiogram obtained for the simultaneous detection of copper and lead at the modified electrode is depicted in Figure 4.21a and it shows that the peak appearing at – 0.43 V (between copper and lead) on the unmodified bare electrode, due to copper and lead interaction (Figure 4.21b), is suppressed. Also as one can see from the stripping chronopotentiograms in Figure 4.21, that the stripping response for copper obtained at the PEI modified electrode is slightly enhanced compared to that at the unmodified bare SPCE. This confirms the results obtained with CV.

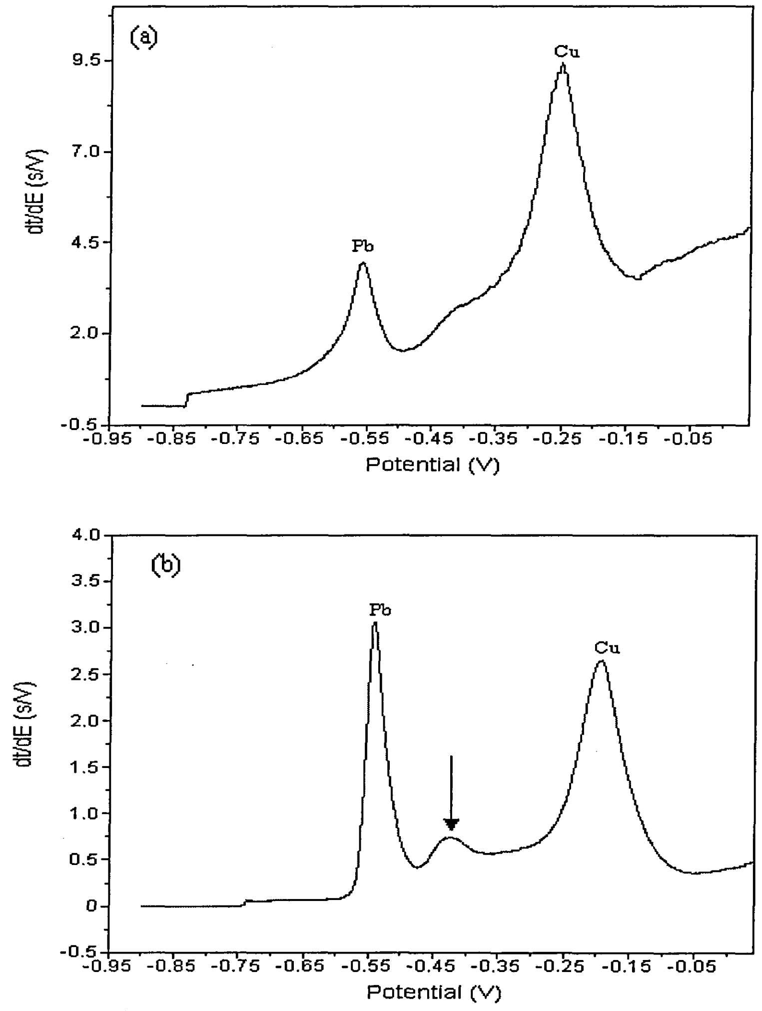


Figure 4.21: Stripping chronopotentiograms for 300 μg l^{-1} lead and copper at (a) 0.5 % PEI modified electrode, and (b) unmodified bare SPCE. Deposition potential = -0.9 V, final potential = 0.3 V, deposition time = 120 s, constant current = 3 μA.

Figure 4.22 depicts the stripping chronopotentiometric responses for increasing concentrations of copper and lead on the screen-printed PEI modified electrode. Linear calibration curves (see Figures 4.23 and 4.24) using a deposition potential of – 0.9 V and a deposition time of 120 s were obtained for the simultaneous detection of copper (II) and lead (II) in the range 0 to 1000 $\mu g\ l^{-1}$, with sensitivities of 1.53 ± 0.04 $ms\ l\ \mu g^{-1}$ and 0.42 ± 0.02 $ms\ l\ \mu g^{-1}$, respectively. The calibration curves obtained for copper and lead at the unmodified bare electrode are included in the graphical plots in Figures 4.23 and 4.24 for comparison. Slope (sensitivity) values of 0.59 ± 0.04 $ms\ l\ \mu g^{-1}$ and 0.36 ± 0.01 $ms\ l\ \mu g^{-1}$ were obtained from the linear calibration curves of copper and lead respectively at the unmodified bare electrode.

Detection limits of 34 $\mu g\ l^{-1}$ and 59 $\mu g\ l^{-1}$ were estimated for the simultaneous detection of copper and lead, respectively at the screen-printed PEI modified electrode. At the unmodified bare SPCE, detection limits of 90 and 63 $\mu g\ l^{-1}$ were estimated for the simultaneous detection of copper and lead. The stripping responses of copper and lead at the screen-printed PEI modified electrode were also reproducible with ten repetitive measurements of 300 $\mu g\ l^{-1}$ copper and lead (120 s deposition) yielding relative standard deviations (R.S.D) of 9.6 % and 6.3 %, respectively.

For single-component analysis, a copper calibration curve sensitivity of 1.20 ± 0.04 $ms\ l\ \mu g^{-1}$ was obtained at the screen-printed PEI modified electrode while a value of 1.07 ± 0.04 $ms\ l\ \mu g^{-1}$ was obtained at the unmodified bare SPCE. Calibration curve sensitivities of 0.78 ± 0.04 $ms\ l\ \mu g^{-1}$ and 0.56 ± 0.04 $ms\ l\ \mu g^{-1}$ were obtained for lead (II) at screen-printed PEI modified electrode and unmodified bare SPCE.

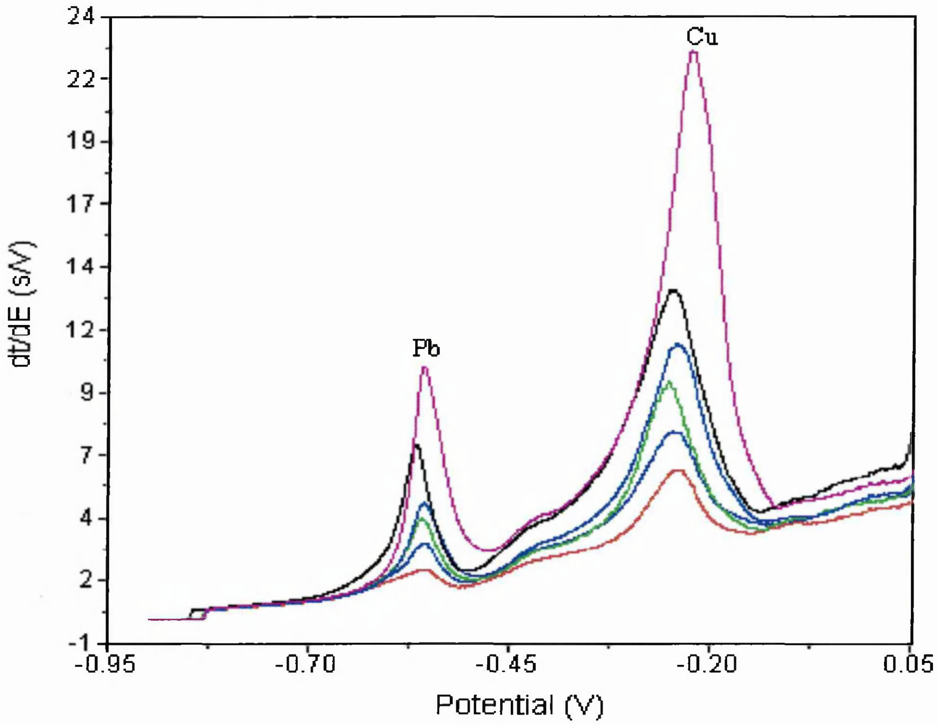


Figure 4.22: Stripping chronopotentiograms for increasing concentrations (100, 200, 300, 400, 500 and 1000 μg l^{-1}) of copper (II) and lead (II) in 0.1 M HCl obtained on PEI modified electrode. Deposition potential = - 0.9 V, final potential = 0.3 V, deposition time = 120 s, constant current = 3 μA.

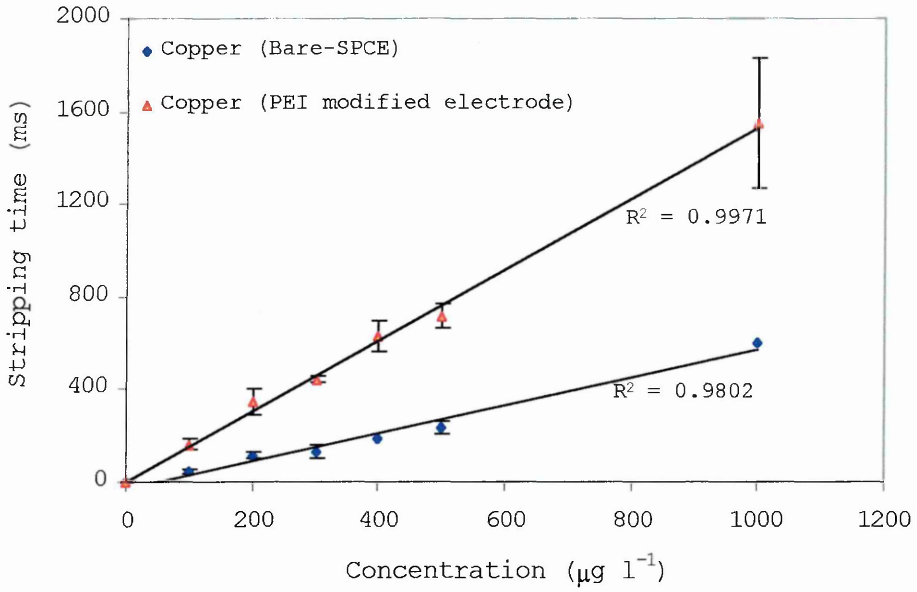


Figure 4.23: Calibration plot for increasing concentrations of copper (II) in 0.1 M HCl. Deposition potential = - 0.9 V, final potential = 0.3 V, deposition time = 120 s, constant current = 3 μA.

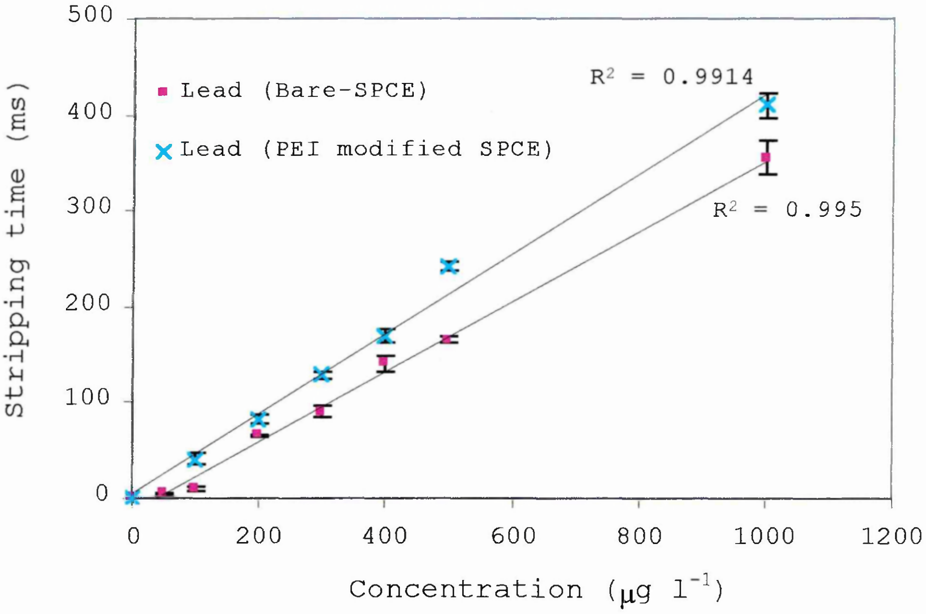


Figure 4.24: Calibration plot for increasing concentrations of lead (II) in 0.1 M HCl. Deposition potential = - 0.9 V, final potential = 0.3 V, deposition time = 120 s, constant current = 3 μA.

4.4 CONCLUSION

A range of chemically modified electrodes have been prepared and tested for metal ion detection. This concept allowed construction of sensitive electrochemical sensors for the metal ions. The results obtained so far seem promising for the detection of lead and copper on modified electrodes.

Initially Poly (ester sulphonic acid) (PESA) and Nafion were investigated as surface modifiers (drop-coating the carbon working surface with reagent), but only nafion was used further because of the enhanced sensitivity for lead at Nafion modified electrode compared to bare carbon electrode. This enhancement is probably due to the ion-exchange property of Nafion and this reflected in the accumulation of lead ion from the bulk solution. The inclusion of the compound, DMTD, in the Nafion fillm on the electrode surface did not positively enhanced the stripping response of lead as expected because of the probable dissolution of the DMTD from the Nafion film on the electrode.

The screen-printed DMTD modified electrode described was suitable for the single stripping chronopotentiometric determination of lead as it is characterised by a low detection limit (20 $\mu g\ l^{-1}$) even if it is not as good as values attainable with BFSPCE (12 $\mu g\ l^{-1}$) and MFSPCE (1 $\mu g\ l^{-1}$) using deposition potential of – 0.9 V for 120 s. The main advantage of this electrode is that it is mercury free and is more sensitive than bare SPCE.

It was possible to prepare a disposable bulk modified Bi_2O_3 sensor for lead and cadmium by modifying the screen-printing graphite-carbon ink with Bi_2O_3 and then printing the mixture onto the working surface of the SPCE. The advantage of the screen-printed Bi_2O_3 modified electrode is that there is no need to add bismuth (III) ions to the working solution in order to generate bismuth film.

The peak appearing at – 0.43 V due to copper and lead interaction on the unmodified bare electrode was suppressed when the PEI modified electrode was employed. Also the stripping response for copper obtained at the PEI modified electrode is slightly enhanced compared to that at the unmodified bare SPCE.

The suitability of the modified electrodes described here in this work to stripping analysis of real samples would be demonstrated in Chapter 5 for water samples, soil and sediment samples extracts so as to evaluate their performances in more complex matrices than aqueous sample solutions. The results obtained with the developed sensors for the various supplied samples would be compared to reference values.

CHAPTER 5

• 5 SAMPLE EVALUATIONS

5.1 INTRODUCTION

A range of electrochemical devices and methods has been developed during the last decade or so, but very few of these have been put into use to solve real life problems. There is a great distance from developing a method used in a laboratory tested on synthetic aqueous samples, to measurements on real samples on-site. Due to this, the screen-printed electrochemical sensor systems developed and described in Chapter 3 and 4 were tested on soil and sediment samples extracts so as to evaluate their performances in more complex matrices than aqueous sample solutions. The performance of the sensor devices in wastewater samples was also evaluated. The results obtained with the developed sensors for the various supplied samples were validated against the ICP-MS results.

Treated soil samples and reference materials (homogenised, dried soils/sediments and corresponding extracts) provided by Universitat Autonoma Barcelona (Spain) and Técnicas de Protección Ambiental S.A. (Spain) were used in the laboratory to optimise the performance of the screen-printed electrochemical sensor devices. The soil samples obtained from different contaminated sites in Spain and reference materials were treated with three different extractants. These are water, acetic acid and aqua regia. The water extraction is a compulsory test in evaluating the metal release from contaminated soil under rain conditions. The acetic acid extraction is carried out to determine the first fraction of the BCR (Community Bureau of Reference) sequential extraction procedure (Rauret *et al.*, 1999; Quevauviller, 2002). The extract obtained here, constitutes the mobile fraction of the heavy metals in soil (ion exchangeable and bound to carbonates). The aqua regia extraction provides the total content of heavy metals in soil samples and is known as pseudo-total content.

The developed screen-printed electrochemical sensor devices were evaluated outside the laboratory environment during the Senspol Technical Meeting on "Sensors for

Characterization and Monitoring of Contaminated Sites" (Sevilla, 6 - 9 November 2002) organised to allow sensors and analytical techniques from different European laboratories to be evaluated under field conditions. The screen-printed electrochemical sensors were used in on-site characterisation of a contaminated metal mining site located in Aznalcollar, Spain. Treated soil sample extracts, reference material extracts and wastewater samples were provided at the site and tested for heavy metal ions. The sample extracts were also brought back to the laboratory in order to carry out further analysis.

5.2 EXPERIMENTAL

5.2.1 Apparatus, reagents and screen-printed electrodes

Plate 5.1 shows the portable electrochemical measuring system used in carrying out some of the sample analysis. It is a hand-held, battery-powered PalmSens potentiostat/ galvanostat (Palm Instruments BV, The Netherlands), interfaced to a Compaq iPAQ pocket PC. The other apparatus, reagents and in-house fabricated screen-printed electrodes used have been described fully in Chapter 3 and 4.

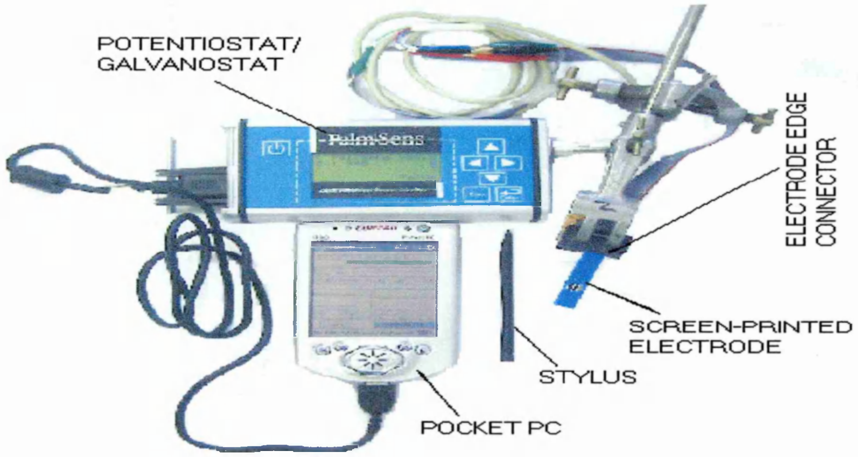


Plate 5.1: Electrochemical measuring system with a disposable screen-printed electrode and the battery-powered electrochemical potentiostat/galvanostat interfaced to a pocket PC.

5.2.2 Risk assessments and codes of practice

Experiments were performed in compliance with Departmental codes of practice and C.O.S.H.H regulations; waste was disposed of by approved routes.

5.2.3 Soil/sediment sample treatment

The sampling and sample treatment using conventional methods were carried out by Grup de Tècniques de Separació (Universitat Autonoma Barcelona, GTS-UAB, Spain) and by Técnicas de Protección Ambiental S.A. (TPA, Madrid, Spain). The description of the treatment (extraction process) applied to soil and sediment samples is detail below:

STEP 1

A 1 g portion of the solid sample was placed in a 65 ml stoppered extraction tube where 40 ml of bi-distilled water were added and then shaked for 16 hours in a rotary rack shaker. Then the mixture was centrifugated for phase separation and the aqueous phase filtered with filters of 0.22 μm pore size (Millex ®-GS, Millipore Corporation, Bedford MA).

STEP 2

The solid remaining from STEP 1 was shaked for 16 hours with 40 ml of 0.11 M acetic acid (pH 2.85). Centrifugation and filtration was carried out as in STEP 1.

STEP 3

A 0.25 g portion of the solid sample was placed in a microwave vessel and 9 ml of aqua regia (6 ml HCl, 2 ml HNO_3 and 1 ml water) were added and then left closed for 10 hours to predigest before microwave treatment. After that, total digestion proceeded by first ramping to 200 °C for 4 minutes and then maintaining for 6 minutes using pressure between 160 - 180 psi. The vessel was cooled down to room temperature before opening and the remaining solution brought up to 100 ml final volume. The solution was filtered with Millipore filters of 0.22 μm pore size.

All the extracts, the treated soils and the aqueous fractions from three steps, were kept refrigerated at 4 °C prior to the analyses being carried out.

5.2.4 Water sample treatment

Aliquots of water samples were filtered with a 0.22 μm pore size filters.

5.2.5 Reference material and real sample analysis

The concentrations for lead (II), copper (II) and cadmium (II) in the wastewater samples, aqueous soil or sediment extracts, acetic acid soil or sediment extracts and soil or sediment aqua regia extracts were quantified by the use of standard additions method following an initial screening of the target species in the samples. CCSCP and SWASV measurements were conducted by diluting the water samples, soil and sediment sample extracts in the supporting electrolyte. The diluted wastewater samples, soil and sediment extract samples were analysed by spiking with appropriate concentrations of standard metal ion solutions.

Stripping chronopotentiometric and voltammetric measurements of the sample solutions were carried out by placing a 100 μl sample drop on the three-electrode strip. Each electrochemical measurement was carried out in triplicates with a new electrode strip in non-dearated and unstirred solution. The optimised experimental conditions for the stripping measurements are tabulated in Tables 5.1 to 5.4.

Table 5.1: SWASV experimental conditions used for determination of lead, cadmium and copper in samples at mercury film screen-printed carbon electrode (MFSPCE).

Experimental conditions	**Optimised conditions**
Supporting medium	0.1 M HCl + 0.5 M ammonium acetate (lead, cadmium, copper)
Mercury (II) concentration (mg l^{-1})	80
Deposition potential (V)	- 1.0
Deposition time (s)	120
Frequency (Hz)	50
Step potential (mV)	5
SW Amplitude (mV)	25

Table 5.2: CCSCP experimental conditions employed in quantifying the concentration of lead, cadmium and copper in samples at MFSPCE.

Experimental conditions	**Optimised conditions**
Supporting medium	0.1 M HCl (lead), 0.1 M HCl + 0.5 M ammonium acetate (lead, cadmium), 2 M HCl (copper), 1 M ammonium acetate + 0.5 M HCl (copper)
Mercury (II) concentration (mg l^{-1})	10
Deposition potential (V)	- 0.9 (lead, copper) and – 1.0 (simultaneous analysis of cadmium and lead)
Deposition time (s)	120
Stripping constant current (µA)	1 or 5
Maximum time for measurement (s)	3 - 10

Table 5.3: Experimental conditions used for the CCSCP determination of lead and cadmium in samples at bismuth film screen-printed carbon electrode (BFSPCE).

Experimental conditions	**Optimised conditions**
Supporting medium	0.1 M HCl (lead), 0.1 M HCl + 0.5 M ammonium acetate (cadmium, lead)
Bismuth (III) concentration (μg l^{-1})	500
Deposition potential (V)	- 0.9 (lead) and – 1.0 (simultaneous analysis of cadmium and lead)
Deposition time (s)	120
Stripping constant current (μA)	1
Maximum time for measurement (s)	3 - 10

Table 5.4: CCSCP experimental conditions used for determination of lead and copper in samples at unmodified and modified screen-printed carbon electrode (SPCE).

Experimental conditions	**Optimised conditions**
Supporting medium	0.1 M HCl (lead, copper)
Deposition potential (V)	- 0.9 (lead, copper)
Deposition time (s)	120
Stripping constant current (μA)	1 or 3
Maximum time for measurement (s)	3 - 10

Results data obtained for the reference material and samples were validated against those obtained independently from Autonomous University of Barcelona, Spain using inductively coupled plasma-mass spectrometry (ICP-MS - model PQ EXCELL VG ELEMENTAL).

5.3 RESULTS

The concentrations of metal ions in the samples were determined by the extrapolation of the standard addition plot constructed using the standard addition method. Results data obtained using the different electrode surfaces (MFSPCE, BFSPCE, bare SPCE, nafion modified SPCE, screen-printed DMTD modified electrode, screen-printed Bi_2O_3 modified electrode and screen-printed PEI modified electrode) are presented in the following sections.

5.3.1 Determination of cadmium, copper and lead in certified reference material (CRM 601, a lake sediment) using MFSPCE

The results in Table 5.5 are for the square-wave anodic stripping voltammetric (SWASV) and constant current stripping chronopotentiometric (CCSCP) determination of lead, cadmium and copper in acetic-acid extracted reference material at MFSPCE. These measurements were carried out with the aim of validating the screen-printed electrochemical sensor device against a reference material certified with the BCR protocol. The results of both CCSCP and SWASV determination are verified by using the data obtained for ICP-MS analysis.

The cadmium result obtained with SWASV and CCSCP were in close agreement with the ICP-MS result. The result obtained for lead using the CCSCP coupled to the MFSPCE was also in agreement with the ICP-MS result. Copper and lead concentrations determined with SWASV coupled to the MFSPCE were much higher than the concentrations obtained with ICP-MS. This could be due to errors introduced during the preparation of both the metal ion standards and subsequent standard addition analysis.

Typical stripping voltammograms for the reference material extract is presented in Figure 5.1. The stripping voltammetric measurements yielded well defined stripping peaks for the reference material sample and for the standard additions of various concentrations of lead (II), cadmium (II) and copper (II) solutions.

Table 5.5: Comparing results for the determination of metal ions in acetic acid extracted certified reference sample (CRM 601, a lake sediment) using different analytical methods.

SAMPLE	Method	Copper (mg l^{-1})	Lead (mg l^{-1})	Cadmium (mg l^{-1})
CRM 601	SWASV-MFSPCE	14.12 ± 0.56	3.92 ± 0.88	4.60 ± 0.5
	CCSCP-MFSPCE	nq	2.72 ± 0.36	4.60 ± 0.16
	ICP-MS	10.20 ± 1.04	2.24 ± 0.36	4.36 ± 0.36

nq = sample not quantified
SWASV = squave-wave anodic stripping voltammetry
CCSCP = constant current stripping chronopotentiometry
ICP-MS = inductively coupled plasma mass spectrometry
MFSPCE = mercury film screen-printed carbon electrode

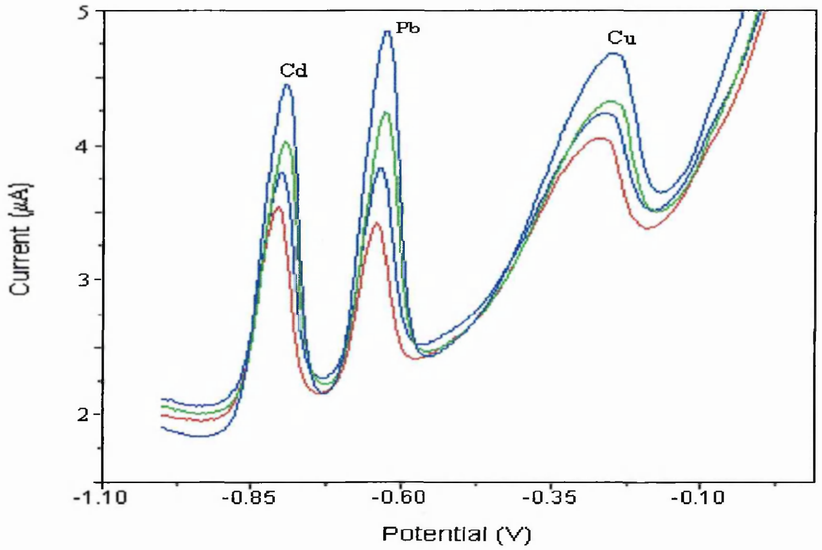


Figure 5.1: Stripping voltammograms for determination of cadmium, lead and copper in certified reference material extract using standard additions method. Responses are for sample and standard additions of 10, 30 and 50 μg l^{-1} of each metal ion on MFSPCE. Deposition potential = - 1.0 V, deposition time = 120 s, SW frequency = 50 Hz, step potential = 5 mV, SW amplitude = 25 mV.

5.3.2 CCSCP coupled to MFSPCE for determination of copper in acetic acid extracted reference material and soil samples using 2 M HCl as supporting medium

The adequacy of the CCSCP method coupled to the MFSPCE for the determination of copper in environmental samples using 2 M HCl as supporting medium was verified with the analysis of acetic acid extracted reference material and soil samples. Results for the CRM 601 certified reference sample are given in Table 5.6. The result obtained also for 1 M ammonium acetate containing 0.5 M HCl was included, so as to provide an alternative to the use of 2 M HCl as supporting medium when a medium with a higher pH is needed for simultaneous determination of copper and other metal ions.

The comparison between concentration data obtained for different acetic acid extracted soil samples using CCSCP coupled to the integrated mercury film screen-printed three-electrode device and ICP-MS (the reference method) is presented in Figure 5.2. A relative high correlation coefficient value obtained between the two methods of determination ($r^2 = 0.9712$) confirms the validity of the proposed method (CCSCP coupled to MFSPCE) for copper detection in environmental soil samples using 2 M HCl as supporting medium. Figure 5.3 shows the stripping chronopotentiograms obtained for one of the acetic acid extracted sample and the subsequent standard additions of different concentrations of copper (II) solution.

Table 5.6: Results for the acetic acid extractable copper content in reference sample using the different methods.

SAMPLE	ICP-MS (mg kg^{-1})	^{a}CCSCP - MFSPCE (mg kg^{-1})	^{b}CCSCP - MFSPCE (mg kg^{-1})
CRM 601	9.80 ± 0.30	10.38 ± 0.90	11.11 ± 1.05

[a] experiment carried out with 2 M HCl, 10 mg l^{-1} Hg (II) *in situ* plated, 120 s, 1 μA.
[b] experiment carried out with 1 M ammonium acetate + 0.5 M HCl, 10 mg l^{-1} Hg (II) *in situ* plated, 120 s, 3 μA.

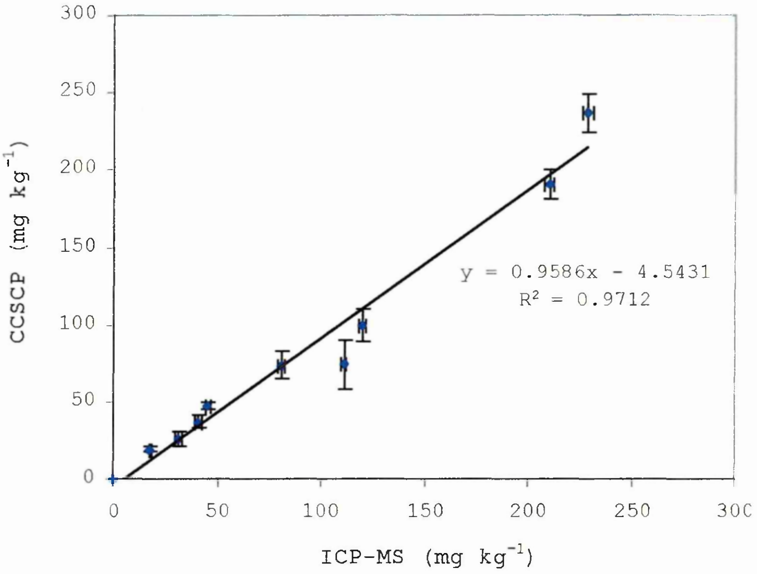


Figure 5.2: Correlation plot between CCSCP-MFSPCE and ICP-MS for the determination of copper in soil samples.

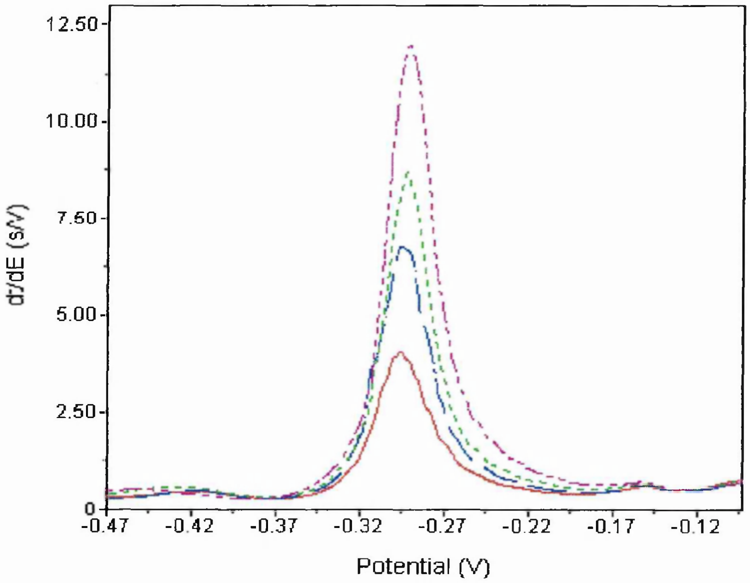


Figure 5.3: Stripping chronopotentiograms for determination of copper in a soil sample extract on MFSPCE. Responses are for sample and standard additions of 50, 100 and 200 μg l^{-1} of copper (II). Deposition potential = - 0.9 V, deposition time = 120 s, constant current = 1 μA, supporting medium = 2 M HCl.

5.3.3 Field evaluation of the bismuth film, mercury film and bare SPCEs coupled to CCSCP for the determination of lead and copper in soil samples

In Table 5.7 are the results obtained at Aznalcollar mining area for the aqua regia extracted soil samples. The results data for the developed screen-printed electrochemical sensors (BFSPCE, MFSPCE and bare SPCEs) demonstrated that the sensors show promise as screening tools for rapid monitoring of lead and copper in environmental matrices at the contaminated sites. The results obtained using the mercury film SPCE provided the closest results to the reference values generated with ICP-MS. The data generated for lead and copper at BFSPCE and bare SPCE (Table 5.7), respectively were not as accurate as expected. This is due to time constraints caused by sharing the portable electrochemical instrument with others during the field test and also due to the samples matrix effects.

Table 5.7: Data obtained for the *in situ* analysis of lead and copper in aqua regia extracted soil samples at Aznalcollar using BFSPCE, MFSPCE and bare SPCE.

SAMPLE Aqua regia extract	**Lead (II) Reference value** (mg kg^{-1})	**Copper (II) Reference value** (mg kg^{-1})	**Lead (II) BFSPCE** (mg kg^{-1})	**Lead (II) MFSPCE** (mg kg^{-1})	**Copper (II) Bare SPCE** (mg kg^{-1})
SA83c	872 ± 7.5	nq	335 ± 25	1080 ± 55	nq
SA84c	977 ± 8.4	348.7 ± 0.9	312.6 ± 30	640 ± 35	1152 ± 100
SA86c	47 ± 5	nq	nd	116 ± 10	nq
SA88c	179 ± 4.6	72 ± 1	nd	nd	200 ± 18
SA89c	13 ± 5.5	nq	nd	41 ± 7	nq
SA90c	40 ± 5.4	20 ± 1.2	nd	212 ± 20	1836 ± 120
SA91c	1116 ± 17.5	nq	370.1 ± 40	1631 ± 130	nq
SA92c	1111 ± 17.5	nq	598.4 ± 45	1091.2 ± 80	nq

nd = not detected because sample extract concentration is low, nq = sample not quantified

5.3.4 The applications of the bismuth film, mercury film and bare SPCEs coupled to CCSCP for the determination of lead (II), cadmium (II) and copper (II) in wastewater samples and extracted soil samples

Tables 5.8 to 5.14 present the results data obtained during a working visit to the laboratory of Grup de Técniques de Separació (Universitat Autonoma Barcelona, GTS-UAB, Spain) and also the subsequent analyses of samples brought back from the visit to Aznalcollar (Spain). The different sample matrices were analysed for lead (II), cadmium (II) and copper (II) using the optimised conditions for CCSCP coupled to bare SPCE, BFSPCE and MFSPCE summarised in Tables 5.2 to 5.4 (Section 5.2.5).

Table 5.8 summarises the data obtained for copper in different soil samples extracted with water, acetic acid and aqua regia. Apart from few results (data for sample SA81a and SA80c), the concentrations of copper determined with the bare SPCE coupled to CCSCP were within ± 30 % of the reference value results generated with ICP-MS. Also the CCSCP results in Table 5.9 for simultaneous determination of lead (II) and copper (II) at bare SPCE shows that the concentrations data are within ± 30 % of the reference value results generated with ICP-MS. This illustrates the applicability of the proposed method and sensor device to soil extract screening analysis.

The concentrations of lead and cadmium determined for wastewater samples are tabulated in Table 5.10. There is good agreement between the concentration data obtained with CCSCP coupled to bismuth film SPCE and mercury film SPCE and the standard reference ICP-MS method. Figure 5.4 shows typical stripping chronopotentiograms and standard addition plot for the determination of lead and cadmium in the wastewater sample on bismuth film screen-printed carbon electrode (BFSPCE). The concentrations of lead and cadmium determined in soil samples extracted with acetic acid and aqua regia are tabulated in Table 5.11 and 5.12. The non-detection of cadmium for some of the soil samples was because of the very low concentrations of cadmium in the extracts.

Table 5.8: The concentrations of copper (II) determined in extracted soil samples obtained from the Aznalcollar site using the bare SPCE.

SAMPLE	Copper (II) Reference value (mg kg^{-1})	Copper (II) Bare SPCE (mg kg^{-1})
Aqueous extract		
SA80a	22.2 ± 0.3	27.1 ± 2
SA81a	2.4 ± 0.1	3.3 ± 1
SA83a	nd	nd
SA85a	nd	nd
SA91a	nd	nd
Acetic acid extract		
SA80b	1482 ± 55	1521 ± 110
SA81b	97 ± 2	113 ± 7
SA83b	47 ± 1	63 ± 5
SA85b	39 ± 1	57 ± 6
SA91b	72 ± 1	97 ± 10
Aqua regia extract		
SA80c	4062 ± 70	5584 ± 500
SA81c	3816 ± 68	3426 ± 311
SA83c	666 ± 1	473 ± 40
SA85c	254 ± 1	202 ± 25
SA91c	498 ± 17	518 ± 50

nd = sample not detected

Table 5.9: Simultaneous determination of lead (II) and copper (II) in extracted soil samples brought back from the contaminated site in Aznalcollar, Spain using the bare SPCE.

SAMPLE	**Reference value** (mg kg^{-1})		**CCSCP - Bare SPCE** (mg kg^{-1})	
	Copper (II)	Lead (II)	Copper (II)	Lead (II)
SA83b	47 ± 1	119 ± 1	51 ± 4	121 ± 7
SA83c	666 ± 1	872 ± 8	646 ± 46	789 ± 36
SA91b	72 ± 1	109 ± 1	82 ± 8	87 ± 4
SA91c	518 ± 17	1116 ± 17	482 ± 49	1423 ± 78

Table 5.10: Simultaneous determination of lead (II) and cadmium (II) in wastewater samples obtained from Aznalcollar, Spain.

SAMPLE	**ICP-MS** (µg l^{-1})	**BFSPCE** (µg l^{-1})	**MFSPCE** (µg l^{-1})
Lead 693	890 ± 20	835 ± 50	1233 ± 60
Cadmium 693	620 ± 40	1184 ± 35	658 ± 35
Lead 694	740 ± 40	1022 ± 60	1165 ± 45
Cadmium 694	630 ± 9	711 ± 45	712 ± 35

BFSPCE = bismuth film screen-printed carbon electrode
MFSPCE = mercury film screen-printed carbon electrode
ICP-MS = inductively coupled plasma mass spectrometry

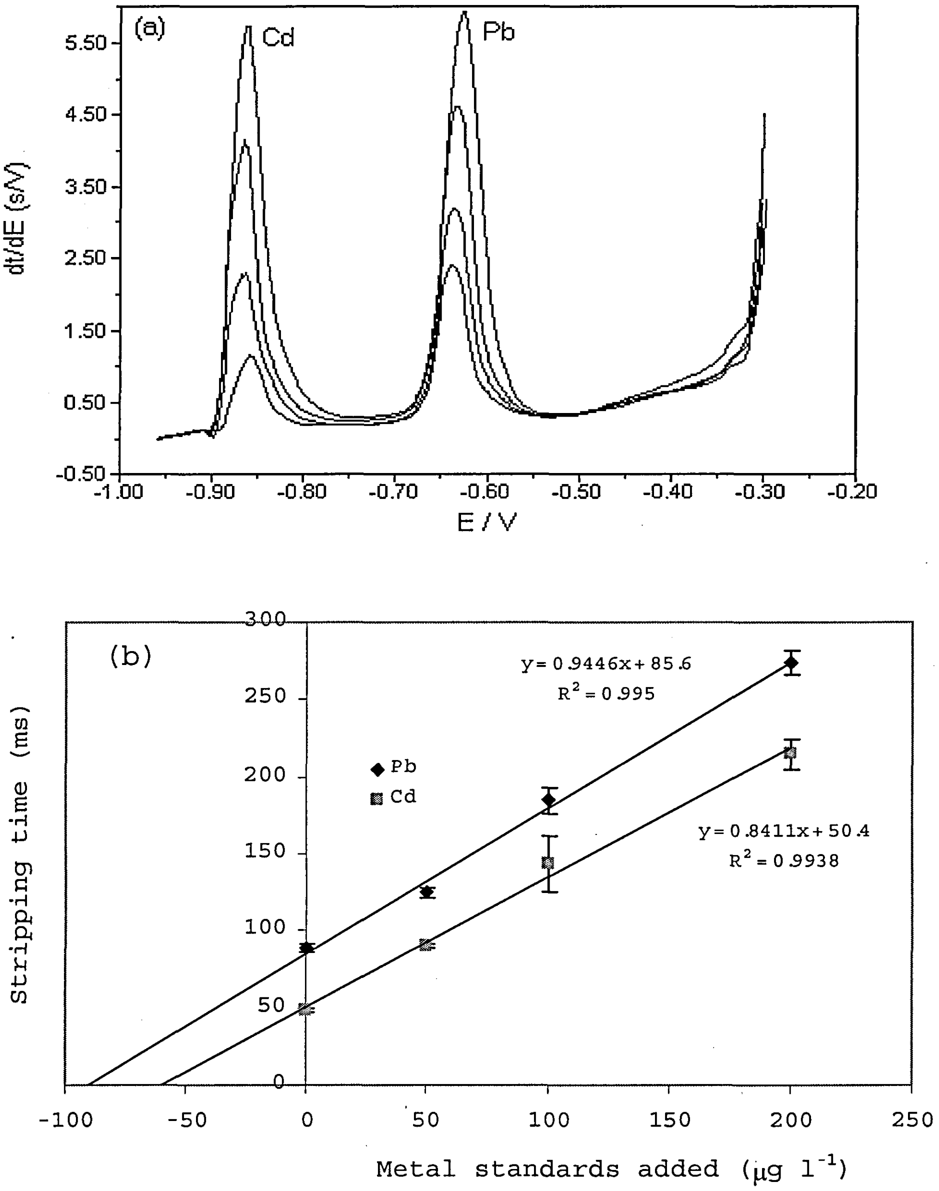


Figure 5.4: (a) Stripping chronopotentiograms and (b) standard addition plot for the determination of lead and cadmium in wastewater sample on BFSPCE. Standard solutions of lead (II) and cadmium (II) were added in 50, 100 and 200 μg l^{-1} increments to the sample. Deposition potential = - 1.0 V, deposition time = 120 s, constant current = 1 μA, supporting medium = 0.1 M HCl + 0.5 M ammonium acetate.

Table 5.11: Determination of lead (II) and cadmium (II) in acetic acid extracted soil samples from different contaminated sites in Spain.

SAMPLE	**Metal ion**	**ICP-MS**	**CCSCP**	
		(mg kg^{-1})	**BFSPCE** (mg kg^{-1})	**MFSPCE** (mg kg^{-1})
TP1	lead	250 ± 8	203 ± 11	256 ± 14
	cadmium	85 ± 1	70 ± 5	100 ± 6
PX	lead	165 ± 3	140 ± 8	190 ± 12
	cadmium	57 ± 1	50 ± 3	61 ± 3
C20	lead	4.46 ± 0.06	3.79 ± 0.2	4.12 ± 0.2
	cadmium	nd	nd	nd
C22	lead	4.29 ± 0.05	4.21 ± 0.2	4.35 ± 0.3
	cadmium	nd	nd	nd
SA79b	lead	1.27 ± 0.04	0.33 ± 0.02	1.28 ± 0.08
	cadmium	nd	nd	nd
SA80b	lead	6.20 ± 0.2	1.35 ± 0.05	5.60 ± 0.3
	cadmium	nd	nd	nd
SA83b	lead	3.15 ± 0.04	2.10 ± 0.13	2.80 ± 0.2
	cadmium	nd	nd	nd
SA84b	lead	1.54 ± 0.03	0.85 ± 0.05	1.23 ± 0.08
	cadmium	nd	nd	nd
SA91b	lead	2.85 ± 0.04	1.35 ± 0.1	2.03 ± 0.2
	cadmium	nd	nd	nd

nd = sample not detected
BFSPCE = bismuth film screen-printed carbon electrode
MFSPCE = mercury film screen-printed carbon electrode
ICP-MS = inductively coupled plasma mass spectrometry
CCSCP = constant current stripping chronopotentiometry

Table 5.12: Concentration data for lead (II) in aqua regia extracted soil samples obtained from the Aznalcollar mining area in Spain.

SAMPLE	ICP-MS (mg kg^{-1})	CCSCP BFSPCE (mg kg^{-1})	CCSCP MFSPCE (mg kg^{-1})
SA79c	29.3 ± 0.2	16.8 ± 1.4	30.9 ± 1.2
SA80c	36.3 ± 0.2	21.9 ± 1.2	38.5 ± 1.9
SA81c	51.1 ± 0.2	29.9 ± 2	41.5 ± 2
SA82c	51.5 ± 0.2	25 ± 1.5	47.3 ± 2
SA83c	872 ± 8	464 ± 25	933 ± 70
SA84c	977 ± 8	609 ± 3	1150 ± 80
SA85c	292 ± 5	nd	265 ± 15
SA91c	1116 ± 18	324 ± 30	906 ± 60
SA92c	1111 ± 18	339 ± 30	1198 ± 80
Ref 721c	245 ± 9	87 ± 4	242 ± 10
Ref 722c	1853 ± 31	1853 ± 80	1710 ± 60

nd = sample not detected
BFSPCE = bismuth film screen-printed carbon electrode
MFSPCE = mercury film screen-printed carbon electrode
ICP-MS = inductively coupled plasma mass spectrometry

There is a fairly good agreement between some of the results obtained with ICP-MS and CCSCP determinations at SPBFE and SPMFE. As for the low concentration data obtained with CCSCP at SPBFE for lead (II) in some of the acetic acid and aqua regia extracted soil samples, the inhibiting effect of copper present in the soil extract samples is the cause. The interference study carried out on aqueous solutions in Section 3.3.4.4 (Chapter 3) points to this and also describes how the introducing ferricyanide into the test solution can aid the elimination of this effect. The reference values for copper in the various samples determined with ICP-MS (reference method) can be found in the Appendix section (Appendix 1.2).

Table 5.13 and 5.14 show the results obtained by adding 0.1 mM ferricyanide to the different sample extract solutions. The concentration data obtained, closer to the lead (II) reference values generated with ICP-MS, illustrate the benefit of adding ferricyanide when analysing the different sample extracts. The stripping chronopotentiometric response and standard addition plot illustrating this benefit are also shown in Figure 5.5 and 5.6. The lead stripping response for the sample was much higher when ferricyanide was present in the test solution than when it was not. The reference values for copper in the various samples can be found in the Appendix section (Appendix 1.3).

Table 5.13: Results data for lead (II) determination on BFSPCE showing the benefit of adding 0.1 mM ferricyanide to the acetic acid extracts of soil samples from Aznalcollar mining area, Spain.

SAMPLE	**Dilution factor** (x 39)	**ICP-MS** ($\mu g\ l^{-1}$)	**CCSCP - BFSPCE** (without ferriyanide) ($\mu g\ l^{-1}$)	**CCSCP - BFSPCE** (with ferricyanide) ($\mu g\ l^{-1}$)
SA79b	10	127 ± 4	33 ± 2	109 ± 5
SA80b	50	124 ± 4	27 ± 1	111 ± 7
SA81b	5	56 ± 2	nd	49 ± 2
SA83b	25	126 ± 1.5	86 ± 5	130 ± 4
SA84b	20	77 ± 1.4	43 ± 2.5	62 ± 4
SA85b	2.5	58 ± 1.3	nd	55 ± 2
SA88b	10	70 ± 0.8	nd	62 ± 3
SA91b	25	114 ± 1.4	54 ± 4	132 ± 6
SA92b	5	71 ± 1.5	nd	77 ± 5

nd = sample not detected
BFSPCE = bismuth film screen-printed carbon electrode
CCSCP = constant current stripping chronopotentiometry

Table 5.14: Concentration data for lead (II) determination on BFSPCE showing the benefit of adding 0.1 mM ferricyanide to the aqua regia extracts of soil samples from Aznalcollar mining area, Spain.

SAMPLE	**Dilution Factor** (x 390)	**ICP-MS** (μg l^{-1})	**CCSCP - BFSPCE** (without ferriyanide) (μg l^{-1})	**CCSCP – BFSPCE** (with ferricyanide) (μg l^{-1})
SA79c	500	59 ± 0.1	17 ± 1.2	48 ± 3
SA80c	500	73 ± 0.4	22 ± 2	62 ± 3
SA81c	500	102 ± 0.1	30 ± 2	96 ± 7
SA82c	500	103 ± 0.1	36 ± 2	98 ± 6
SA83c	25	97 ± 0.9	nd	86 ± 4
SA84c	20	122 ± 0.9	58 ± 0.6	93 ± 3
SA85c	10	82 ± 1.8	nd	74 ± 5
SA91c	50	56 ± 0.9	16 ± 2.3	58 ± 4
SA92c	50	63 ± 1	19 ± 2	69 ± 3
Ref 721c	10	66 ± 1	24 ± 1.6	50 ± 4

nd = sample not detected
BFSPCE = bismuth film screen-printed carbon electrode
CCSCP = constant current stripping chronopotentiometry

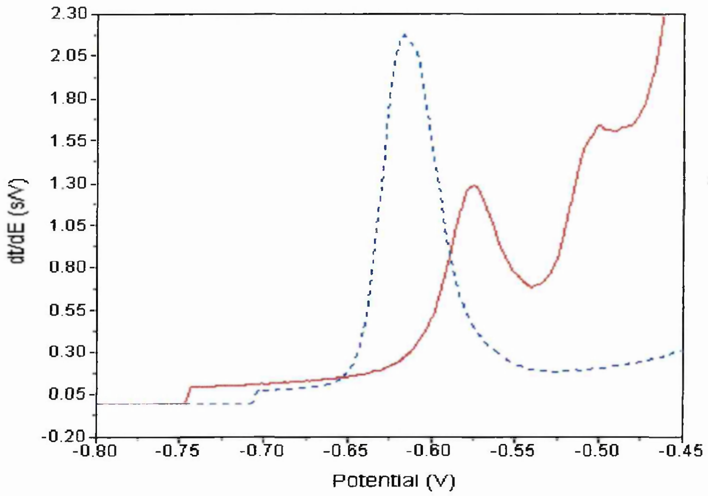


Figure 5.5: Stripping chronopotentiograms for the SA80b soil sample extract on BFSPCE. Lead stripping responses obtained without the addition of 0.1 mM ferricyanide (solid line) and with the addition of 0.1 mM ferricyanide (broken line). Deposition potential = - 0.9 V, deposition time = 120 s, constant current = 1 μA.

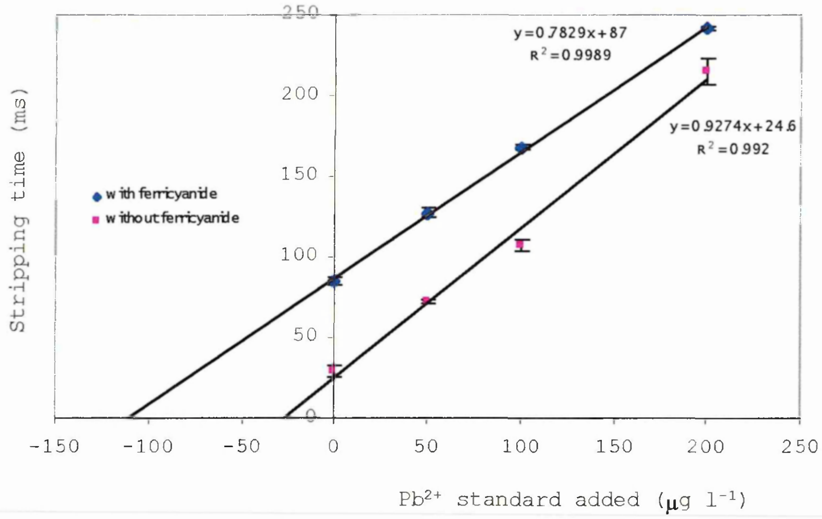


Figure 5.6: Standards additions plot showing the benefit of adding ferricyanide to the analytical solutions for the determination of lead in the SA80b soil sample extract on BFSPCE. Deposition potential = - 0.9 V, deposition time = 120 s, constant current = 1 μA, supporting medium = 0.1 M HCl.

5.3.5 The determination of lead (II) and copper (II) in wastewater samples and extracted soil samples using the different modified electrodes

In order to demonstrate the applicability of the different modified electrodes described in Chapter 4 to environmental samples, CCSCP coupled to the modified electrodes were applied to the measurement of lead (II) in water samples, acetic acid and aqua regia extracted soil samples as well as the simultaneous measurement of lead (II) and copper (II) in acetic acid and aqua regia extracted soil samples. The concentration values summarised in Table 5.15 and 5.16 show that there are good agreements between the results obtained with the different modified electrodes and the reference method (ICP-MS).

Table 5.15: The determination of lead (II) in water and soil samples from the Aznalcollar mining area and other sites in Spain using the Nafion modified SPCE, the screen-printed DMTD modified electrode and Bi_2O_3 modified electrode.

SAMPLE	Reference method (mg l^{-1} or mg kg^{-1})	CCSCP (DMTD modified SPCE) (mg l^{-1} or mg kg^{-1})	CCSCP (Bi_2O_3 modified SPCE) (mg l^{-1} or mg kg^{-1})	CCSCP (Nafion modified SPCE) (mg l^{-1} or mg kg^{-1})
693 (water sample)	0.89 ± 0.02	0.72 ± 0.06	0.91 ± 0.03	nq
694 (water sample)	0.74 ± 0.04	0.91 ± 0.07	0.60 ± 0.03	nq
C20 (acetic acid soil extract)	4.46 ± 0.06	nq	nq	3.6 ± 0.3
C22 (acetic acid soil extract)	4.29 ± 0.05	nq	nq	4.1 ± 0.2
TP1 (acetic acid extracted soil)	250 ± 8	213 ± 20	167 ± 4	nq
Ref 722c (aqua regia extracted soil)	1853 ± 31	2013 ± 80	nq	nq

DMTD = 2,5- Dimercapto-1, 3, 4- thiadiazole, Bi_2O_3 = bismuth (III) oxide, nq = sample not quantified

Table 5.16: Concentration data for the determination of lead (II) and copper (II) in extracted soil samples obtained from the Aznalcollar mining area, Spain using the screen-printed PEI modified electrode.

SAMPLE	**Reference method** (mg kg^{-1})		**CCSCP - PEI modified electrode** (mg kg^{-1})	
	Copper (II)	Lead (II)	Copper (II)	Lead (II)
SA83b*	47 ± 1	119 ± 1	59 ± 6	135 ± 10
SA83c*	666 ± 1	872 ± 8	729 ± 61	1039 ± 80
SA91b	72 ± 1	109 ± 1	90 ± 6	96 ± 7
SA91c	518 ± 17	1116 ± 17	497 ± 4	1379 ± 90

PEI = polyethylenimine, * b = acetic acid extractable sample, *c = aqua regia extractable sample

5.4 CONCLUSION

For environmental samples, the usefulness of CCSCP coupled to screen-printed electrodes (SPEs) for the determination of lead and cadmium in water samples and lead, copper and cadmium in extracts obtained by solid-liquid phase extraction of soil (or sediments) samples was evaluated.

Overall, the results show that the developed screen-printed electrochemical sensors are capable of measuring copper, lead and cadmium in water samples and in soil (or sediments) samples. After comparison to ICP-MS, the data generated with the electrochemical sensors revealed fairly accurate measures of lead, copper and cadmium in water samples and extracted soil samples. The mobile lead, copper and cadmium ions in the soils or sediments were determined after sequential extraction had been carried out with water (free copper), acetic acid (exchangeable copper, lead and cadmium) and aqua regia (pseudo total lead, copper).

Results obtained for samples analysed both in the laboratory and in the field (Aznalcollar) with CCSCP using BFSPCE, bare SPCE, MFSPCE, Nafion modified

SPCE, screen-printed DMTD, Bi_2O_3 and PEI modified electrodes were comparable to ones obtained with ICP-MS, apart from a few which were not. These were due to errors introduced during the measurement process and the presence of copper in the sample at a significant level. The copper interference on the stripping response of lead (II) was suppressed using 0.1 mM ferricyanide.

CHAPTER 6

• 6 DISCUSSION

6.1 INTRODUCTION

The basic idea of the work carried out in this thesis was to fabricate single-use metal ion sensor incorporating a three-electrode configuration for on-site analysis and coupling it to a potential – time or current – potential excitation waveform. This task was successfully fulfilled with envisaged device used for detection of heavy metal ions in aqueous synthetic solutions and also contaminated soil and water samples. The motivations to produce this kind of sensors were driven by both practical and economic interests. These include cost, speed, simplicity, sensitivity, reproducibility, robustness and applicability in the field.

The development of the screen-printed electrochemical sensors for the detection of heavy metal ions was carried out in stages so that the final devices demonstrated the desired characteristics. The characterisation and application of the developed screen-printed electrochemical sensors will be discussed in the following sections.

6.2 FABRICATION AND CHARACTERISATION OF SCREEN-PRINTED ELECTRODE

The electrochemical properties (e.g., potential window, electron transfer kinetics, background response, quality and the reproducibility of the reference electrode potential) of the in-house fabricated SPEs were studied so that valuable insights into the electroanalytical behaviour can be obtained. The results obtained thereof are discussed in two parts:

- Characterisation of screen-printed carbon working electrode -
- Characterisation of screen-printed Ag/AgCl reference electrode

6.2.1 Voltammetric characterisation of the working electrode

SPCEs were first examined with a view to using them for electrochemical sensing. This was done because the performance of a working electrode (WE) is strongly affected by the composition of material used in producing it. The characteristics of an ideal WE should be low ohmic resistance, chemical and electrochemical inertness over a broad range of potentials, high hydrogen and oxygen overvoltage resulting in a wide potential window and low residual current (Moreno-Baron *et al.*, 2003). Hence, our interest in using the SPCE demanded examining the potential window of the proposed electrode and an understanding of the electron-transfer processes at the SPCE surfaces. The latter involved evaluating the heterogeneous rate constant, k^o, using the increase in peak separation (ΔE_p) obtained from the current – potential curves, as this serves as an index of the ease of electron transfer at the SPCE surface. Also estimation of the SPCE's active surface area was carried out.

Because the working range available for the detection of electroactive species can be affected by several factors including supporting electrolyte decomposition and electrode dissolution (Somasundrum, 1994), the potential window of the SPCE was examined. The different negative potential limits seen in Figure 2.3 (Chapter 2) for the different supporting electrolyte medium can be ascribed principally to the influence of pH on the potential of the hydrogen evolution reaction. Under acidic condition (0.1 M HCl, pH 1.3), the SPCE was operable in the potential range – 0.7 V to ~ + 0.8 V, while in slightly acidic condition (0.5 M ammonium acetate + 0.1 M HCl, pH 4.6), the SPCE was operable in the potential range – 1.1 V to ~ + 1.1V. The most negative potential signals the commencement of hydrogen evolution on the electrode and the most positive potential is for the oxidation of the hydroxide ions on the electrode. The potential range obtained for the SPCE in 0.1 M HCl supporting medium was similar to that obtained by Wang *et al.* (1998b), using SPCE printed with the same type of ink material (Acheson).

The electrochemical characterisation of the SPCE was carried out with potassium hexacyanoferrate (II) (or potassium ferrocyanide, $K_4Fe(CN)_6$) using CV and chronoamperometry. For SPCEs, there are a lot of data in the literature concerning the oxidation of $K_4Fe(CN)_6$, particularly regarding the electrode kinetics of this reaction, which is strongly dependent on the state of species and on the electrode surface (Brett *et al.*, 2001).

It was found that the presence of inhibitory organic layer in the carbon ink used for screen-printing had an effect on the electron transfer reactivity and overall analytical performance of the resulting SPCE. The SPCE did not display the ideal reversible behaviour of a fully conductive electrode (GCE) that might be expected for potassium ferrocyanide. This unattractive behaviour suggested a lower graphite-carbon loading compared to GCE. The hydrophobic character of the SPCE surface resulted in the quasi-reversible behaviour of the redox couple at the SPCE whereas the same substance measured at the GCE exhibited a fair reversibility.

From the cyclic voltammograms obtained on SPCEs for potassium ferrocyanide at different scan rates, the $\Delta E_{p,}$ increased as the scan rate increased and this indicates that the redox reaction at the electrode is quasi-reversible. The electron transfer reaction was quasi-reversible because the rate of electron transfer was too slow to keep the $Fe(CN)_6^{4-}$/ $Fe(CN)_6^{3-}$ couple in equilibrium as the potential was changed.

The ΔE_p value of 260 mV obtained at a low scan rate (20 mV s^{-1}) for 0.5 mM ferrocyanide in 0.1 M KCl at the SPCE (see Figure 2.4, Chapter 2), indicated the poor reversibility of the electrochemical reaction on the electrode. This value is considerably greater than the theoretical anodic peak - cathodic peak separation for a reversible one-electron transfer reaction, which is 57 mV and the peak separation value of 95 mV obtained with the GCE used here in this work. The value obtained with SPCE is an indicator of the charge transfer inhibition within the carbon electrode material (Stiene and Bilitewski, 2002). Wang *et al.* (1998b) reported values of 224, 205, 126, 63, and 58 mV for 0.05 mM ferrocyanide in 0.1 M KCl (20 mV s^{-1}) at Acheson SPCE, Ercon SPCE, Dupont SPCE, Gwent SPCE and GCE, respectively.

The difference in charge transfer inhibition between the GCE and the SPCE is due to the chemical composition of electrode material (Morrin *et al.*, 2003). GCE is made with pure carbon whereas the screen-printable ink used for fabricating SPCE consists of graphite-carbon particles, organic solvents and a polymer binder (Wang *et al.*, 1996b; Seddon *et al.*, 1997; Wang *et al.*, 1998b; Stiene and Bilitewski, 2002). The organic solvents and polymer binders are added so as to achieve good printing conditions, but this limits the conductivity and also modifies the electrochemical behaviour by covering the individual graphite-carbon particles.

Apart from electron transfer kinetics, uncompensated resistance also exhibit the same variation of $\Delta E_{p,}$ with scan rate as observed in this work (BAS Capsule 274, 1988b). This means that uncompensated resistance could have contributed to the large shift in peak separation between the anodic and cathodic responses obtained for ferrocyanide as scan rate is increased at SPCE.

The electrical resistance between the electrical contact point and the working electrode area was also measured. An electrical resistance value of 3.53 ± 0.12 kΩ was obtained for the SPCE and a value of 5 ± 0.1 Ω for the GCE used in this work. Compared to GCE, the characteristically high electrical resistance value for SPCE implies that there are resistive elements in the carbon ink used for screen-printing.

Using the increase in peak separation (ΔE_p) obtained from the current – potential curves as scan rate is increased, the ease at which electron transfer take place on the SPCE surface, the heterogeneous electron transfer rate (k°), was assessed. Estimations were made of k^o using the method described by Nicholson (1965), with the working curves that relate ΔE_p to a kinetic parameter (ψ). This led to a calculated value of 1.36 x 10^{-4} cm s^{-1} for SPCE using the α value of 0.38. The k° value obtained for SPCE is in the range expected for quasi-reversible reactions (Erlenkotter *et al.*, 2000). Palchetti (1998) obtained a value of 7.4 x 10^{-4} cm s^{-1}, for SPCEs used for detecting heavy metal ions while Grennan *et al.* (2001) reported a value of 2.2 x 10^{-3} cm s^{-1} for SPCEs to be used in an amperometric biosensor system. For the GCE used in this work, a k° value of 2.69 x 10^{-3} cm s^{-1} was obtained. This value is lower than the values reported by Grennan *et al.* (2001) (1.2 x 10^{-2} cm s^{-1}) and Morrin *et al.* (2003) (5.9 x 10^{-2} cm s^{-1}). The decrease in the electron transfer rate value obtained for the SPCE compared to GCE can be attributed to, as mentioned before, the presence of polymeric binder in the screen-printing graphite-carbon ink.

6.2.1.1 Determination of the working area of the SPCE

Accurate knowledge of electrode's actual area taking into account the surface roughness is necessary for evaluating sensors and many electrochemical experiments. Chronoamperometry was employed for the determination of electrode area using potassium ferrocyanide (0.05 mM), a well-characterised anion as a redox probe. A fixed potential of + 0.8 V (vs. screen-printed Ag/AgCl reference electrode) was applied to the working electrode for 120 s. The chronoamperometry response data was transformed from Current vs. time to Current vs. $\text{time}^{1/2}$ (Figure 2.9), so as to obtain a linear relationship. Thus, the surface area of the SPCE calculated using the slope (1 x 10^{-6}) of the linear response and the Cottrell equation (see Equation 1.18, Chapter 1) rearranged for area (A), was found to be 0.146 ± 0.017 cm^2.

The electrochemical active area for the SPCE surface was also examined with CV using equation 1.12 (see Chapter 1). The values, 0.059 ± 0.006 cm^2 and 0.061 ± 0.002 cm^2 were calculated using slope values obtained from plots of anodic peak currents versus square root of scan rate (10 – 75 mV s^{-1}, see Figure 2.7) and for various concentrations of potassium ferrocyanide (0.5 mM – 5 mM at 50 mV s^{-1}, Figure 2.8), respectively. The apparent geometrical area of the SPCE estimated by simple measurement was found to be 0.130 cm^2 (0.65 cm x 0.2 cm).

Comparing these values, intuitively one might expect these values to be the same but they do differ here. The actual surface area of an electrode can be orders of magnitude greater than the electrochemical active area because the electrode surface might contain some non-conducting substances and this could have caused the electrode area determined by the CV method to be smaller. Since the SPCE surface is rough, the electrochemical active area should be larger compared to the geometric area, as the geometric area measurement is only a flat projection of the surface. In the experiments carried out, the chronoamperometric result was closer to the apparent geometric area while that of the CV is about half of the apparent geometric area obtained by simple measurement. Desmond *et al.* (1998) reported a CV value, which was a third of the overall geometrical area.

6.2.2 Characterisation of the screen-printed reference electrode

The on-board screen-printed Ag/AgCl reference electrode was found to be suitable for the precise control of the working electrode potential in aqueous media containing chloride ions. The pseudo reference electrode printed with silver/silver chloride ink is basically silver containing a small amount of silver chloride. Therefore, any medium solutions need to be supplemented with a constant concentration of chloride, as the potential of the screen-printed Ag/AgCl reference electrode is dependent on the concentration of chloride in the solution.

The effect of different chloride concentrations on the potential of the screen-printed Ag/AgCl reference electrode was examined by potentiometric measurements. This involved measuring the potential difference between the screen-printed reference electrode and a saturated calomel reference electrode (SCE). A logarithmic response for readings taken 15 minute after immersing the electrode strip in chloride solutions (concentration between 0.001 M and 1 M KCl) was obtained with a slope value of 60.2 ± 1.2 mV per decade. This value compares favourably with the slope value 55.46 mV per decade, reported by Desmond *et al.* (1998) and the theoretical Nernstian slope value, 59.1 mV per decade at 25 °C (Ahmad *et al.*, 2001).

The reproducibility of the screen-printed Ag/AgCl reference electrode potentials between different sensor strips was of high quality. A value of – 37.8 ± 4.8 mV was obtained when the potential differences between different screen-printed Ag/AgCl electrodes and a SCE, versus time were measured. This value was the mean potential difference for 6 electrodes versus SCE after 15 minutes in a saturated KCl solution. The measurement was performed over a 15 minute period because it is envisaged that electrochemical measurements involving the use of the screen-printed Ag/AgCl reference electrode would be over this time scale. Desmond *et al.* (1996; 1997; 1998) obtained values of – 55, 49.1 and – 49.7 mV, respectively by carrying out the same experiment. These values and the one obtained in this study, are within ± 11 mV of the theoretical (potential difference) value of – 44 mV expected for the following half-cells Ag | AgCl | KCl $_{\text{(saturated, aqueous)}}$ and Hg | Hg_2Cl_2 | KCl $_{\text{(saturated, aqueous)}}$ (Desmond *et al.*, 1997; 1998).

For a chloride concentration of 0.1 M, chosen to be used in subsequent measuring supporting medium, a potential difference between screen-printed Ag | AgCl | 0.1 M KCl pseudo reference electrode and a commercial Ag | AgCl | 3 M NaCl reference electrode of – 67 ± 1.5 mV was obtained. This value is within 20 mV of the theoretical value, - 87 mV (Erlenkotter *et al.*, 2000; Olschewski *et al.*, 2000). Erlenkotter *et al.* (2000) obtained a potential difference of – 72 mV while Reeder and Heineman (1998) reported a shift of - 82 mV due to the chloride concentration of the reference electrode changing from 3 to 0.1 M. The comparison between the value obtained in this study and the ones quoted by other researchers show that the screen-printed reference electrode is suitable to be used for electrochemical measurements.

6.3 HEAVY METAL IONS DETECTION ON SCREEN-PRINTED ELECTRODES

Using conventional electrochemical cells and classical bulky electrodes represent a problem for decentralised measurements, also large volume of sample solutions are needed. However, improvements can be achieved by their replacement with miniaturised and planar electrodes that can be used as drop on sensor (Palchetti *et al.*, 2001).

The coupling of electrochemical techniques with disposable screen-printed electrodes (SPEs), have been reported for the detection of heavy metals (Wang and Tian, 1992; 1993a; 1993b; Somasundrum and Bannister, 1993; Wang *et al.*, 1993; Neuhold *et al.*, 1995; Desmond *et al.*, 1996; 1998; Palchetti *et al.*, 1999; Honeychurch *et al.*, 2000; Choi *et al.*, 2001; Zen *et al.*, 2002; Masawat *et al.*, 2003; etc.). However, not a lot have involved the use of an integrated three-electrode system (screen-printed working, counter and reference electrodes), printed onto a planar substrate. Most are cases where two-electrode screen-printed system were used (Wang and Tian, 1992; 1993a; 1993b; Wang *et al.*, 1993; Palchetti *et al.*, 1999) or where the screen-printed working electrode is placed alongside conventional silver/silver chloride (Ag/AgCl) reference electrode and platinum counter electrode in a conventional electrochemical cell (Somasundrum and Bannister, 1993; Neuhold *et al.*, 1995; Palchetti *et al.*, 1999; Honeychurch *et al.*, 2000; etc.).

In this work, the detections of heavy metal ions were carried out on the SPE surface containing the integrated carbon working electrode, Ag/AgCl reference electrode and carbon counter electrode. To greatly enhance the analytical power and scope of the bare SPCE, the formation of another metal layer onto the electrode surface, favourable to the deposition of metal to be analysed was applied. This involved a nucleation and growth process of metal nuclei, with the deposit acting as nucleation site for the analyte metal (Bagel *et al.*, 2001). Mercury and bismuth ions were used *in situ* for the formation of metal layer (metallic film) onto the SPCE surface. Measurements on bare SPCE were also examined.

6.3.1 Mercury film screen-printed carbon electrode – Stripping voltammetric and chronopotentiometric detection of lead, cadmium and copper

In this work, the applicability and suitability of mercury film screen-printed carbon electrode (MFSPCE) to voltammetric and chronopotentiometric stripping analysis of heavy metal ions was examined. The MFSPCE was produced by *in situ* deposition of mercury (II) with the target analytes onto the bare SPCE. The mercury film formed on the SPCE strips behaved satisfactorily and allowed the detection of lead

(II), cadmium (II) and copper (II) with very close characteristics to what can be expected on mercury drop electrodes.

In situ plating of the bare SPCE was the chosen mode of forming mercury film on the electrode because it simplified and shortened the experimental procedure. Using SWASV, an optimised mercury (II) ion concentration of 80 mg l^{-1} was used for co-deposition with lead (II), cadmium (II) and copper (II) onto the SPCE, while with CCSCP, a mercury ion concentration of 10 mg l^{-1} was used for co-deposition with lead (II) and cadmium (II). A lower concentration was used for CCSCP measurements compared to SWASV because the concentration of mercury (II) governed the oxidation rate of the amalgamated lead and cadmium, and thus the sensitivity of the stripping signals obtained. The decrease in stripping chronopotentiometric peak areas (or stripping times) obtained upon increasing the mercury concentration is due to a fast oxidation rate at high mercury (II) concentration. This is in agreement with the findings of other researchers and with established theory (Adeloju *et al.*, 1996; Chow *et al.*, 1996), which states that the mercury (II) concentration is inversely proportional to the stripping peak area in stripping chronopotentiometry.

A 0.1 M HCl (pH 1.3) was chosen as the supporting electrolyte to use for separate lead (II) analysis on the basis of sensitivity and as well as the need for chloride ions in the test solution for stabilising the potential of the on-board screen-printed Ag/AgCl reference electrode (Desmond *et al.*, 1997; 1998; Choi *et al.*, 2001). For analysis involving cadmium, a supporting medium with pH greater than 3.5 was used due to the reduced sensitivity caused by the problem of hydrogen evolution at low pH. The sensitivity for cadmium was improved significantly in less acidic medium. Hence, a medium containing 0.1 M HCl and 0.5 M ammonium acetate was used because HCl is normally free from metal ion impurities compared to potassium chloride (KCl) (Kadara *et al.*, 2003) and ammonium acetate is added to increase the pH to around 4.6. Also the ammonium acetate was added with a view of increasing the viscosity of the medium for copper detection with CCSCP. Reasons for this would be discussed later (see Section 6.3.1.1).

With a view to sample analysis, the interference from surface-active substances and coexisting ions on the stripping chronopotentiometric responses of lead (II) and cadmium (II) were investigated. Although it is widely observed with ASV of amalgam-forming metal ions that surface active substances could decrease the stripping response (Wang and Taha, 1990; Komorsky-Lovrić and Branica, 1993), it was decided useful in this work to examine the extent to which these substances can affect the stripping chronopotentiometric response at MFSPCE as there is conflicting evidence in the literature as to the extent to which surface-active substances can interfere in stripping chronopotentiometric measurements (Wang and Tian, 1993a; Sahlin and Jagner, 1996; Town, 1999).

Since a constant applied current is used to effect oxidation, surface-active substances, which may affect the reversibility of oxidation in ASV, should have had no effect on the stripping time in CCSCP (Town, 1999). But in the experiments carried out in this work, a huge increase in the response of 50 $\mu g\ l^{-1}$ lead (II) was observed in the presence of Triton X-100 while in the presence of SDS and BSA, there was a slight increase. This increase in lead response is probably due to slower reoxidation of the amalgamated lead by free mercury ions that have not been used up in the formation complex surface structures. Wang and Tian (1993a) reported 1 and 18 % depression of lead response in the presence of SDS and albumin, respectively while in the presence of Triton X-100, a 12 % enhancement of the response was obtained. For Sahlin and Jagner (1996), the presence of Triton X-100 made no difference to the lead response using both CCSCP and PSA. However, Town (1999) found that Triton X-100 reduced the stripping response of lead.

Another major drawback of stripping analysis is that responses are often complicated by the formation of intermetallic compounds between metals deposited in the mercury electrode (Wang, 1985; Zen *et al.*, 2001). For example, copper, a common trace metal found in a variety of analytical samples, is notorious for interacting with other metals by forming intermetallic species as well as causing reduction in the other metal's stripping peak. Due to this, the effect of copper as well as cadmium on the stripping peak response of lead (II) was evaluated at the MFSPCE using CCSCP. The lead stripping response obtained was suppressed in the presence of 20 fold excess of copper (II). This suppression observed can be attributed to a surface phenomenon caused by the solubility of copper in the mercury film being exceeded at parts of the electrode surface. This results in the direct plating of copper and lead onto copper surfaces. The reason put forward here was adapted from similar suggestions made by Ostapczuk and Kublik (1977) for the suppression of the stripping peak of cadmium in the presence of copper at an *in situ* plated mercury film electrode.

The effect of 20 fold excess of cadmium (II) on lead (II) was found to be negligible. On the effect of copper and lead on the stripping response of 50 μg l^{-1} cadmium (II), there was a slight increase in the stripping response for cadmium in the presence of lead while in the presence of copper; there was a decrease in the cadmium stripping response. Similar effect was reported for anodic stripping voltammetric measurements of cadmium in the presence of copper (Ostapczuk and Kublik, 1977; Wise *et al.*, 1983).

The performance characteristics of the developed MFSPCE summarised in Table 6.1, did not suffer in comparison to other results reported for lead and cadmium detection at mercury film plated SPCEs considering the conditions applied in this work. For instance, Wang and Tian (1992) obtained limits of detection of 0.03 and 0.05 μg l^{-1} for lead and cadmium using SWASV while for PSA measurements, limits of detection of 0.3 and 0.4 μg l^{-1} were estimated for lead and cadmium, respectively. A longer deposition time of 600 s and a stirring step was employed in both cases. Wang *et al.* (1993) reported detection limits of 0.1 μg l^{-1} for SWASV measurements of lead using a deposition time of 240 s and 0.3 μg l^{-1} for PSA measurements of lead using a deposition time of 120 s. Employing DPASV with no stirring of solutions involved during the deposition step, Desmond *et al.* (1998) reported high detection limits of 71 and 64 μg l^{-1} for cadmium and lead, respectively using a longer deposition time of 300 s. Palchetti *et al.* (1999), using a stirring step as part of the measurement protocol, quoted detection limits of 0.4 and 1 μg l^{-1} using SWASV for lead and cadmium measurements and 0.6 and 0.4 μg l^{-1} for PSA measurements of lead and cadmium, respectively. A longer deposition time of 180 s was employed with the SWASV measurements while a shorter deposition time of 75 s was used for PSA measurements.

Table 6.1: Analytical data obtained using MFSPCE for stripping analysis of lead and cadmium (II) as reported in the present work.

Working electrode	**Stripping technique**	**Medium**	**Linear range (μg l^{-1})**	**Detection limit (μg l^{-1})**	**RSD (%)**
SPE plated with in situ with 80 ppm mercury (II)	SWASV	0.1 M HCl + 0.5 M ammonium acetate (pH 4.6)	Pb = 0 – 150 Cd = 0 -100 (120 s deposition)	Pb = 1 Cd = 2 (120 s deposition)	Pb = 6.7 Cd = 6.9
SPE plated with in situ with 10 ppm mercury (II)	CCSCP	0.1 M HCl (Pb) 0.1 M HCl + 0.5 M ammonium acetate (Cd)	Pb = 0 – 1000 Cd = 0 - 1000 (120 s deposition)	Pb = 1 Cd = 3 (120 s deposition)	Pb = 5.8 Cd = 6.9

6.3.1.1 Mercury film SPCE - Stripping chronopotentiometric measurement of copper

Although, ASV coupled to an integrated three-electrode system has been reported for the detection of copper (Desmond *et al.*, 1996; 1998; Choi *et al.*, 2001), the same cannot be said as far as we know about the use of constant current stripping chronopotentiometry (CCSCP) coupled to an integrated screen-printed three-electrode system apart from the work reported in this thesis and published in literature (Kadara *et al.*, 2003).

In this section, results obtained in the development of an electrochemically-based device for decentralised measurement of copper in extracted environmental samples using CCSCP are discussed. Despite the fact that the methodical principle and most of the experimental parameters are more or less an adaptation of already existing protocol, the emphasise in this work, was on dealing with the ways in reproducibly generating a stripping chronopotentiometric response for copper on SPCEs coated *in situ* with 10 mg l^{-1} of mercury (II). Because the dynamic linear range obtainable with SWASV for copper on mercury film SPE was narrow, CCSCP technique with its reasonably broad linear range (Beinrohr *et al.*, 1999) provided an opportunity to develop a screening device, which can be used over a broad concentration range for determining copper in environmental matrices. Also with this, there is no need to massively dilute the sample as much as occurs with SWASV, so as to obtain a linear standard addition plot.

The difficulty in obtaining a stable and well-defined stripping chronopotentiometric peak for copper on the screen-printed three-electrode system coated *in situ* with mercury film was resolved by using an increased concentration of supporting electrolyte in the test solution. The use of concentrated electrolytes as the supporting medium ensured that the background signal obscuring the copper stripping response on the SPCE (plated *in situ* with 10 mg l^{-1} mercury (II)) was suppressed and this allowed copper detection to be carried out. The large background signal obtained in the potential range - 0.4 to - 0.1 V is probably due to the inherent sensitivity of the CCSCP technique to the surface reaction occurring when the electrode material is anodised or exposed to dissolved oxygen. With SWASV measurements, this effect was negligible.

The well-defined peak obtained with 2 M HCl as well as 2 M KCl (see Figure 3.24 and 3.25, Chapter 3) in this work, showed that both of the highly concentrated chloride medium performed a role in reducing the residual dissolved oxygen, exposed to the SPCE working surface. Jagner *et al.* (1995) suggested that a concentrated electrolyte medium should be used for the determination of copper so that the dissolved oxygen concentration is low and the viscosity of the test solution, high. This is because both these properties reduce the chemical oxidation (stripping) rate. The same authors (Jagner and co-workers, 1995) and Green *et al.* (1997) also indicated that a concentrated chloride medium ensures that all copper measured is stripped as copper (I) chloride and not as a mixture of copper (II) and copper (I), as obtained with lower chloride concentrations (1 – 100 mM). Thus, the main chloride medium used for subsequent experiments was 2 M HCl, because it is normally free from metal ion impurities.

Other medium compositions were also examined, with studies carried out with ammonium acetate containing HCl because the pH of the medium composition would be suited to the simultaneous CCSCP detection of copper, lead and cadmium compared to a medium containing only HCl. The stripping peak for copper obtained using 1 M ammonium acetate containing 0.5 M HCl was much more stable and well-defined (see Figure 3.26b, Chapter 3) compared to one generated at a lower concentration (0.5 M ammonium acetate + 0.1 M HCl). The reason for this is, the increase in viscosity and the reduction in concentration of dissolved oxygen in the test solution (Jagner *et al.*, 1995).

Comparing the analytical data (detection limit, linear range, precision) obtained in this work to that reported in literature for mercury film screen-printed electrode, the data obtained did not suffer all that in comparison. The detection limits estimated for copper with the integrated screen-printed three-electrode system developed in this work, 6 μg l^{-1} (2 M HCl as supporting electrolytes) and 20.5 μg l^{-1} (1 M ammonium acetate containing 0.5 M HCl as supporting electrolytes) for a deposition time of 120 s, were much lower than the value of 123 μg l^{-1} reported by Desmond *et al.* (1998) for an integrated screen-printed three-electrode system using a deposition time of 300 s. Similar limit of detection to that obtained for copper detection using 2 M HCl (6 μg l^{-1}) as supporting electrolyte, was also estimated for SWASV measurement of copper in 0.1 M HCl + 0.5 M ammonium acetate (pH 4.6) in the work carried out in this thesis. Palchetti *et al.* (1999) obtained a detection limit of 8 μg l^{-1} (deposition time of 180 s) using SWASV. A SPCE preplated with mercury film was used alongside conventional reference and counter electrodes for the SWASV measurements. The same authors (Palchetti *et al.*, 1999) also obtained a detection limit of 0.8 μg l^{-1} (deposition time of 75 s) for PSA measurements using a screen-printed two-electrode system (screen-printed working and reference electrodes). Although the latter detection limit value was lower than obtained in this work, the measurement protocol used involved a stirring step for deposition, which is not the case in this work. The use of screen-printed three-electrode system used here alleviated the need to manipulate the electrodes into position as experienced most times with the use of conventional electrodes which most of the other researchers used in combination with their mercury film screen-printed carbon electrodes.

6.3.2 Bismuth-film screen-printed electrodes as metal ion sensors

Due to the toxicity of mercury, alternative electrode materials are highly desired for both centralised and field applications. With this in mind, bismuth, a "green" element with very low toxicity (Wang, 2002), was added to the test solutions in this work in the form of bismuth (III) so as to act as a source for bismuth film to be plated on the SPCE. The generation of the film *in situ* was selected mainly due to its simplicity (Vytras *et al.*, 2002).

The bismuth film SPCE (BFSPCE) in conjunction with CCSCP provided convenient detection of heavy metals, in particular, the determination of lead and cadmium at the ppb concentration levels. The use of bismuth film, plated *in situ* on the electrode resulted in an increase in the sensitivity of the stripping response of lead and cadmium compared to that attainable on bare SPCE. It is well known that bismuth forms binary or multi-component "fusing" alloys with lead and cadmium (Wang *et al.*, 2001b). Such alloy formation appears to be responsible for attractive and unique stripping performance of the bismuth film electrode.

An optimised bismuth (III) concentration of 500 $\mu g\ l^{-1}$ was chosen with respect to the concentration level of the model analytes (lead (II) and cadmium (II) ions) for co-deposition onto the bare screen-printed carbon electrode (SPCE). Flechsig *et al.* (2002), Hocevar *et al.* (2002a; 2002b) and Kefala *et al.* (2003) employed bismuth ions at the same concentration level for co-deposition with the target metal ions. The reoxidation peaks for cadmium and lead on the BFSPCE appeared at potentials of ~-

0.84 V and ~-0.61 V (compared to ~-0.76 V and ~-0.57 V on MFSPCE and ~-0.76 V and ~-0.63 V on SPCE), respectively (vs. screen-printed Ag/AgCl reference electrode). A time of 2 minutes was chosen for deposition, as this is a compromise between length of analysis and sensitivity. On the basis of sensitivity, resolution and reproducibility, a E_{dep} of – 1.0 V was employed for the simultaneous measurement of lead and cadmium. This is because at potentials more negative than – 1.0 V, the stripping peak for lead became distorted and less well defined probably due to hydrogen evolution and to interferences emanating from the electrode surface. For separate analysis, a E_{dep} of – 1.1 V was employed for cadmium and – 0.9 V for lead (II).

Using these conditions, the linear concentration range, detection limit and precision of measurements for the detection of lead (II) and cadmium (II) on BFSPCE were obtained. The calibration plot linear ranges derived for the detection of both metal ions, the detection limits and precisions are summarised in Table 6.2 alongside the analytical data obtained by other researchers using bismuth film working electrode for stripping analysis of lead and cadmium.

The linear ranges derived from the stripping chronopotentiometric measurements of the metal ions at the BFSPCE for a 120 s deposition time, was much wider than reported by Wang *et al.* (2000; 2001a), employing forced convection (stirring) while depositing the metal ions. Taking into consideration that measurements in this work were carried out without a stirring step, it can be expected that using an increased deposition time while stirring, would result in a lower detection limit comparable with those reported in the literature and tabulated in Table 6.2. However, this is not aim of the work carried out here which was to develop simple, portable and rapid sensing devices for metal ions detection in environmental matrices.

Table 6.2: Comparison of analytical data obtained using bismuth film electrode for stripping analysis of metal ions as reported in the literature and achieved in the present work.

Working electrode	Stripping technique	Medium	Linear range (μg l^{-1})	Detection limit (μg l^{-1})	RSD (%)	Reference
GCE and CPE coated *in situ* with 400 ppb Bismuth (III)	SWASV	0.1 M acetate buffer (pH 4.5)	0 – 150, 40 - 200 (120 s deposition)	Pb = 1.1 (120 s deposition, stirred)	Pb = 4.4 Cd = 2.4	Wang *et al.* (2000)
SPCE preplated with 100 ppm bismuth (III)	SWASV	0.1 M acetate buffer (pH 4.5)	10 – 100 (120 s deposition, stirred)	Pb = 0.3 (600 s deposition, stirred)	Pb = 7.4	Wang *et al.* (2001a)
GCE coated *in situ* with 500 ppb bismuth (III)	CCSCP	0.1 M acetate buffer (pH 4.5)	20 – 200 (120 s deposition, stirred)	Pb = 0.8 Cd = 0.2 (300 s deposition, stirred)	Pb = 2.5 Cd = 1.5	Hocevar *et al.* (2002a)
Electrically heated CPE plated *in situ* with 500 ppb bismuth (III)	SWASV	0.1 M acetate buffer (pH 4.5)	20 – 140 (120 s deposition, unstirred)	Pb = 3.16 (120 s deposition, unstirred)	-	Flechsig *et al.* (2002)
CPE plated *in situ* with 2.5 x 10 $^{-5}$ M (5.2 ppm) bismuth (III)	PSA	0.05 M HCl + 0.15 M KCl or 0.2 M acetate buffer (pH 4.5)	0 – 20, 0 – 75 (60s, 30 s deposition, stirred)	Pb = 0.5 (600 s deposition, stirred)	-	Vytras *et al.* (2002)
GCE plated *in situ* with 500 ppb bismuth (III)	SWASV	0.1 M acetate buffer (pH 4.5)	0 – 10, 2 – 20, 5 – 50, 10 -100 (120s, 180 s deposition, stirred)	Pb = 0.2 Cd = 0.2 (600 s deposition, stirred)	Pb = 3.2 Cd = 3.0	Kefala *et al.* (2003)
SPCE plated *in situ* with 500 ppb bismuth (III)	CCSCP	0.1 M HCl + 0.5 M ammonium actetate (pH 4.6)	20 – 300 (120 s deposition, unstirred	Pb = 10 Cd = 8 (120 s deposition, unstirred)	Pb = 4.5 Cd = 5.1	This work (see Section 3.3.4, Chapter 3)
SPCE plated *in situ* with 500 ppb bismuth (III)	CCSCP	0.1 M HCl	0 – 300 (120 s deposition, unstirred	Pb = 12 (120 s deposition, unstirred)	Pb = 5.6	This work (see Section 3.3.4, Chapter 3)

Although, higher detection limit compared to the MFSPCE described in this thesis was obtained, the BFSPCE can be envisaged as an attractive alternative for mercury free detection of lead and cadmium. With the exception of the work carried out in this thesis using BFSPCE (which parts of it has been accepted for publication in Analytical and Bioanalytical Chemistry Journal, Kadara and Tothill, 2004), the application of bismuth-coated screen-printed electrodes has only once been reported for the detection of metal ions. Wang *et al.* (2001a) reported its use for stripping voltammetric measurements of lead. The SPCE preplated with bismuth film was used in-conjunction with conventional reference and counter electrodes. For the studies carried out on bismuth film electrode in this thesis, an integrated screen-printed three-electrode system (working, counter and reference electrodes) was used, with the carbon working electrode plated *in situ* with bismuth film. The application of this BFSPCE for the detection of lead and cadmium could be said to be the first reported use of a disposable integrated sensor plated with bismuth film for the determination of heavy metals.

To study the possible interferences on CCSCP measurements of lead and cadmium at BFSPCE, different concentrations of copper and other co-existing ions (chromium, copper, iron, lead, cadmium and sulphate ions) were evaluated. This was done because of the inherent problem in electrochemical stripping analysis caused by the potential interaction between metal ions that have been co-deposited onto the electrode surface (Wise *et al.*, 1983; Wang, 1985). Copper (II) had the most significant effect. It was found to inhibit the stripping response of lead (II) and cadmium (II) at the BFSPCE. This is probably due to the competition between bismuth and copper for surface sites on the electrode as well as the formation of an intermetallic compound between copper-lead and copper-cadmium. Neither cadmium nor lead compete with bismuth for the surface site but rather form a binary alloy with bismuth. Also lead and cadmium can generally be determined together using stripping methods because their atoms do not interact in the metal layer formed during electrochemical reduction/deposition (Wagner *et al.*, 2003). To successfully circumvent the detrimental effect of copper (II), the selective masking of copper was carried out using ferricyanide in this work. Complete recovery of the lead stripping response in the presence of copper was obtained when 0.1 mM ferricyanide was added to the test solution. Other authors (Marques and Chierice, 1991; Crowley and Cassidy, 2002) have used cyanide and ferrocyanide to mask the effect of copper through the formation of a copper complex.

6.3.3 Bare screen-printed carbon electrode - stripping chronopotentiometric detection of lead (II), cadmium (II) and copper (II)

The feasibility of using the bare SPCE for detecting lead, cadmium and copper was investigated. The analysis carried out by electrochemical reduction and accumulation of lead (II), copper (II) and cadmium onto a bare SPCE was possible but brought about severe limitations. The bare SPCE responded to lead, cadmium and copper at concentrations too high to provide an interest in determination of the metals in lowly concentrated samples. The practical detection limits estimated were in the high µg l^{-1} concentration level. The detection limit obtained for the separate analysis of lead, cadmium and copper using CCSCP were 35, 45 and 59 µg l^{-1}, respectively for a deposition time of 120 s.

Honeychurch *et al.* (2000; 2002a) reported detection limits of 2.5 and 8.2 µg l^{-1} for DPASV measurements of lead and copper, respectively using a deposition time of 150 s while stirring the solutions via a magnetic bar. Although the detection limits obtained in the work carried out in this thesis for lead and copper detection on bare SPCE were lower, the bare SPCEs were used as drop-on sensors, so there is no need for stirring and also analysis performance is much simpler.

The difficulty in obtaining well-defined stripping chronopotentiometric peak for copper on the bare SPCE using 0.1 M HCl as supporting electrolyte in the copper oxidation range (– 0.3 to 0 V) was solved by electrochemically treating the electrode. This was done because in the copper oxidation range, the baseline exhibits a high background response over the potential interval from – 0.4 to 0.1 V. Similar effect was observed for the *in situ* plated MFSPCE described earlier. This background signal distorted the copper response. The large signal observed for the baseline and the distortion of the copper response, can be explained by the inherent sensitivity of the CCSCP technique to the surface reactions on the working electrode. When the electrode was pretreated at a potential of – 0.9 V for 120 s using 0.1 M HCl and then dried, the interfering signal was suppressed and undistorted copper stripping chronopotentiograms could be obtained.

As copper (II) is a common ion in variety of analytical samples, its effect on the stripping responses of lead was studied. The stripping response of lead (II) obtained on bare SPCE in the presence of copper was different from when it was on its own. For a 10 fold excess of copper (II), the lead stripping response was reduced down to 2 % of the original lead stripping response obtained in the absence of copper. Tibbetts *et al.* (2000) and Wagner *et al.* (2001) also reported marked decrease in lead signal on GCE when copper was present in solution with lead.

In order to reduce this effect, ferricyanide was added to the test solution as used for copper interference at BFSPCE to mask (via selective complexation) the copper in the solution. From the results obtained (see Table 3.3 and 3.4, Chapter 3), the copper interference on the stripping response of lead was reduced significantly when 0.1 mM ferricyanide was added to the test solution. In the literature, other methods have been employed to overcome the effect of copper. These includes the use of a copper electrode to force total lead-copper interaction (Tibbetts *et al.*, 2000), the use of potassium iodide to mask the copper interference (Honeychurch *et al.*, 2001a) and the use of a solid electrode modified by 2,2'-bipyridyl in a polyaniline matrix to form stable complexes of copper ions (Wagner *et al.*, 2001; 2002).

The effect of other metal ions on copper stripping response was also studied and it was found that the addition of lead caused the diminution of the copper response relative to a solution containing only copper. Also there was a small peak found between lead and copper signals. This behaviour can be explained in terms of the formation of a lead-copper solid solution and/or an intermetallic compound during the accumulation step, which gets oxidised and forms an additional peak between both metals during the stripping step (Tibbetts *et al.*, 2000; Wagner *et al.*, 2003).

6.4 DETECTION WITH MODIFIED SCREEN-PRINTED ELECTRODES

The use of chemically modified electrodes for trace metal analysis has been extensively reported in the literature (Dong and Wang, 1988; Arrigan *et al.*, 1994a; Arrigan *et al.*, 1994b; Wang *et al.*, 1996a; Chen *et al.*, 1999, etc.). Nevertheless, the number of physico-chemically modified sensing elements that have found real use in practical analyses is few (Stulik, 1999). This is because the modified sensors are often insufficiently stable both chemically and mechanically.

To achieve the aim of developing modified electrodes, which can be used for enhancing the stripping chronopotentiometric measurements of heavy metal ions at SPCE, a number of modifiers were coated onto the SPCE surface and also included in the ink matrix used in fabricating the SPCE.

6.4.1 Polyester sulphonic acid (PESA) and Nafion modified SPCE

PESA and Nafion were the first modifying reagents examined. They were chosen because they exhibit attractive permselective, ion-exchange and anti-fouling properties (Wang and Golden, 1989).

The PESA and Nafion modified electrodes were prepared by drop-coating 2 μl of the polymer (Nafion and PESA) solutions onto the SPCE electrode surface. The thickness of the each film formed on the electrode was studied and optimised. Lead (II) in 0.1 M HCl was used as the model analyte. The accumulation and reduction of the lead (II) ion was carried out simultaneously by applying a deposition potential of – 0.9 V (vs. screen-printed Ag/AgCl reference electrode) to the PESA and Nafion modified SPCEs. Attempts at accumulating lead (II) at open-circuit through ion-exchange and then reducing it at – 0.9 V, did not improve the sensitivity of the stripping response. Crowley and Cassidy (2002) reported a similar outcome for lead detection on Nafion modified GCE with the sensitivity greater when accumulating and reducing simultaneously at close-circuit.

The lead (II) stripping response obtained at the PESA modified electrode by simultaneously accumulating and reducing lead (II) was much smaller than those for the bare SPCE and Nafion modified SPCE. This could have been due to the thickness of the polymer film on the SPCE surface. Attempts at drop-coating lower amounts of the PESA solution onto the SPCE surface did not yield any improvement. Similar effect has been reported for PESA films by Wang and Taha (1990) and Brett and Fungaro (2000b), but with mercury film incorporated into the polymer matrix. They observed a lower peak response with PESA/mercury film electrode compared to bare mercury film electrode and this can be attributed to the diffusional barrier brought about by the PESA film affecting the uptake of metal ion from the test solution. In light of this observation, further work with the PESA modified electrode was not pursued.

The ability of the Nafion in accumulating lead (II) from the bulk solution was shown by the higher stripping response obtained on the Nafion modified SPCE compared to the bare SPCE unmodified SPCE. The reason of this improvement could be that the Nafion film to some extent repelled anions present in the test solution and therefore lowered the effective chloride activity at the screen-printed carbon working electrode surface. As well, the presence of Nafion on working electrode surfaces prevents adsorption or precipitation incidents (Ugo and Moretto, 1995).

In order to increase further the sensitivity, a complexing agent, Dimercapto-1,3,4-thiadiazole (DMTD), was incorporated within the Nafion matrix so as to preconcentrate the lead ions by both ion-exchange and complexation interactions. Nafion has been employed as an active matrix for incorporating ligands, which is then used for metal ion analysis (Dong and Wang, 1988; Chen *et al.*, 1999). However, contrary to expectations, there was no much of a difference (peak area or stripping time) between the stripping responses of lead (II) on the Nafion modified SPCE and the Nafion-DMDT modified SPCE. So further studies focussed on the Nafion modified SPCE.

The detection limit obtained for lead (II) at the Nafion modified SPCE, 22 μg l^{-1} following a deposition time of 120 s (or 18 μg l^{-1} for a deposition time of 180 s), was much lower than the one obtained for lead detection at bare SPCE (35 μg l^{-1}) using the same conditions. Although Palchetti *et al.* (2000) quoted slightly lower value, for lead at Nafion coated SPCE, 15 μg l^{-1}, their work involved performing measurement using a medium-exchange procedure and accumulating for 600 s at open circuit. The detection limit estimated in this work can be improved on by increasing the depositing time for detecting much lower concentration of lead (II).

6.4.2 Screen-printed DMTD modified electrode

DMTD, which has been used as a spectrophotometric reagent for lead and copper determinations (Ahmed and Mamun, 2001; Ahmed *et al.*, 2002) was examined as a modifier to include in the graphite-carbon ink used in producing the SPCE. DMTD has four donor sites and can coordinate as bidentate ligand (Huang *et al.*, 2001). As far is known, there have been no reports on the application of DMTD for the modification of SPCEs for the trace detection of lead (II). However there are a small number of articles published on the use of DMTD in conjunction with electrochemical stripping analysis (Li *et al.*, 1988; Maxwell and Smyth, 1996). Initial studies carried out by coating the SPCE with DMTD was promising, hence, screen-printed DMTD modified electrodes (5 % w/w) were produced by mixing 9.5 g of graphite carbon ink with 0.5 g of DMTD and then screen-printing the mixture. The complexing properties of DMTD were not compromised by its incorporation within the graphite-carbon ink or by the printing and drying processes. The use of DMTD as a modifier greatly increased the sensitivity of lead detection compared to the bare SPCE (unmodified). A lead (II) detection limit of 20 μg l^{-1} was estimated for the DMTD modified electrode compared to 35 μg l^{-1} for the bare SPCE.

The performance of the newly developed screen-printed DMTD modified electrode is based on the preconcentration of lead (II) from aqueous solution onto the surface of the modified electrode by the formation of complexes with the modifier. On the DMTD modified electrode, lead (II) was preconcentrated and reduced simultaneously on the surface of the electrode at – 0.9 V (vs. on-board Ag/AgCl reference electrode). Then, the reduced lead-DMTD complex was anodically stripped with a constant stripping current of 1 μA from – 0.9 V to 0.1 V. The reactions at the electrode are as follows:

(1) Preconcentration process

$$(Pb^{2+})_{solution} + (DMTD)_{surface} \longrightarrow (Pb^{2+}-DMTD)_{adsorption}$$

$$(Pb^{2+}-DMTD)_{adsorption} + 2e \longrightarrow (Pb^{0}-DMTD)_{adsorption}$$

(2) Stripping process

$$(Pb^{0}-DMTD)_{adsorption} \longrightarrow (Pb^{2+})_{solution} + (DMTD)_{surface} + 2e$$

6.4.3 Screen-printed bismuth oxide modified electrode

The focus of the work carried out here, was to prepare a Bi_2O_3 modified electrode by directly modifying the screen-printing graphite-carbon ink with solid oxide and then printing the mixture onto the working surface of the SPCE so that it can serve as a source of bismuth film *in situ* generated for stripping analysis of heavy metals. Bulk modified electrodes prepared using Bi_2O_3 and HgO have already been described for metal ion analysis and these electrodes exhibit good performance (Seo *et al.*, 1998; Choi *et al.*, 2001; Krolicka *et al.*, 2002; Lee *et al.*, 2002; Pauliukaite *et al.*, 2002).

Evaluating the potential window of the screen-printed Bi_2O_3 modified electrode using 0.1 M HCl + 0.5 M ammonium acetate solution (pH 4.6), the potential range (-1.2 to - 0.33 V) obtained was sufficient for detecting both lead (II) and cadmium (II) because their oxidation peak potentials falls into this range. Within the potential range obtained for 0.1 M HCl, lead could be detected but not cadmium. This was due to the early commencement of the hydrogen evolution on the electrode, which obscured the cadmium-stripping peak. The results obtained with the use of the screen-printed Bi_2O_3 modified electrode for lead (II) and cadmium (II) detection indicated that the excellent results obtained at the bismuth film SPCE (*in situ* plated) can be replicated to an extent. The only downside is that the simultaneous detection of cadmium (II) and lead (II) was not possible and this was due to the lead stripping peak being distorted at a deposition potential of – 1.0 V. The advantage of this screen-printed Bi_2O_3 modified electrode is that there is no need to add bismuth (III) ions to the solution in order to generate bismuth film.

6.4.4 Screen-printed PEI modified electrode

0.5 % Polyethylenimine (PEI) was incorporated into the graphite-carbon ink matrix and then screen-printed onto the working surface of the SPCE. The purpose for using PEI to modify the SPCE was to reduce the interactions between lead and copper on the electrode surface. But when copper (II) and lead (II) were simultaneously measured in 0.1 M HCl using CV, the copper oxidation peak area obtained with the PEI modified electrode was more than twice that obtained with the unmodified bare SPCE. The lead oxidation peak in the presence of copper on the modified electrode was smaller compared to that obtained at the unmodified bare SPCE.

The result obtained here indicates that the PEI incorporated into the electrode plays a role in preconcentrating slightly more of the copper onto the electrode surface. However, work reported by Osipova *et al.* (1997; 1998; 2000) have shown that the PEI is used to weaken the effect of copper on lead, cadmium and silver. This effect was also observed when stripping chronopotentiometric measurements were performed on lead and copper in the same solution. The peak that appeared on bare SPCE between lead and copper at – 0.43 V was eliminated when PEI modified electrode was used. The interaction between lead and copper as discussed earlier has significant effect on the magnitude of the responses of both lead and copper. Hence, the inclusion of PEI, as a modifier into the working electrode ink matrix can help to lower this intermetallic interaction.

With the PEI modified electrode, the CCSCP response for copper obtained was slightly enhanced compared to that at the unmodified bare SPCE and this correlates with the results obtained with CV.

6.5 SAMPLE ANALYSIS

The analysis of sediment and soil extract solutions for copper, lead and cadmium using stripping analysis is significantly more challenging than a water matrix. Due to the need for metal detection in soil or its extracts, there have been a lot of request recently by environmental companies and pollution monitoring agencies for decentralised monitoring equipments that can produce reliable results in field conditions.

The potential of the screen-printed electrochemical sensors in determining lead, copper and cadmium in environmental samples were examined for the BFSPCE, MFSPCE, bare SPCE, Nafion modified SPCE, screen-printed DMTD modified carbon electrode, screen-printed PEI modified carbon electrode and screen-printed bismuth oxide carbon electrode.

The adequacy of CCSCP and SWASV coupled to the screen-printed three-electrode system (mercury film SPCE) for the determination of lead, cadmium and copper was first verified by the use of a reference material (CRM 601, a lake sediment) certified with the BCR protocol. Concentrations of the metal ions in the acetic-acid fraction of the reference material were determined by standard addition method. The concentration value obtained for the acetic-acid extractable lead, copper and cadmium fractions using CCSCP were close to the ICP-MS analysis result (see Table 5.5 and 5.6, Chapter 5) as well as the indicative values (2.28 ± 0.44 mg kg^{-1} for lead, 10.5 ± 0.8 mg kg^{-1} for copper and 4.45 ± 0.67 mg kg^{-1}) quoted in published literature

(Quevauviller, 2002) as the concentration for lead, copper and cadmium in CRM 601 reference material extracted with acetic-acid.

Successful progression to measuring the concentrations of these metal ions in various soils, sediments and water samples, showed that the different screen-printed electrochemical sensors coupled to CCSCP are excellent tools for the screening of metal ions. Mobile lead (II), copper (II) and cadmium (II) ions in soils or sediments were determined after sequential extraction with water (free copper), acetic acid (exchangeable copper, lead and cadmium) and aqua regia (pseudo total lead, copper). The last 2 are well-established methodologies for the determination of labile ions and the pseudo total content in soil and sediment samples respectively, while the first extraction was included because it is a compulsory test in several EU countries regulations.

In addition to determining lead, copper and cadmium in wastewater samples, soil and sediment samples extracts obtained from contaminated sites, the sensing devices were employed at a site (Aznalcollar, Spain) contaminated by metal mining activities in order to demonstrate their capabilities in addressing environmental problems in the field. Results obtained for samples analysed both in the laboratory and in the field with CCSCP using BFSPCE, bare SPCE, MFSPCE, Nafion modified SPCE, screen-printed DMTD, Bi_2O_3 and PEI modified electrodes were comparable to ones obtained with ICP-MS, apart from a few which were not. These were due to practical problems, the most serious being the undesirable effect of copper present in significant proportion in the samples.

The effect of copper on the results for lead was addressed when 0.1 mM ferricyanide was included into the measurement protocol. This inclusion of ferricyanide into the measurement protocol came about from the *in situ* measurement of metal ions at the Aznalcollar contaminated site. The results obtained there allowed for the further optimisation of the sensing devices in the lab subsequently so as to produce sensors which can measure various real and complex matrices as required for on-site measurements.

As the results obtained for the samples have shown, the performances of the sensors have helped in speeding up the development of portable devices as they permit simple and rapid measurements of metal ions.

CHAPTER 7

• 7 CONCLUSIONS & FUTURE WORK

7.1 CONCLUSION

The work described in this thesis has detailed the development process undertaken for the production of disposable electrochemical screen-printed sensors for heavy metals detection in environmental matrices. The process included the fabrication of the electrodes and characterisation, which enabled various sensing devices to be established and used for metal ion detection and determination. Below is a summary of the findings:

- The electrodes fabricated from thick film inks provided a convenient base for electrochemical metal ion sensors as well as being cheap to produce.

- The characterisation of the screen-printed electrode (SPE) showed that its surface structure and electrochemical activity is dependent on the electrode material. The screen-printed carbon electrode (SPCE) did not display the ideal reversible behaviour that might be expected for potassium ferrocyanide. This unattractive behaviour suggests a low graphite-carbon loading.

- Although, the SPCE was not ideally suited for the study of electrochemical kinetics, this does not preclude their use in electroanalysis. Under acidic condition (pH 1.2), the SPCE is operable in the potential range – 0.7 V to ~ + 0.8 V, while in slightly acidic condition (pH 4.6), the SPCE is operable in the potential range – 1.1 V to ~ + 1.1 V.

- The screen-printed reference electrode was suitable for the precise control of the working electrode potential in aqueous media if supplemented with a constant concentration of chloride (0.1 M), as the potential of the screen-printed silver/silver chloride reference electrode is dependent on the concentration of chloride in the solution.

- The fabricated three-electrode configuration system coupled with SWASV and CCSCP provided a means of a relatively inexpensive on-site detector for trace levels of lead (II), copper (II) and cadmium (II). Detection of these metals was carried out on mercury film screen-printed carbon (MFSPCE), bismuth film screen-printed carbon electrode (BFSPCE), bare SPCE and the various modified SPCEs using the optimised procedures developed for measurements.

- The preconcentration of lead, copper and cadmium metallic phase from aqueous solutions was favoured when co-depositing with either mercury or bismuth film onto the bare electrode surface.

- The bare SPCE acted as supports for the *in situ* plating of bismuth and mercury films. With the optimised working conditions, the results obtained indicate that the screen-printed electrochemical sensors are sensitive and reproducible enough for the CCSCP and SWASV determination of lead, copper and cadmium in the parts per billion range.

- The linear concentration range obtainable with SWASV was narrow compared to CCSCP. Hence, CCSCP was the preferred measurement technique used due to the fact that it's reasonably broad linear range provided an opportunity to develop a screening device, which can be used over a broad concentration range for determining lead, copper and cadmium in samples, with no need to massively dilute the sample solution.

- Limits of detection below 20 $\mu g\ l^{-1}$ were obtained for lead (II) and cadmium (II) detection at BFSPCE and MFSPCE coupled to CCSCP. The reproducibility of the measurements (RSD, %), which also contributed to interest in the electrochemical system, was below 15 % for both film electrodes.

- Although the performance of the BFSPCE used for CCSCP measurements measured up to those at the MFSPCE, the only downside is the undesirable effect of copper. To eliminate the detrimental effect of copper (II), ferricyanide was used to complex the copper (II).

- The sensitivity of measurement on bare SPCE was lower compared to that on MFSPCE and BFSPCE, but that did not hinder the detection of lead (II), copper (II) and cadmium in test solutions with high concentrations. Detection limits were 35, 45 and 59 $\mu g\ l^{-1}$ for lead, cadmium and copper, respectively.

- A range of chemically modified electrodes have been prepared and tested for heavy metal ion detection. This concept allowed construction of sensitive electrochemical sensors for the metal ions.

- The screen-printed DMTD modified electrode described was suitable for the single determination of lead as it is characterised by a low detection limit (20 $\mu g\ l^{-1}$) even if it is not as good as values obtained for BFSPCE (10 $\mu g\ l^{-1}$) and MFSPCE (1 $\mu g\ l^{-1}$). The main advantage of this electrode is that it is mercury free and is more sensitive than the bare SPCE.

- The other modified electrodes (Nafion modified SPCE, screen-printed bismuth oxide modified electrode and screen-printed polyethyleneimine modified electrode) showed promise for the detection of lead, cadmium and copper, with detection limits lower than that attainable on the bare SPCE.

- For environmental samples, the usefulness of CCSCP coupled to screen-printed electrodes (SPEs) for the determination of lead and cadmium in water samples and lead, copper and cadmium in extracts obtained by solid-liquid phase extraction of soil (or sediments) samples was evaluated. Overall, the results show that the developed screen-printed electrochemical sensors are capable of measuring copper, lead and cadmium in water samples and in soil (or sediments) samples.

- The results of the sample analysis carried out in this work could help in speeding up the development of these devices.

- The outcome of this thesis should be more rapid uptake of these electrochemical sensor devices in the toolkits of consultants and contractors working in integrated water-soil management. This will therefore speed up the optimisation of remedial measures and allow better quality control of interventions to manage pollution. These sensors will bring savings in time and cost to current methods and extra useful information in solving environmental problems from heavy metal contamination.

7.2 FUTURE WORK

- Although the application of the integrated screen-printed electrodes for electrochemical sensing has been highlighted in this work, further work needs to be carried out in improving the characteristics of the graphite-carbon based ink used in printing the carbon-working electrode. This could be achieved through developing custom-made inks with the right electrochemical characteristics for the detection of heavy metals.

- The design of the electrode could be changed from its single electrode format into a microdisk array format. Combining the attractive features of the microelectrode array and screen-printing technology could produce highly reproducible microdisk arrays with enhanced deposition efficiency and large "collective" response.

- With the deposition of metal ions at the surface of the carbon electrode surface made much more possible by the presence of modifying agents that can specifically bind to the considered analyte (metal ion), more novel ligands and ion-exchangers that could increase the sensitivity and reduce the detection limits of analysis need to be examined. This could involve the direct bulk modification of the graphite-carbon ink with chemical reagents (printable ligands with metal complexing properties).

- Application of the BFSPCE and screen-printed DMTD modified electrode to evaluating concentrations of metal ions in field conditions.

REFERENCES

Adeloju, S.B., Sahara, E. and Jagner, D. (1996). Anodic Stripping Potentiometric Determination of Cu, Pb, Cd, and Zn in Natural Waters on a Novel Combined Electrode System. *Analytical Letters* **29(2)**, 283 – 302.

Adeloju, S.B. and Hadjichari, A. (1999). Simultaneous Determination of nickel and cobalt in natural water and sediment samples on an in-situ plated mercury film electrode by adsorptive cathodic stripping voltammetry. *Analytical Sciences* **15**, 95-100.

Ahmad, R., Cartwright, M. and Taylor, F. (2001). *Analytical Methods for Environmental Monitoring,* Pearson Education Limited.

Ahmed, M.J. and Mamun, M-A. (2001). Spectrophotometric determination in industrial, environmental, biological and soil samples using 2,5-dimercapto-1,3,4-thiadiazole. *Talanta* **55**, 43-54.

Ahmed, M.J., Jahan, I. and Banoo, S. (2002). A simple spectrophotometric method for the determination of copper in industrial, environmental, biological and soil samples using 2,5-dimercapto-1,3,4-thiadiazole. *Analytical Sciences* **18,** 805-810.

Alloway, B.J. (1990). *Heavy Metal in Soils*, John Wiley and Sons.

Argyraki, A., Ramsey, M.H. and Potts, P.J. (1997). Evaluation of portable x-ray fluorescence instrumentation for in situ measurements on lead on contaminated land. *Analyst* **122**, 743-749.

Arrigan, D.W.M., Svehla, G., Alderman, J. and Lane, W.A. (1994a). Ionophore/ionophore films on glassy carbon electrodes for accumulation voltammetry - Investigation of a Lead (II) ionophore. *Analyst* **119**, 287 – 291.

Arrigan, D.W.M., Svehla, G., Harris, S.J. and McKervey, M.A. (1994b). Use of Calixarenes as modifiers of carbon paste electrode for Voltammetric Analysis. *Electroanalysis* **6**, 97 - 106.

Arrigan, D.W.M. (1994). Voltammetric determination of trace metals and organics after accumulation at modified electrodes. *Analyst* **119**, 1953-1965.

Bachmann, T.T. (1999). Development of disposable sensors for rapid multianalyte detection: Acetylcholinesterase and microbial biosensors. *PhD thesis*, Institute of Technical Biochemistry, University of Stuttgart, Germany.

Bagel, O., Lagger, G., Girault, H.H., Brack, D, Loyall, U. and Schafer, H. (2001). Mercury free determination of lead by differential pulse anodic stripping voltammetry onto silver-plated rotogravėd carbon electrodes. *Electroanalysis* **13(2)**, 100-103.

Baird, C. (1999). *Environmental Chemistry,* 2nd Ed. W.H Freeman and Company.

Bard, A.J. and Zoski, C.G. Voltammetry Retrospective. *Analytical chemistry* (2000). **72(9)**, 346A-352A.

Bard, A.J. and Faulkner, L.R. (2001). *Electrochemical methods – Fundamentals and applications.* 2nd Edition, John Wiley & Sons, Inc., New York.

BAS Capsule 141. (1988a). Determination of heterogeneous electron transfer rate constant. *Preliminary notes and applications from Bioanalytical Systems, Inc.*

BAS Capsule 274. (1988b). Calculation of the standard heterogeneous charge transfer rate contant (ks) for Dimeric Iron Fulvalene complexes using cyclic voltammetry. *Preliminary notes and applications from Bioanalytical Systems, Inc.*

Beinrohr, E., Csemi, P., Manova, A. and Dzurov, J. (1994). Absolute analysis of trace metals through galvanostatic stripping chronopotentiometry with signal accumulation. *Fresenius J. Anal. Chem.* **349**, 625-632.

Beinrohr, E., Čaktr, M., Dzurov, J., Kottaš, P. and Kozakova, E. (1996). Calibrationless determination of mercury by flow-through stripping coulometry. *Fresenius J. Anal. Chem.* **356**, 253-258.

Beinrohr, E., Čakrt, M., Dzurov, J., Jurica, L. and Broekaert, J.A.C. (1999). Simultaneous calibrationless determination of zinc, cadmium, lead, and copper by flow-through stripping chronopotentiometry. *Electroanalysis* **11(15)**, 1137-1144.

Bernick, M.B., Kalnicky, D.J., Prince, G. and Singhui, R. (1995). Results of field-portable x-ray fluorescence analysis of metal contaminants in soil and sedimnent. *Journal of Hazardous Materials* **43**, 101-110.

Bersier, P.M., Howell, J and Bruntlett, C. (1994). Advanced Electroanalytical Techniques Versus Atomic Absorption Spectrometry, Inductively Coupled Plasma Atomic Emission Spectrometry and Inductively Coupled Plasma Mass Spectrometry in Environmental Analysis. *Analyst* **119**, 219 – 232.

Blake, U.A., Blake II, R.C., Khosraviani, M. and Pavlov, A.R. (1998). Metal Ion Immunoassays. *Analytica Chimica Acta* **376**, 13 – 19.

Blake, D.A., Jones, R.M., Blake II, R.C., Pavlov, A.R., Darwish, I.A. and Yu, H. (2001). Antibody-based sensors for heavy metal ions. *Biosensors and Bioelectronics* **16**, 799-809.

Bond, A.M., Mahon, P.J., Schiewe, J. and Vicente-Beckett, V. (1997). An inexpensive and renewable pencil electrode for use in field-based stripping voltammetry. *Analytica Chimica Acta* **345**, 67 – 74.

Bonfil, Y., Brand, M. and Kirowa-Eisner, E. (1999). Determination of Sub-µg/l Concentrations of Copper by Anodic Stripping Voltammetry at the Gold Electrode. *Analytica Chimica Acta* **387,** 85-95.

Bonfil, Y. and Kirowa-Eisner, E. (2002). Determination of nanomolar concentrations of lead and cadmium by anodic-stripping voltammetry at the silver electrode. *Analytica Chimica Acta* **457**, 285-296.

Bontidean, I., Berggren, C., Johansson, G., Csoregi, E., Mattiasson, B., Lloyd, J.R., Jakeman, K.J. and Brown, N.L. (1998). Detection of heavy metal ions at femtomolar levels using protein-based biosensors. *Analytical Chemistry,* **70(19),** 4162 – 4169.

Bott, A.D. (2000). Controlled current techniques. *Current Separations* **18(4),** 125-127.

Brett, C.M.A. and Fungaro, D.A. (2000a). Modified electrode voltammetric sensors for trace metals in environmental samples. *J. Braz. Chem. Soc.* **11(3),** 298–303.

Brett, C.M.A. and Fungaro, D.A. (2000b). Poly(ester sulphonic acid) coated mercury thin film electrodes: characterisation and application in batch injection analysis stripping voltammetry of heavy metal ions. *Talanta* **50**, 1223–1231.

Brett, C.M.A., Angnes, L. and Liess, H-D. (2001). Carbon film resistors as electrodes: Voltammetric properties and application in electroanalysis. *Electroanalysis* **13 (8-9)**, 765-769.

Chave, P. (2001). *The EU Water Framework Directive – An introduction.* IWA Publishing, Cornwall, U.K.

Chen, Z., Pourabedi, Z., Hibbert, D.B. (1999). Stripping voltammetry of Pb (II), Cu (II), and Hg (II) at a Nafion-coated glassy carbon electrode modified by neutral ionophores. *Electroanalysis* **11(13)**, 964-968.

Choi, J-Y., Seo, K., Cho, S-R., Oh, J-R., Kahng, S-H. and Park, J. (2001). Screen-printed anodic stripping voltammetric sensor containing HgO for heavy metal analysis. *Analytica Chimica Acta* **443**, 241-247.

Chow, C.W.K., Kolev, S.P., Davey, D.E. and Mulcahy, D.E. (1996). Determination of Copper in Natural Waters by Batch and Oscillating Flow Injection Stripping Potentiometry. *Analytica Chimica Acta* **330**, 79 – 87.

Compagnone, D., Lupu, A.S., Ciucu, A., Magearu, V., Cremisini, C. and Palleschi, G. (2001). Fast Amperometric FIA procedure for heavy metal detection using enzyme inhibition. *Analytical letters* **34(1)**, 17-27.

Concerted Actions CLARINET, NICOLE, ETCA and SENSPOL - Joint Statement. (2000). *Concerted Actions of the EC Environment and Climate Research and Development Programme: Management of Contaminated Land for the Protection of Water Resources.* http://www.clarinet.at/ (accessed on 09/01/2001).

Crowley, K. and Cassidy, J. (2002). Trace analysis of lead at a Nafion-modified electrode using square-wave anodic stripping voltammetry. *Electroanalysis* **14(15-16)**, 1077-1082.

Cui, G., Yoo, J.Y., Lee, J.S., Yoo, J., Uhm, J.H., Cha, G.S. and Nam, H. (2001). Effect of pre-treatment on the surface and electrochemical properties of screen-printed carbon paste electrodes. *Analyst* **126**, 1399-1403.

Daniele, S., Bragato, C. and Baldo, M.A. (1997). An approach to the calibrationless determination of copper and lead by anodic stripping voltammetry at thin mercury film microelectrodes. Application to well water and rain. *Analytica Chimica Acta* **346**, 145 - 156.

Darwish, I.A. and Blake, D.A. (2001). One-step competitive immunoassay for cadmium ions: Development and validation for environmental water samples. *Analytical Chemistry* **73**, 1889-1895.

Desmond, D., Lane, B., Alderman, J., Hall, G., Alvarez-Icaza, M., Garde, A., Ryan, J., Barry, L., Svehla, G., Arrigan, D.W.M. and Schniffner, L. (1996). An ASIC-Based System for Stripping Voltammetric Determination of Trace Metals. *Sensors and Actuators B* **34**, 466 – 470.

Desmond, D., Lane, B., Alderman, J., Glennon, J.D., Diamond, D. and Arrigan, D.W.M. (1997). Evaluation of miniaturised solid state reference electrodes on a silicon based component. *Sensors and Actuators B* **44**, 389-396.

Desmond, D., Lane, B., Alderman, Arrigan, D.W.M. and Glennon, J.D. (1998). An Environmental Monitoring System for Trace Metals Using Stripping Voltammetry. *Sensors and Actuators B* **48**, 409 – 414.

Dong, S. and Wang, Y. (1988). Anodic stripping voltammetric determination of trace lead with a Nafion/crown-ether film electrode. *Talanta* **35(10)**, 819-821.

Driscoll, J.N., Marshall, S.K., Wood, C. and Spittler, T. (1991). A mult-functional portable XRF instrument for measurement of heavy metals and radioactivity in mixed waste sites. *Am. Lab.* **23**, 25-36.

Erlenkotter. A., Kottbus, M. and Chemnitius, G.C. (2000). Flexible Amperometric Transducers for Biosensors Based on a Screen-Printed Three-Electrode System. *Journal of Electroanalytical Chemistry* **481**, 82 – 94.

Esteban, M. and Casassas, E. (1994). Stripping Electroanalytical Techniques in Environmental Analysis. *Trends in Analytical Chemistry* **13(3)**, 110 – 117.

Estela, J.M., Tomas, C., Cladera, A. and Cerda, V. (1995). *Critical Reviews in Analytical Chemistry* **25(2)**, 91 – 119.

Ewing, G.W. (1994). *Analytical Instrumentation Handbook,* 2nd Edition, Revised & Expanded.

Fennouh, S., Casimiri, V., Geloso-Meyer, A. and Burstein, C. (1998). Kinetic study of heavy metal salt effects on the activity of L-lactate dehydrogenase in solution or immobilised on an oxygen electrode. *Biosensors and Bioelectronics* **13**, 903-909.

Fisher, A.C. (1996). *Oxford Chemistry Primers: Electrode Dynamics,* Oxford University Press, New York.

Fisher, E. and van den Berg, C.M.E. (1999) Anodic stripping voltammetry of lead and cadmium using a mercury film electrode and thiocyanate. *Analytica Chimica Acta* **385,** 273-280.

Flechsig, G-U., Korbout, O., Hocevar, S.B., Thongngamdee, S., Ogorevc, B., Grundler, P. and Wang, J. (2002). Electrically heated bismuth-film electrode for voltammetric stripping measurements of trace metals. *Electroanalysis* **14(3)**, 192-196.

Galan-Vidal, C.A., Munoz, J., Dominguez, C. and Alegret, S. (1995). Chemical Sensors, Biosensors and Thick-Film Technology. *Trends in Analytical Chemistry* **14(5)**, 225 – 231.

Galan-Vidal, C.A., Munoz, J., Dominguez, C. and Alegret, S. (1998). Glucose biosensor strip in a three-electrode configuration based on composite and biocomposite materials applied by planar thick flim technology. *Sensors and Actuators B – Chemical* **52**, 257-263.

Galus, Z. (1976). *Fundamentals of Electrochemical Analysis.* Ellis Horwood, Chichester.

Gil, E.D. and Ostapczuk, P. (1994). Potentiometric stripping determination of mercury (II), selenium (IV), copper (II) and lead (II) at a gold film electrode in water samples. *Analytica Chimica Acta* **293**, 55-65.

Gopel, W., Hesse, J. and Zemel, J.N. (1993). Sensors: *A Comprehensive Survey* Vol. 8, VCH Publishers.

Grabarczyk, M. and Korolczuk, M. (2003). Determination of labile chromium in water samples by catalytic adsorptive stripping voltammetry in on-line system. *Electroanalysis* **15(5-6)**, 524-528.

Green, A.M., Clark, A.C., Scollary, G.R. (1997). Determination of free and total copper and lead in wine by stripping potentiometry. *Fresenius J. Anal. Chem.* **358,** 711-717.

Grennan, K., Killard, A.J. and Smyth, M.R. (2001). Physical Characterisation of a screen-printed electrode for use in an amperometric biosensor system. *Electroanalysis* **13(8-9)**, 745-750.

Greulach, U. and Henze, G. (1995). Analysis of Arsenic (V) by CSV. *Analytica Chimica Acta* **306**, 217 - 223.

Gunzler, H. and Williams, A. (2001). *Handbook of Analytical Techniques.* Vol. 2, Wiley – VCH Verlag GmbH, D-69469 Weinheim (FRG).

Gyurcsányi, R.E., Cristalli, A., Nagy, G., Nagy, L., Corder, C., Pendley, B.D., Ufer, S., Nagle, H.T., Neuman, M.R. and Lindner, E. (2001). Analytical performance characteristics of thin and thick film amperometric microcells. *Fresenius J. Anal. Chem.* **369**, 286-294.

Her Majesty Industrial Pollution Inspectorate. (1999). *Croner's Environmental Management,* Croner Publications Ltd.

Hocevar, S.B., Wang, J., Deo, R.P., Ogorevc, B. (2002a). Potentiometric stripping analysis at bismuth-film electrode. *Electroanalysis* **14(2)**, 112-115.

Hocevar, S.B., Ogorevc, B., Wang, J., Pihlar, B. (2002b). A study on operational parameters for advanced use of bismuth film electrode in anodic stripping voltammetry. *Electroanalysis* **14(24)**, 1707-1712.

Honeychurch, K.C., Hart, J.P. and Cowell, D.C. (2000). Voltammetric Behaviour and Trace Determination of Lead at a mercury-free screen-printed carbon electrode. *Electroanalysis* **12(3)**, 171 – 177.

Honeychurch, K.C., Hart, J.P. and Cowell, D.C. (2001a). Voltammetric studies of lead at a 1-(2-Pyridylazo)-2-Naphthol modified screen-printed carbon electrode and its trace determination in water by stripping voltammetry. *Analytica Chimica Acta* **431**, 89 – 99.

Honeychurch, K.C., Hart, J.P., Cowell, D.C. and Arrigan, D.W.M. (2001b). Voltammetric studies of lead at calixarene modified screen-printed carbon electrodes and its trace determination in water by stripping voltammetry. *Sensors and Actuators B* **77**, 642-652.

Honeychurch, K.C., Hawkins, D.M., Hart, J.P. and Cowell, D.C. (2002a). Voltammetric behaviour and trace determination of copper at a mercury-free screen-printed carbon electrode. *Talanta* **57**, 565-574.

Honeychurch, K.C., Hart, J.P., Cowell, D.C. and Arrigan, D.W.M. (2002b). Voltammetric behaviour and trace determination of cadmium at a calixarene modified screen-printed carbon electrode. *Electroanalysis* **14(3)**, 177-185.

Honeychurch, K.C. and Hart, J.P. (2003). Screen-printed electrochemical sensors for monitoring metal pollutants. *Trends in Analytical Chemistry* **22(7-8)**, 456-469.

Huang, L., Tang, F., Hu, B., Shen, J., Yu, T. and Meng, Q. (2001). Chemical reactions of 2,5-Dimercapto-1,3,4-thiadiazole (DMTD) with metallic copper, silver, and mercury. *J. Phys. Chem. B* **105**, 7984-7989.

Jagner, D., Sahlin, E. and Renman, L. (1995). Experimental and computational study of species formed during electrochemical stripping oxidation of copper in chloride media – Determination of copper (II) in the ng/l range by stripping potentiometry. *Talanta* **42**, 1447 – 1455.

Kabata-Pendias, A. (1995). Agricultural problems related to excessive trace metal contents of soils. In: Salomons, W., Forstner, U. and Mader, P. (Eds.) *Heavy metals: Problems and Solutions.* Springer-Verlag, Berlin Heidelberg, pp 3-18.

Kadara, R.O., Newman, J.D. and Tothill, I.E. (2003). Stripping chronopotentiometric detection of copper using screen-printed three-electrode system – application to acetic acid bioavailable fraction from soil samples. *Analytica Chimica Acta* **493**, 95-104.

Kadara, R.O. and Tothill, I.E. (2004). Stripping chronopotentiometric measurements of lead (II) and cadmium (II) in soils extracts and wastewaters using a bismuth film screen-printed electrode assembly. *Anal. Bioanal. Chem.* **378**, 770-775.

Kebbekus, B.B. and Mitra, S. (1998). *Environmental Chemical analysis,* Blackie Academic & Professional.

Kefala, G., Economou, A., Voulgaropoulos, A. and Sofoniou, M. (2003). A study of bismuth-film electrodes for the detection of trace metals by anodic stripping voltammetry and their application to the determination of Pb and Zn in tapwater and human hair. *Talanta* **61**, 603-610.

Kennedy, J.H. (1990). *Analytical Chemistry Principles,* 2nd Ed. W.B. Saunders Company.

Khoo, S.O. and Guo, S.X. (2002). Rapidly renewable and reproducible mercury film coated carbon paste electrode for anodic stripping voltammetry. *Electroanalysis* **14(12)**, 813-822.

Khosraviani, M., Pavlov, A.R., Flowers, G.C. and Blake, D.A. (1998). Detection of Heavy Metals By Immunoassay: Optimisation and Validation of A Rapid, Portable Assay For Ionic Cadmium. *Environmental Science Technology* **32**, 137 – 142.

Kirowa-Eisner, E., Brand, M. and Tzur, D. (1999). Determination of sub-nanomolar concentrations of lead by anodic stripping voltammetry at the silver electrode. *Analytica Chimica Acta* **385**, 325-335.

Komorsky-Lovric, S. and Branica, M. (1993). Comparison of potentiometric stripping analysis and square-wave voltammetry with respect to the influence of Triton X-100. *Analytica Chimica* Acta **276**, 361-366.

Kopanica, M. and Novotny, L. (1998). Determination of Traces of As (III) by ASV in Solutions, Natural Waters and Biological Material. *Analytica Chimica Acta* **368**, 211 – 218.

Kovacs, G.T.A., Storment, C.W. and Kounaves, S.P. (1995). Microfabricated heavy metal ion sensor. *Sensors and Actuators B* **23**, 41-47.

Krolicka, A., Pauliukaite, R., Svancara, I., Metelka, R., Bobrowski, A., Norkus, E., Kalcher, K. and Vytras, K. (2002). Bismuth-film-plated carbon paste electrodes. *Electrochem. Commun.* **4**, 193-196.

Kroll, A., Amezaga, J.M., Younger, P.L. and Wolkersdorfer, C. (2002). Regulation of Mine Waters in the European Union: The Contribution of Scientific Research to Policy Development. *Mine Water and the Environment* **21**, 193-200.

Kukla, A.L., Kanjuk, N.I, Starodub, N.F. and Shirshov, Y.M. (1999). Multienzyme electrochemical sensor array for determination of heavy metal ions. *Sensors and Actuators B* **57**, 213-218.

Kutner, W., Wang, J., L'Her, M. and Buck, R.P. (1998). Analytical aspects of chemically modified electrodes: classification, critical evaluation and recommendations. *Pure & Appl. Chem.* **70(6)**, 1301-1318.

Lacatusu, R. (1998). Appraising levels of soil contamination and pollution with heavy metals. *European Soil Bureau-Reseach Report* **4**, 393-402.

Lau, O-W. and Cheng, O-M. (1998). Determination of zinc in environmental samples by anodic stripping voltammetry. *Analytica Chimica Acta* **376**, 197-207.

Lee, T., Chung, K-C. and Park, J. (2002). Anodic stripping voltammetric characteristics of carbon composite electrode with mercuric precursors. *Electroanalysis* **14**, 833-838.

Leth, S., Maltoni, S., Simkus, R., Mattiasson, B., Corbisier, P., Klimant, I. Wolfbeis, O.S. and Csoregi, E. (2002). Engineered bacteria based biosensors for monitoring bioavailable heavy metals. *Electroanalysis* **14(1)**, 35-42.

Li, C., James, B.D., Rumble, J. and Magee, R.J. (1988). Adsorptive stripping voltammetry of some trace elements in biological samples. I. Cadmium and Zinc. *Mikrochim. Acta (Wien)* **111**, 175-182.

Locatelli, C. and Torsi, G. (1999). Cathodic and anodic stripping voltammetry: simultaneous determination of As-Se and Cu-Pb-Cd-Zn in the case of very high concentration ratios. *Talanta* **50**, 1079 – 1088.

Manahan, S.E. (2001). *Environmental Chemistry*, 5th Ed. Lewis Publishers.

Marques, A.L.B. and Chierice, G.O. (1991). Elimination of the copper-zinc interference in anodic stripping voltammetry by addition of complexing agent. *Talanta* **38** (7), 735-739.

Masawat, P., Liawruangrath S. and Slater, J.M. (2003). Flow injection measurement of lead using mercury-free disposable gold-sputtered screen-printed carbon electrodes (SPCE). *Sensors and Actuators B* **91**, 52-59.

Maxwell, T.J. and Smyth, W.F. (1996). A study of stripping voltammetric behaviour of selected metal chelates and its application to automated analysis of river waters. *Electroanalysis* **8(8-9)**, 795-802.

McCarron, T. (1999). Electrochemistry ions in solutions. http://www.tannerm.com (accessed on 25/04/2003).

McNaughtan, A., Meney, K. and Grieve, B. (1999). Electrochemical issues in Impedance Tomography. *In Conference proceedings of Ist World Congress on Industrial Process Tomography* (Buxton, Greater Manchester, April 14-17, 1999), 344-347.

Misiego, A.S., Garcia-Monco Carra, R.M., Carracedo, P.A. and Majado Torre, M.E. (2003). Determination of Cr (in small quantities) by adsorptive stripping voltammetry: a comparative study of square-wave versus differential pulse. *Anal. Bioanal. Chem.***376**, 1119-1125.

Moreno-Baron, L., Merkoci, A. and Alegret, S. (2003). Graphite-epoxy composite as an alternative material to design mercury free working electrodes for stripping voltammetry. *Electrochimica Acta* **48**, 2599-2605.

Morrin, A., Killard, A.J. and Smyth, M.R. (2003). Electrochemical characterisation of commercial and home-made screen-printed carbon electrodes. *Analytical Letters* **39(9)**, 2021-2039.

Muraviev, D. and Valiente, M. (1998). *Environmental Technologies Concerted Action: Decontamination of Acidic Mine Waters from Heavy Metals With Conversion of Pollutants to Fertilisers.* http://www.iatms10.iatm.tuwien.ac.at/~etca/projcoop/p3.htm (accessed on 04/04/2001).

Neuhold, C.G., Wang, J., do Nascimento, V.B. and Kalcher, K. (1995). Thick film voltammetric sensors for trace copper based on a cation-exchanger-modified surface. *Talanta* **42**, 1791-1798.

Nicholson, R.S. (1965). Theory and application of cyclic voltammetry for measurement of electrode reaction kinetics. *Analytical Chemistry* **37(11)**, 1351-1355.

Olschewski, H., Erlenkotter, A., Zaborosch, C. and Chemnitius, G-C. (2000). Screen-printed enzyme sensors for L-lysine determination. *Enzyme and Microbial Technology* **26**, 537-543.

Olsen, K.B., Wang, J., Setiadji, R. and Lu, J. (1994). Field Screening of chromium, cadmium, zinc, copper, and lead in sediments by stripping analysis. *Environ. Sci. Technol.* **28**, 2074-2079.

Osborne, M.D., Seddon, B.J., Dryfe, R.A.W., Lagger, G., Loyall, U., Schafer, H. and Girault, H.H. (1996). Excimer laser-induced electrochemical activity in carbon ink films. *J. Electroanal. Chem.* **417**, 5-15.

Osipova, E.A., Kamenev, A.I., Sladkov, V.E. and Shkinev, V.M. (1997). Voltammetric Determination of copper (II), lead (II), and cadmium (II) in aqueous solutions of polyethyleneimine using electrodes made of carbon materials. *J. Anal. Chem.* **52(3)**, 242-247.

Osipova, E.A., Sladkov, V.E., Kamenev, A.I. and Shkinev, V.M. (1998). Voltammetric stripping analysis of metal concentrates with polymers soluble in aqueous solutions. *Fresenius J. Anal. Chem.* **361**, 311-313.

Osipova, E.A., Sladkov, V.E., Kamenev, A.I., Shkinev, V.M. and Geckeler, K.E. (2000). Determination of Ag(I), Hg(II), Cu(II), Pb(II), Cd(II) by stripping voltammetry in aqueous solutions using complexing polymers in conjunction with membrane filtration. *Analytica Chimica Acta* **404**, 231-240.

Ostapczuk, P. and Kublik, Z. (1977). Voltammetric and Potentiometric comparison of tendencies of cadmium and zinc to intermetallic compound formation with silver, copper and gold in mercury. *J. Electroanal. Chem.* **83**, 1-17.

Palchetti, I. (1998). Screen-Printed Sensors for Heavy Metal Detection. *PhD thesis*, Biotechnology Centre, Cranfield University, U.K.

Palchetti, I., Cagnini, A., Mascini, M. and Turner, A.P.F. (1999). Characterisation of Screen-Printed Electrodes for Detection of Heavy Metals. *Mikrochimica Acta* **131**, 65–73.

Palchetti, I., Cagnini, A., Mascini, M. and Turner, A.P.F. (2000). Disposable Screen-printed Electrodes (SPE) Mercury-free for Lead Detection. *Analytical letters* **33** (7), 1231–1246.

Palchetti, I., Marrazza, G. and Mascini, M. (2001). New procedures to obtain electrochemical sensors for heavy metal detection. *Analytical letters* **34** (6), 813-824.

Pauliukaite, R., Metelka, R., Svancara, I., Krolicka, A., Bobrowski, A., Vytras, K., Norkus, E. and Kalcher, K. (2002). Carbon paste electrodes modified with Bi_2O_3 as sensors for the determination of Cd and Pb. *Anal. Bioanal. Chem.* **374**, 1155-1158.

Phillips, D.J.H. and Rainbow, P.S. (1993). *Bio-monitoring of Trace Aquatic Contaminants,* Elsevier Applied Science.

Pravda, M. (1998). Sensors for Neurochemistry. *PhD thesis*, Vrije Universiteit, Brussel, Belgium.

Prichard, E., MacKay, G.M. and Points, J. (1996). *Trace Analysis: A Structured Approach to Obtaining Reliable Result,* Crown Copyright.

Quevauviller, Ph. (2002). Operationally-defined extraction procedures for soil and sediment analysis. Part 3: New CRMs for trace-element extractable contents. *Trends in Analytical Chemistry* **21(11)**, 774-785.

Rauret, G., Lopez-Snachez, J.F., Sahuquillo, A., Rubio, R., Davidson, C., Ure, A. and Quevuaviller, Ph. (1999). Improvement of the BCR three-step sequential extraction procedure prior to the certification of new sediment and soil reference materials. *J. Environ. Monit.* **1**, 57-61.

Reeder, G.S. and Heineman, W.R. (1998). Electrochemical Characterisation of a Microfabricated Thick-film Carbon Sensor for Trace Determination of Lead. *Sensors and Actuators B* **52**, 58–64.

Reeve, R.N. (1994). *ACOL: Environmental Analysis,* John Wiley and Sons Ltd.

Rogers, K.R. and Mascini, M. (1998). Biosensors for field analytical monitoring. *Field Analytical Chemistry and Technology* **2(6)**, 317-331.

Rogers, K.R. and Mascini, M. (1999). Biosensors for Analytical Monitoring-General Introduction and Review, *USEPA Human Exposure Research Branch* http://www.epa.gov/esd/chemistry/biochem/intro.htm (05/01/2001).

Sahlin, E. and Jagner, D. (1996). Influence of Triton X-100 in stripping potentiometry. *Analytica Chimica Acta* **333**, 233-240.

Satoh, I., Kasahara, T. and Goi, N. (1990). Amperometric biosensing of copper (II) ions with an immobilised apoenzyme reactor. *Sensors and Actuators B* **1**, 499-503.

Satoh, I. (1993). Amperometric biosensing of heavy metal ions using a hybrid type of apoenzyme membrane in flow streams. *Sensors and Actuators B* **13-14**, 162-165.

Scholz, F. (Ed.) (2002). Electroanalytical methods: Guide to experiments and applications. Springer-Verlag Berlin Heidelberg.

Seddon, B.J., Osborne, M.D., Lagger, G., Dryfe, R.A.W., Loyall, U., Schafer, H. and Girault, H.H. (1997). Micro-glassy carbon inks for thick-film electrodes. *Electrochimica Acta* **42(12)**, 1883 – 1894.

Seo, K., Kim, S., Park, J. (1998). Modified composite electrode containing HgO as built-in mercury precursor for anodic stripping analysis of trace metals. *Analytical Chemistry* **70**, 2936-2940.

Shetty, R.S., Deo, S.K., Shah, P., Sun, Y., Rosen, B.P. and Daunert, S. (2003). Luminescence-based whole-cell-sensing systems for cadmium and lead using genetically engineered bacteria. *Anal. Bioanal. Chem.* **376**, 11-17.

Silva, P.R.M., El Khakani, M.A., Chaker, M., Dufresne, A. and Courchesne, F. (2001). Simultaneous determination of Cd, Pb, and Cu metal trace concentrations in water certified samples and soil extracts by means of Hg-electroplated-Ir microelectrode array based sensors. *Sensors and Actuators B* **76**, 250-257.

Sladkov, V.E. and Osipova, E.A. (2001). Effect of polyethyleneimine on the selectivity of determining silver (I) by stripping voltammetry in the presence of copper (II). *J. Anal. Chem.* **56(1)**, 43-46.

Somasundrum, M. and Bannister, J.V. (1993). Amperometric Determination of Copper Using Screen-Printed Electrodes. *Sensors & Actuators B* **15(1-3)**, 203 – 208.

Somasundrum, M. (1994). Electrochemistry of metal complexes and their use in amperometric systems. *PhD thesis*, Biotechnology Centre, Cranfield University, U.K.

Sterner, O. (1999). *Chemistry, Health and Environment,* Wiley-VCH Verlag GmbH, D-69469 Weinheim (Germany).

Stiene, M. and Bilitewski, U. (2002). Electrochemical characterisation of screen-printed carbonaceous electrodes for the determination of peroxidase activity in novel screen-printed flow-through modules. *Anal. Bioanal. Chem.* **372**, 240-247.

Stringer, R., Labunska, I. and Brigden, K. (2001). Organochlorine and heavy metal contaminants in the environment around the Complejo Petroquimicos Paharitos, Coatzacoalcos, Mexico. *Greenpeace Technical note:* (05/2000), 52-58. http://archive.greenpeace.org/~toxics/reports/primex9april.pdf (accessed on 29/05/2003).

Stulik, K. (1999). Challenges and promises of electrochemical detection and sensing. *Electroanalysis* **11(14)**, 1001-1004.

Tibbetts, D.F., Davies, J. and Compton, R.G. (2000). Sonoelectroanalytical detection of lead at a bare cooper electrode. *Fresenius J. Anal. Chem.* **368**, 412-414.

Tieman, R.S., Igo, D.H., Heineman, W.R., Johnson, J. and Seguin, R. (1991). Fabrication and characterisation of platinum/ceramic electrochemical sensor. *Sensors and Actuators B* **5**, 121-127.

Town, R.M. (1999). Effects of complexants and surfactants on the deposition and stripping steps in chronopotentiometric stripping analysis and anodic stripping voltammetry: implications for operationally defined speciation measurements. *Fresenius J. Anal. Chem.* **363**, 474-476.

Town, R.M. and van Leeuwen, H.P. (2001). Fundamental features of metal ion determination by stripping chronopotentiometry. *J. Electroanal. Chem.* **509**, 58-65

Ugo, P. and Moretto, L.M. (1995). Ion-exchange voltammetry at polymer-coated electrodes: principles and analytical prospects. *Electroanalysis* **7**, 1105-1113.

Ugo, P. and Moretto, L.M., Bertoncello, P. and Wang, J. (1998). Determination of trace mercury in saltwaters at screen-printed electrodes modified with Sumichelate Q10R. *Electroanalysis* **10(15)**, 1017-1021.

University of Bath (2003). Electrochemistry Refresher. http://www.bath.ac.uk/e-learning/electures/lectures/voltammetry/var01.htm (accessed on 12/11/2003).

Volotovsky, V., Nam, Y.J. and Kim, N. (1997). Urease-based bisensor for mercuric ions determination. *Sensors and Actuators B* **42**, 233-237.

Vytras, K., Svancara, I., Metelka, R. (2002). A novelty in potentiometric stripping analysis: Total replacement of mercury by bismuth. *Electroanalysis* **14(19-20)**, 1359-1364.

Wagner, K., Strojek J.W., Koziel, K. (2001). Processes during anodic stripping voltammetry determination of lead in the presesnce of copper on solid electrode modified with 2,2'-bipyridyl in polyaniline. *Analytica Chimica Acta* **447**, 11-21.

Wagner, K., Strojek J.W., Koziel, K. (2002). Electrochemical and quartz crystal microbalance studies of Pb (II) deposition and stripping in the presence of Cu (II) on a gold electrode modified with 2,2'-bipyridyl in polyaniline. *Analytica Chimica Acta* **455**, 69-81.

Wagner, K., Strojek J.W., Koziel, K. (2003). Electrochemical and microgravimetric investigation of lead, cadmium and copper deposition and stripping on solid electrodes. *Electroanalysis* **15(5-6)**, 392-397.

Wang, J. (1985). *Stripping Analysis: Principles, Instrumentation and Applications,* VCH Publishers, Florida.

Wang, J. (1988). *Electroanalytical techniques in clinical chemistry and laboratory medicine.* VCH Publishers, Inc., New York.

Wang, J. and Golden, T. (1989). Permselectivity and ion-exchange properties of Eastman-AQ polymers on glassy carbon electrodes. *Analytical Chemistry* **61**, 1397-1400.

Wang, J. and Taha, Z. (1990). Poly(ester-sulfonic acid)-coated mercury film electrodes for anodic stripping voltammetry. *Electroanalysis* **2**, 383-387.

Wang, J. and Tian, B. (1992). Screen-printed stripping voltammetric and potentiometric electrodes for decentralised testing of trace lead. *Analytical Chemistry* **64(15)**, 1706–1709.

Wang, J. and Tian, B. (1993a). Mercury-free disposable lead sensors based on potentiometry stripping analysis at gold-coated screen-printed electrodes. *Analytical Chemistry* **65(11)**, 1529–1532.

Wang, J. and Tian, B. (1993b). Screen-printed electrodes for stripping measurements of trace mercury. *Analytica Chimica Acta* **274**, 1-6.

Wang, J., Lu, J.M. and Yarnitzky, C. (1993). Screen-printed ultramicroelectrode arrays for on-site stripping measurements of trace metals. *Journal of Electroanalyical Chemistry* **361(1-2)**, 77 - 83.

Wang, J. (1994). Decentralised electrochemical monitoring of trace metals: From disposable strips to remote electrodes- Plenary Lecture. *Analyst* **119**, 763 – 766.

Wang, J., Nascimento, V.B., Lu, J., Park, D.S. and Angnes, L. (1996a). Disposable Nickel Screen-Printed Sensor Based on Dimethylglyoxime-Containing Carbon Ink. *Electroanalysis* **8(7)**, 635 – 638.

Wang, J., Pedrero, M., Sakslund, H., Hammerich, O. and Pingarron, J. (1996b). Electrochemical activation of screen-printed carbon strips. *Analyst* **121**, 345-350.

Wang, J., Tian, B., Lu, J., Wang, J., Luo, D. and MacDonald, D. (1998a). Remote electrochemical sensor for monitoring trace mercury. *Electroanalysis* **10(6)**, 399-402.

Wang, J., Tian, B., Nascimento,V.B. and Angnes, L. (1998b). Performance of screen-printed carbon electrodes fabricated from different carbon inks. *Electrochimica Acta* **43(23)**, 3459-3465.

Wang, J., Tian, B., Wang, J., Lu, J., Olsen, C., Yarnitzky, C., Olsen, K., Hammerstrom, D. and Bennett, W. (1999). Stripping Analysis into the 21 st Century: Faster, Smaller, Cheaper, Simpler and Better. *Analytica Chimica Acta* **385**, 429 – 435.

Wang, J. (2000). *Analytical Electrochemistry.* 2^{nd} Edition, John Wiley and Sons, Inc., Publication, New York.

Wang, J., Lu, J., Hocevar, S.B. and Farias, A.M. (2000). Bismuth-Coated Carbon Electrodes for Anodic Stripping Voltammetry. *Analytical Chemistry* **72(14),** 3218 - 3222.

Wang, J., Lu, J., Hocevar, S.B. and Ogorec, B. (2001a). Bismuth-coated screen-printed electrodes for stripping voltammetric measurements of trace lead. *Electroanalysis* **13(1),** 13–16.

Wang, J., Lu, J., Kirgoz, U.A, Hocevar, S.B. and Ogorevc, B. (2001b). Insights into the anodic stripping voltammetric behaviour of bismuth film electrodes. *Analytica Chimica Acta* **434,** 29-34.

Wang, J. (2002). Real-time electrochemical monitoring: Toward green analytical chemistry. *Acc. Chem. Res.* **35,** 811-816.

Wild, A. (1993). *Heavy metals and Radionuclides.* In: Soils and the Environment. Cambridge University Press.

Wise, J.A., Roston, D.A. and Heineman, W.R. (1983). The effects of copper-zinc and copper-cadmium intermetallic compounds in different systems used for anodic stripping voltammetry. *Analytica Chimica Acta* **154,** 95-104.

Yarnitzky, C., Wang, J. and Tian, B. (2000). Hand-held lead analyser. *Talanta* **51,** 333 – 338.

Zen, J-M., Chung, H-H. and Kumar, A.S. (2000). Determination of lead (II) on a copper/mercury-plated screen-printed electrode. *Analytica Chimica Acta* **421,** 189-197.

Zen, J-M., Lin, H-Y. and Yang, H.H. (2001). Elimination of copper-zinc interference at mercury film electrodes by a Nafion/clay modified layer. *Electroanalysis* **13(6),** 505-508.

Zen, J-M., Yang, C-C. and Kumar, A.S. (2002). Voltammetric behaviour and trace determination of Pb^{2+} at a mercury-free screen-printed silver electrode. *Analytica Chimica Acta* **464,** 229-235.

APPENDIX

Appendix 1.1

Batch to Batch variation study using Stripping Chronopotentiometry and Stripping Voltammetry

The screen-printed electrode batch-to-batch variation was evaluated to find out if similar sensitivity could be obtained on two different batches. Figure A shows the repetitive measurements of 50 µg l^{-1} lead (II) for 2 different batches. Figure B shows the comparison between the two batches when the concentration of mercury is varied for 50 µg l^{-1} lead (II) using constant current stripping chronopotentiometry (CCSCP). The results for both batches of electrodes are comparable. However, the result obtained from experiments carried out using square-wave anodic stripping voltammetry (SWASV) was completely different. Figure C and D illustrate this, with decrease in sensitivity for the lead response on the second batch. Figure D shows that the lead stripping voltammetric response obtained for the second batch was reduced as the concentration of mercury is increased. The difference in sensitivity for the two batches might be due to the particularities of the carbon electrode surface, which the SWASV technique is susceptible to.

With CCSCP, it seems that the carbon electrode surface on which mercury film was plated, does not affect the stripping response in the manner observed with SWASV. The advantage of CCSCP over SWASV is that CCSCP can discriminate against capacitive background current (Town and van Leeuwen, 2001). Hence, the results here emphasise the need to control and optimise the screen-printing procedure for reproducible performance in SWASV detection. The results obtained here made the choice of what technique to use for metal ion detection with the SPCE much easier.

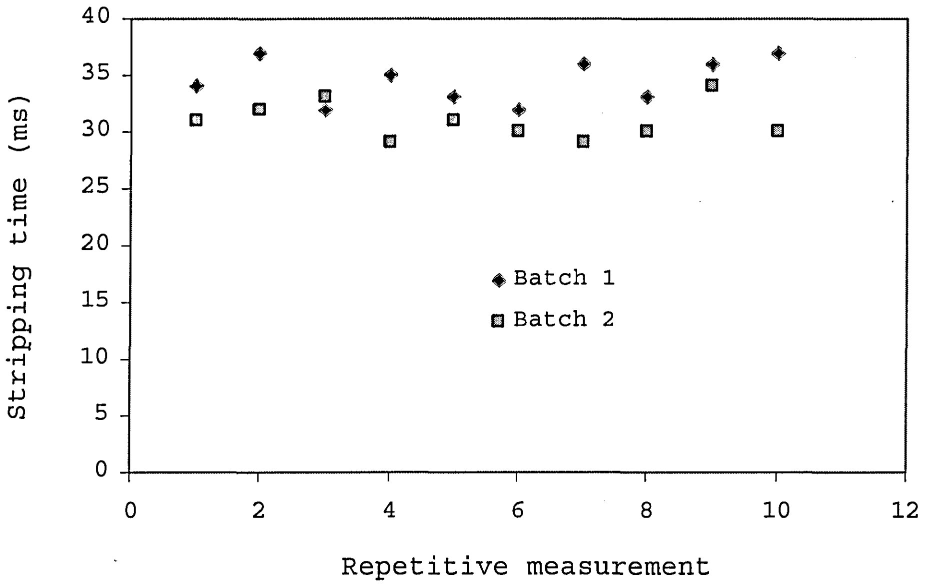


Figure A: Repetitive CCSCP measurements of 50 µg l^{-1} lead (II) in 0.1 M HCl for different batches. Hg^{2+} = 10 mg l^{-1}, deposition time = 120 s, deposition potential = - 0.9 V, constant current = 1 µA.

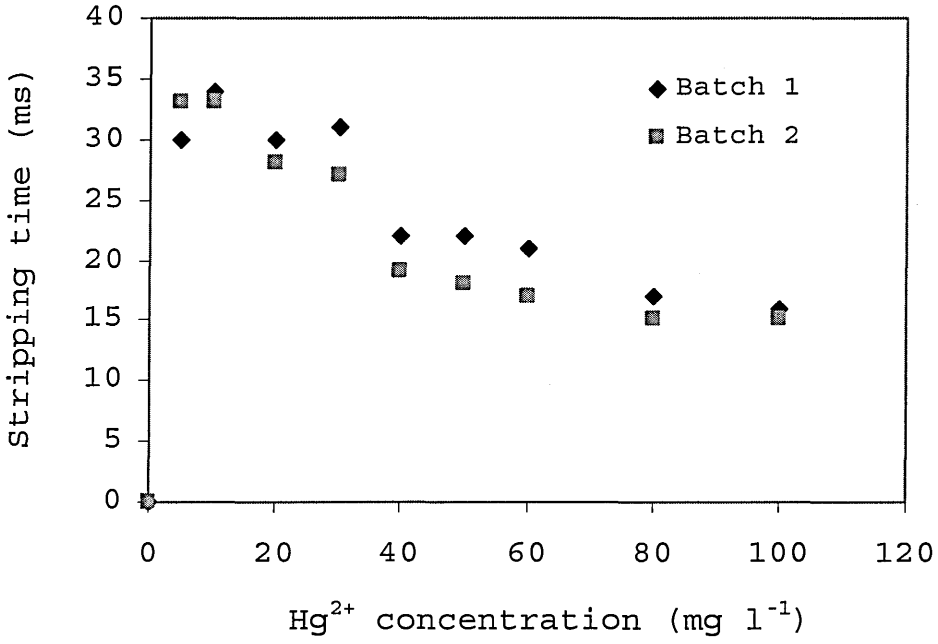


Figure B: Comparison between the two batches when the concentration of mercury co-deposited is varied for 50 µg l^{-1} lead (II) in 0.1 M using CCSCP. Deposition potential = - 0.9 V, constant current = 1 µA.

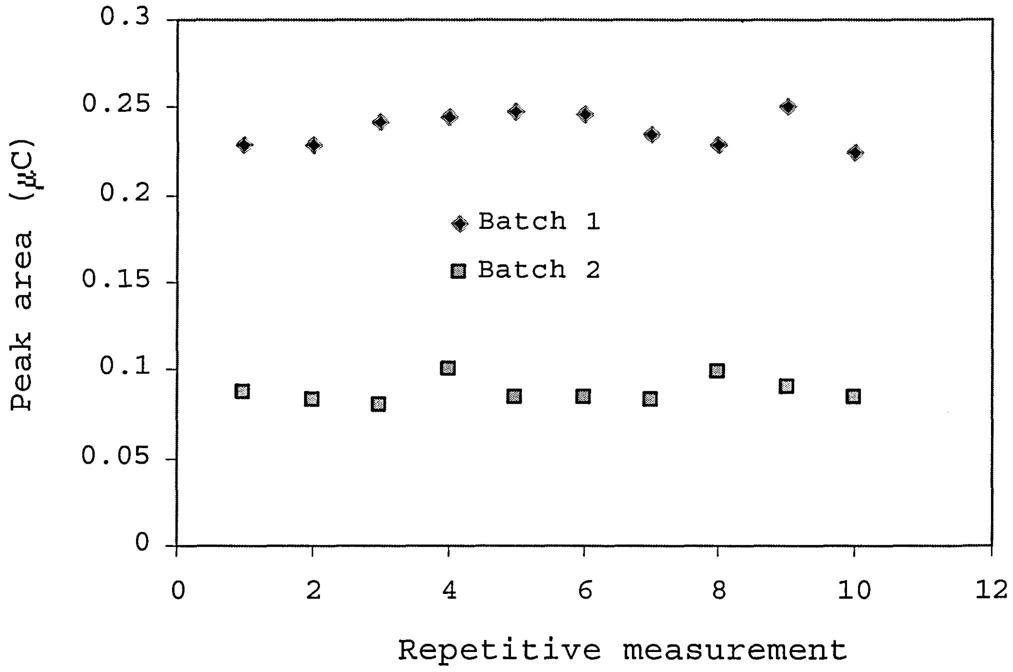


Figure C: Repetitive SWSV measurements of 50 μg l^{-1} lead (II) in 0.1 M HCl. Hg^{2+} = 80 mg l^{-1}, deposition time = 120 s, deposition potential = - 0.9 V

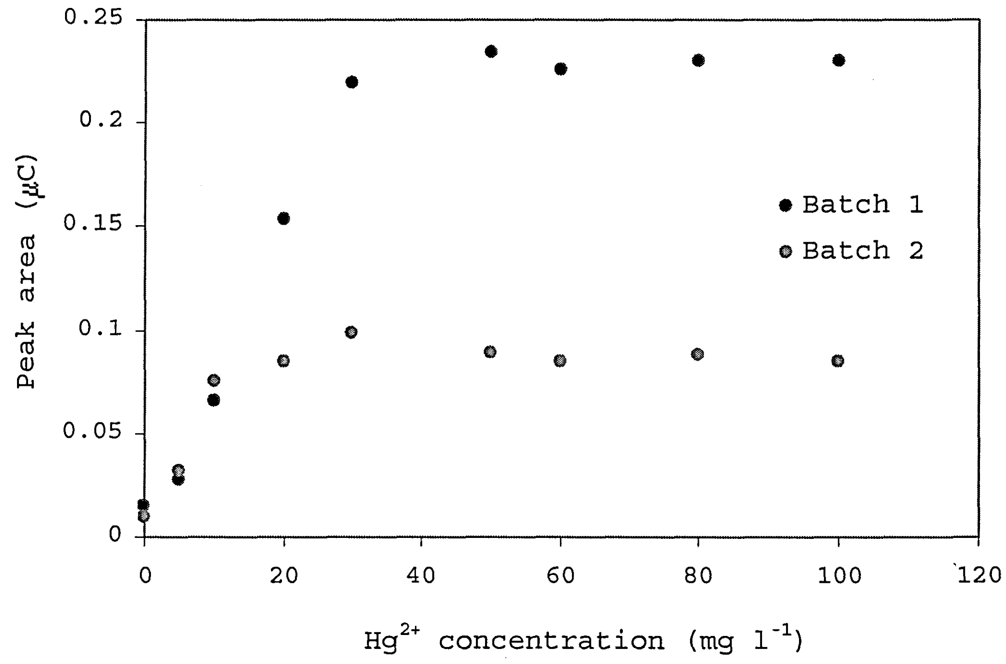


Figure D: Comparison between the two batches when the concentration of mercury co-deposited is varied for 50 μg l^{-1} lead (II) in 0.1 M using SWASV. Deposition time = 120 s, deposition potential = - 0.9 V.

Appendix 1.2

Concentrations of copper (II) determined in soil samples extracts using ICP-MS (carried out independently by Autonomous University of Barcelona, Spain)

SAMPLE Acetic acid extracted soil	Concentration of copper (II) (mg kg^{-1})
TP1	35 ± 2
PX	12 ± 1
C20	0.80 ± 0.01
C22	0.85 ± 0.01
SA79b	5.2 ± 0.1
SA80b	38 ± 1
SA83b	1.24 ± 0.04
SA84b	0.26 ± 0.04
SA91b	1.88 ± 0.04

SAMPLE Agua regia extracted soil	Concentration of copper (II) (mg kg^{-1})
SA79c	4.7 ± 0.2
SA80c	10.3 ± 0.2
SA81c	10 ± 0.2
SA82c	7.8 ± 0.2
SA83c	666 ± 1
SA84c	385 ± 1
SA85c	254 ± 1
SA91c	518 ± 17
SA92c	508 ± 16
Ref 721c	199 ± 15
Ref 722c	419 ± 14

Appendix 1.3

Concentrations of copper (II) determined in the diluted soil samples extracts using ICP-MS (carried out by Autonomous University of Barcelona, Spain)

SAMPLE Acetic acid extracted soil	Concentration of copper (II) ($\mu g\ l^{-1}$)
SA79b	520 ± 2
SA80b	760 ± 15
SA81b	496 ± 10
SA83b	50 ± 2
SA84b	13 ± 1
SA85b	398 ± 7
SA88b	14 ± 1
SA91b	75 ± 3
SA92b	71 ± 2

SAMPLE Agua regia extracted soil	Concentration of copper (II) ($\mu g\ l^{-1}$)
SA79c	9 ± 0.4
SA80c	21 ± 0.4
SA81c	20 ± 0.4
SA82c	16 ± 0.4
SA83c	74 ± 1
SA84c	54 ± 2
SA85c	71 ± 2
SA91c	26 ± 1
SA92c	29 ± 1
Ref 721c	54 ± 1

PUBLICATIONS

Results from this thesis have been published and presented as follows:

Conference Posters

Kadara, R.O., Newman, J.D. and Tothill, I.E. (2001). Electrochemical sensor development for toxic heavy metals in various matrices of contaminated groundwater and soils. *New Directions in Electroanalysis, University of Salford, 22-25, April 2001.*

Kadara, R.O, Bello-Rodriquez, B, Newman, J.D. and Tothill, I.E. (2001). Electrochemical sensor development for toxic heavy metal. *Senspol, University of Alcala, 9-11, May 2001.*

Kadara, R.O. and Tothill, I.E. (2002). Electrochemical sensor development for toxic heavy metals in groundwater and soils. *Senspol Workshop, Kings College, University of London, 4-6, June 2002.*

Kadara, R.O. and Tothill, I.E. (2002). Surface modified screen-printed sensor for lead and cadmium. *Electrochem2002, Preston, Lancs, 1-4, September 2002.*

Oral Presentations

Tothill, I.E., **Kadara, R.,** Bello-Rodriguez, B., Arrigan, D., Valiente, M., Kaifer, M.J. and Pottecher, G. (2002). Development of chemical sensors and biosensors for monitoring of toxic heavy metals in contaminated soils samples. *Senspol Workshop, Kings College, University of London, 4-6, June 2002.*

Kadara, R.O. and Tothill, I.E.. (2002). Electrochemical sensor development for toxic heavy metals in groundwater and soils. *Senspol Technical Meeting, Seville, 6-9, November 2002.*

Proceeding book

Alcock, S.J. and **Kadara, R.O.** (Eds.) (2003). Proceedings 2nd SENSPOL Workshop: Response to New Pollution Challenges. Cranfield University Press, Bedfordshire, UK. ISBN 1 871315 86 7.

Proceeding CD

Sesay, A., Newman, J.D., **Kadara, R.O**. and Alcock S.J. (2003). SENSPOL Survey of Sensor Capabilities: Sensors for the Abatement of Water Pollution from Contaminated Land, Landfills and Sediment. Cranfield University Press, Bedfordshire, UK. CD, 231pp. ISBN 1 871315 85 9.

Research papers

Kadara, R.O., Newman, J.D. and Tothill, I.E. (2003). Stripping chronopotentiometric detection of copper using screen-printed three-electrode system – Application to acetic acid bioavaliable fraction from soil sample extracts. *Analytica Chimica Acta* **493**, 95-104.

Kadara, R.O. and Tothill, I.E. (2004). Stripping chronopotentiometric measurements of lead (II) and cadmium (II) in soils extracts and wastewaters using a bismuth film screen-printed electrode assembly. *Analytical and Bioanalytical Chemistry* **378**, 770-775.

Md Noh., Mohd F.*, **Kadara, R.O.** and Tothill, I.E. Application of cysteine modified screen-printed electrode to chronopotentiometric stripping analysis of cadmium (II) in soil samples extracted with acetic acid. (Submitted)

Kadara, R.O. and Tothill, I.E. Solving the effect of copper on the stripping response of lead obtained on bismuth coated screen-printed electrode. (Submitted)

Kadara, R.O. and Tothill, I.E. The application of bare screen-printed carbon electrode to the simultaneous detection of lead and copper. (In Preparation)

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ELECTROCHEMICAL SENSOR DEVELOPMENT FOR TOXIC HEAVY METALS IN VARIOUS MATRICES OF CONTAMINATED GROUNDWATER AND SOILS

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Background

The pollution of industrial sites is a major problem in many industrial regions throughout the world and the treatment and clean up of these sites requires an innovative problem-solving approach for rapid assessment and remediation of contaminated sites. Key to these remediative processes is the requirement to measure toxic contaminants on-site using simple, field-portable and reliable analytical method.

Experimental

Electrodes used in this work were fabricated using screen-printing techniques and the fabrication process and cycle, are illustrated in Figure 1 and 2. Electrochemical studies were carried out with a computer-controlled potentiostat-10 Autolab Electrochemical analyser.

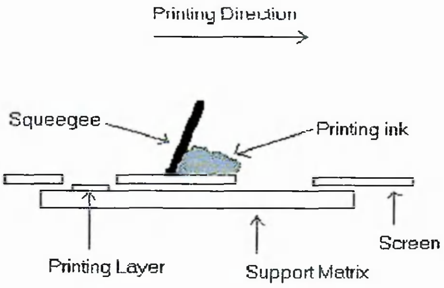
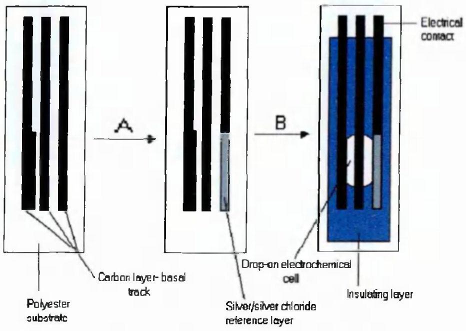


Figure 1: Schematic representation of the screen-printing process.



A= addition of reference layer, B= addition of insulating layer

Figure 2: Schematic representation of fabrication cycle

(1) <u>Mercury-coated screen-printed electrode</u>

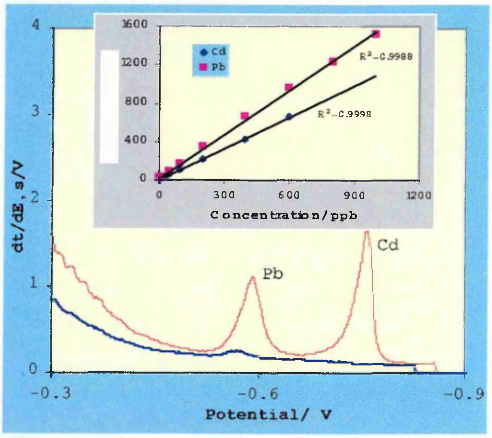


Figure 3: Stripping potentiograms for 40 µg/l lead and 50 µg/l cadmium. Inset: Lead and cadmium calibration curves. Conditions: $[Hg]^{2}$ =10 mg/l, t_{dep}=120 s, E_{dep}=-1.0 V, constant current=1 µA, medium=0.1 M HCl/ 0.5 M NH_4Ac.

(2) <u>Bismuth-coated screen-printed electrode</u>

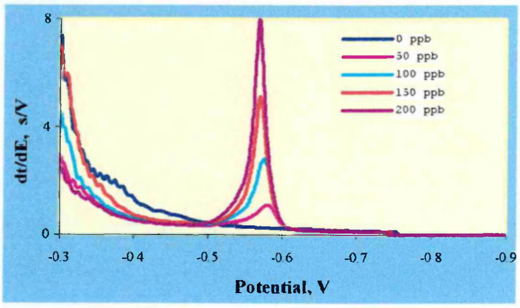


Figure 4: Stripping potentiometric peaks with increasing concentrations of lead. Conditions: $[Bi]^{3+}$=0.5 mg/l, t_{dep}=120 s, E_{dep}=-0.9 V, constant current=1 µA, medium=0.1 M HCl

Results and Discussion

- Detections down to low ppb levels have been achieved for cadmium and lead on mercury-coated screen-printed electrodes (Figure 3).
- Stable stripping response and linearity up to 1 mg/l obtained using stripping potentiometry.
- Good reproducibility for lead detection on bismuth-coated screen-printed electrode (Figure 4).
- Bismuth-coated screen-printed electrode shows promise also for cadmium determination (not shown).

Acknowledgement

We are grateful to the European Union for funding the DIMDESMOTOM project (EVK1-CT-1999-00002).

Reference

1. **Wang, J, Lu, J, Hocevar, S B & Ogorec, B** (2001) *Electroanalysis* **13** (1): 13 – 16
2. **Florou, A B et al,** (2000) *Analytica Chimica Acta* **423** :109

ELECTROCHEMICAL SENSOR DEVELOPMENT FOR TOXIC HEAVY METALS

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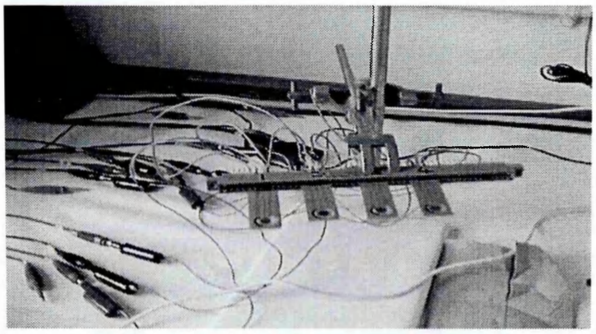
Background

The pollution of industrial sites is a major problem in many industrial regions throughout the world. The treatment and cleanup of these sites requires an innovative problem-solving approach for rapid assessment and remediation of contaminated sites. Key to these remediative processes is the requirement to measure toxic heavy metals on-site using simple, field-portable and reliable analytical method. The aim of this study is to develop improved and robust sensing devices (biosensor and chemical sensor) capable of decentralised testing.

Experimental

- Electrodes used in this work were fabricated using screen-printing technology.
- The electrochemical studies were carried out with a computer-controlled potentiostat-10 Autolab Electrochemical analyser.
- Amperometry, Cyclic Voltammetry (CV) and Potentiometric Stripping Analysis (PSA) were the techniques used.
- CV was used to study the electrochemical behaviour of ferrocyanide on the screen-printed working electrodes.



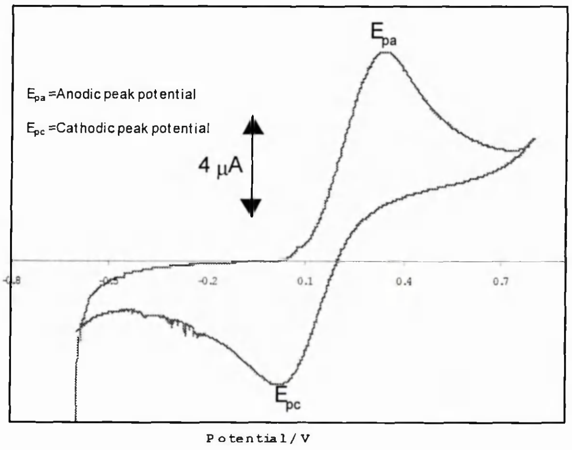
igure 1: Set-up for sensing device with four screen-printed electrodes inserted ito an edge connector, which is then connected to an Autolab analyser.

esults & Discussion

) Screen-Printed Electrode Characterisation

CV characterisation indicates electrochemical reaction on the screen-printed electrodes (SPEs) is quasi-reversible. The peak separation obtained on SPE (260 mV) was larger than the theoretical peak-peak separation (E_{pa}- E_{pc} = 59 mV) for a reversible one electron transfer reaction. (Figure 2).

This implies that the screen-printed electrodes exchange electrons with the bulk solution reversibly under slow conditions and irreversibly at fast conditions.



gure 2: Cyclic voltammograms of 0.5 mM Ferrocyanide in M KCl at 20 mV/s.

(2) Metal Ions Detection

- Detections down to low ppb levels have been achieved for cadmium and lead on mercury-coated screen-printed electrodes with PSA (Figure 3).
- The difference in the uric acid response when the Hg (II) ions were present allowed the inhibitory effect to be observed. The uric acid response decreased when measured in presence of urease due to enzyme catalysis. When the Hg (II) ions were present, the urease activity was inhibited, and that produced an increase in the uric acid response (Figure 4).
- The inhibitory effect of Hg (II) was confirmed and a linear range obtained with concentrations of the metal ion in the low micro-molar region.

(a) Electrochemical sensor development based on stripping analysis

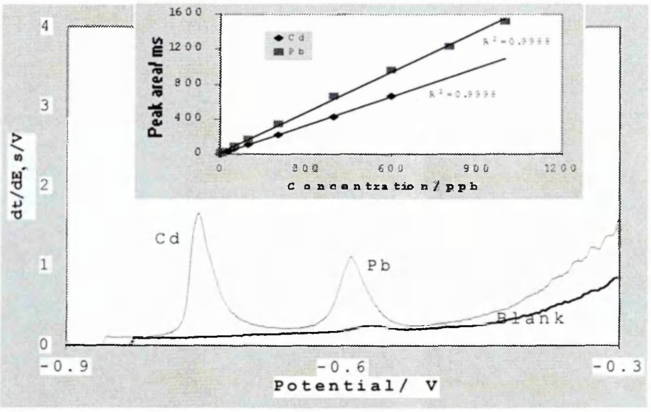


Figure 3: Stripping potentiograms for 40 μg/l lead and 50 μg/l cadmium. Inset: Lead and cadmium calibration curves. Conditions: $[Hg]^{2+}$=10 mg/l, t_{dep}=120 s, E_{dep}=-1.0 V, constant current=1 μA, medium=0.1 M HCl/ 0.5 M NH_4Ac.

(b) Biosensor developmental work based on urease inhibition

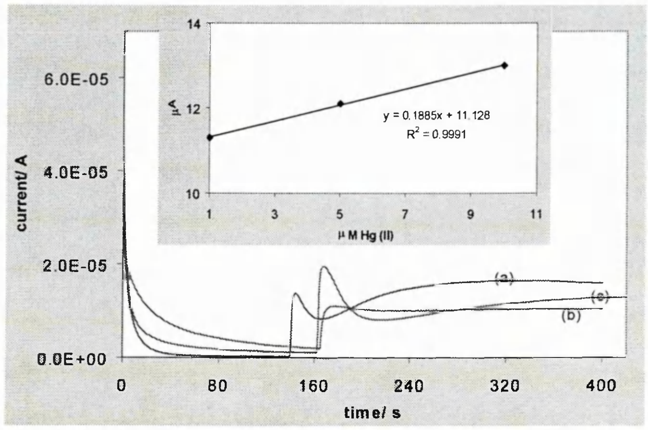


Figure 4: Amperometric signals showing the differences in uric acid response in the absence and presence of metal ions (a) 10 mM uric acid only; (b) 10 mM uric acid analysed with 75 U urease; (c) 10 mM uric acid analysed with urease and 10 μM Hg (II).

Acknowledgement

We are grateful to the European Union for funding the DIMDESMOTOM project (EVK1-CT-1999-00002).

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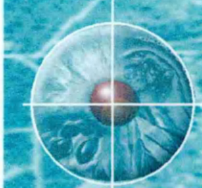

Electrochemical Sensor Development for Toxic Heavy Metals in Groundwater and Soils

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Background

Toxic heavy metals (THM) are well-known pollutants from both natural and anthropogenic sources. Monitoring them in environmental matrices requires a simple, field-portable and reliable method that can detect them directly in the field. The aim of present work is to develop disposable electrochemical sensing device capable of decentralised testing of THM in soils and groundwater samples.

Experiment

Screenprinted carbon electrodes (SPCEs) used in this work were modified by:

(i) coating the working electrode (WE) with 2 μl 0.5 % Nafion® (Nafion®-coated SPCE)

(ii) coating the WE *in situ* with 500 ppb Bi^{3+} (Bi-film SPCE)

(iii) incorporating 2 % solid bismuth oxide into the graphite ink used in screenprinting the SPCE (Bi_2O_3 modified SPCE)

(iv) incorporating 5 % 2,5 dimercapto-1,3,4-thiadiazole (DMDT) into the graphite ink used in screen-printing the SPCE (DMDT modified SPCE)

Electrochemical measurements were carried out with stripping chronopotentiometry (SCP). Conditions: Constant current=1 μA, t_{dep}; 120 s.

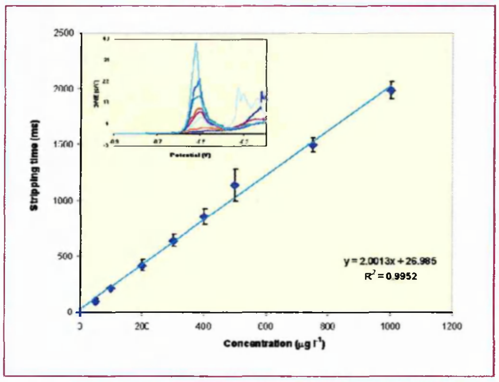

Figure 3. Calibration plot for Pb (II) on DMDT modified SPCE. Inset: Stripping chronopotentiograms for increasing concentrations of Pb (II) in 0.1 M HCl.

Results and Discussion

Figure 1 and 2 show the stripping chronopotentiograms for lead (II) on Nafion® coated SPCE, Bi_2O_3 modified SPCE, DMDT modified SPCE and bare SPCE. Higher peak areas were obtained on the modified electrodes compared to the bare SPCE. Table 1 depicts the results for the analysis of real soil samples from contaminated sites. Comparisons are made between the developed sensors and AAS.

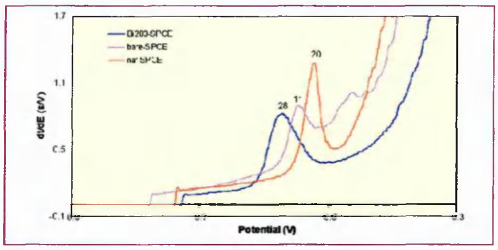

Figure 1. Stripping chronopotentiograms for 50 ppb Pb in 0.1 M HCl. E_{dep}; -0.9 V

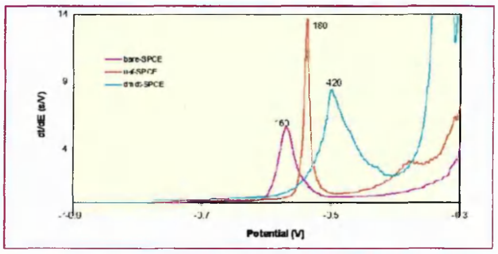

Figure 2. Stripping potentiograms for 200 ppb Pb in 0.1 M HCl. E_{dep}; -0.9 V

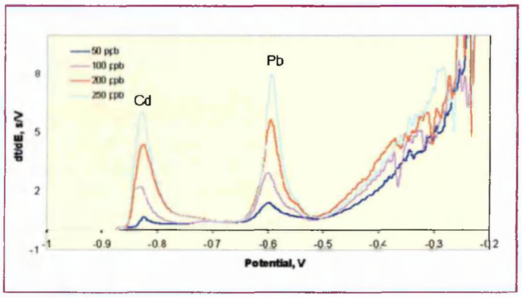

Figure 4. Stripping chronopotentiograms for increasing concentrations of Pb (II) and Cd (II) on bismuth (500 ppb Bi^{3+}) coated film SPCE. E_{dep}; -1.0 V, 0.1 M HCl/0.5 M NH_4Ac

Table 1. Results for the determination of Pb (II) in soil sample (n =3)

Method	AAS	SCP (DMDT modified SPCE)	SCP (Bi_2O_3 modified SPCE)	SCP (Bi^{3+} film SPCE)	SCP (Bare SPCE)
Concentration (ppm)	250	213	167	202	159
% comparison to AAS result	-	85.2	66.8	80.8	63.6
RSD %	2.6	15	7.3	6	7.8

Conclusion

The results obtained so far show promise for the development of on-site electrochemical sensors for THM in contaminated soils and water.

Acknowledgements
The authors of this poster are grateful to the European Union for funding the DIMDESMOTOM project (EVK1-CT-1999-00002).

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Surface Modified Screen-Printed Electrodes for Lead (II) and Cadmium (II) Detection in Soil Samples

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Introduction

Heavy metals like lead (II) and cadmium (II) are not biodegradable and accumulate in the environment, producing toxic effects even at very low concentrations. Thus, monitoring them in the environment requires simple, field-portable and reliable method that can detect directly in the field. The aim of this work is to test out screen-printed modified electrodes for lead and cadmium detection.

Experiment

The screen-printed three-electrode integrated system (Figure 1) was modified by incorporating 2% bismuth (II) oxide (Bi_2O_3) or 5% 2,5 dimercapto-1,3,4-thiadiazole (DMTD) into the graphite ink paste and screen-printed onto the carbon working electrode surface. Electrochemical measurements on the modified screen-printed carbon electrodes (SPCEs) were carried out with cyclic voltammetry (CV) and stripping chronopotentiometry (SCP).

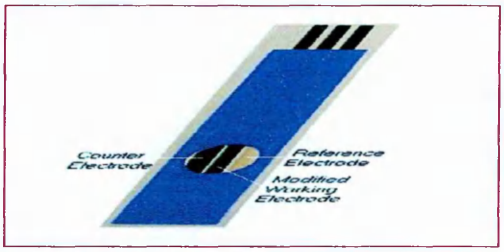

Figure 1. Schematic representation of electrode

Results and Discussion

Figure 2 shows the calibration curves for lead (II) on bare-SPCE and dmtd modified-SPCE using CV. An increase in peak area response of lead (II) was obtained on dmtd-SPCE (peak is much more broader) compared to bare-SPCE. Figure 3 shows the stripping chronopotentiograms for cadmium (II) on bismuth-film SPCE, Bi_2O_3-SPCE and bare SPCE. Higher peak response was obtained on the bismuth modified electrodes compared to the bare SPCE. Hence the Bi_2O_3-SPCE was used to generate a calibration curve (25 - 400 ppb) for cadmium (II) (Figure 4). Table 1 shows the results for the analysis of soil samples obtained on the dmtd (Figure 5, stripping responses for lead in sample using standard addition plot) and Bi_2O_3 modified SPCEs coupled to SCP. The other results obtained with AAS and the other electrodes are for comparison.

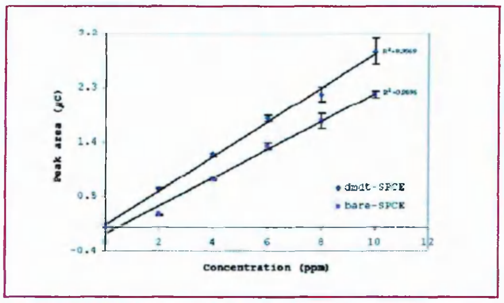

Figure 2. Calibration curves for lead (II) detection using cyclic voltammetry. E_{dep}: -1.0 V (for 30s). Scan rate: 50 mV/s, 0.1 M HCl

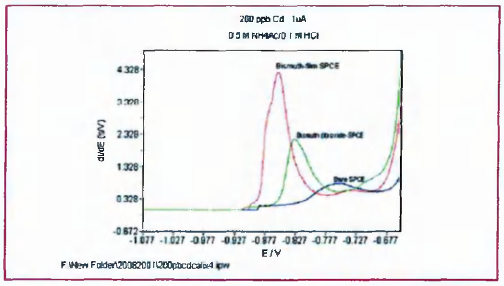

Figure 3. Stripping chronopotentiograms for 200 ppb cadmium (II) on the different electrodes. E_{dep} : -1.0 V, tdep: 120 s, constant current = 1 μA

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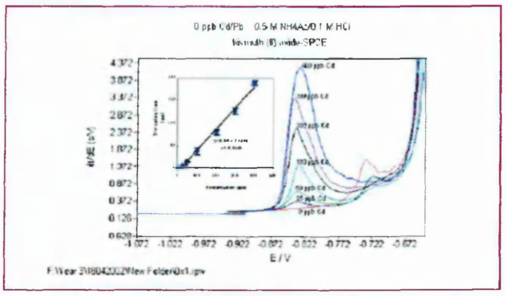

Figure 4. Stripping chronopotentiograms for increasing concentration of cadmium (II). Inset: Calibration plot for cadmium (II).

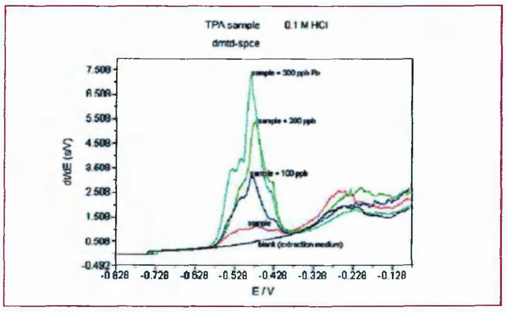

Figure 5. Stripping chronopotentiograms for the determination of lead (II) in soil sample on DMTD modified SPCE. Responses are sample + standard additions of lead (II) ions. E_{dep}: -0.9 V, constant current = 1 μA

Table 1. Results (in ppb) for the determination of lead (II) in soil samples (n = 3)

Sample	AAS	SCP (DMTD modified SPCE)	SCP (Bi_2O_3 modified SPCE)	SCP (Bi-film SPCE)	SCP (Bare SPCE)	SCP (Hg-film SPCE)
TPA 1	**96**	82	64	80	60	98
TPA 2	**118**	123	84	107	67	109
TPA 3	**200**	153	133	170	100	235

Conclusion

- **Cadmium (II) can be detected at much more lower concentration on Bi_2O_3 modified SPCE compared to bare-SPCE**
- **The dmtd modified sensors showed good potential for lead (II) detection in soil samples as the results obtained on them, are comparable to ones obtained on bismuth-film and mercury-film electrodes**
- **There is also good agreement between the values obtained with SCP coupled to dmtd modified SPCE and AAS**
- **The relative standard deviations for lead (II) and cadmium (II) measurements on both dmtd and Bi_2O_3 modified SPCEs are within 10%**
- **The dmtd and Bi_2O_3 modified SPCE present less toxic alternatives to mercury film SPCE**

Acknowledgements

The authors of this poster are grateful to the European Union for funding the DIMDESMOTOM project (EVK1-CT-1999-00002).

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Stripping chronopotentiometric detection of copper using screen-printed three-electrode system—application to acetic-acid bioavailable fraction from soil samples

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Abstract

Single-use sensors, incorporating a three-electrode configuration (graphite carbon-working electrode; carbon-counter electrode and silver/silver chloride-reference electrode), have been fabricated on a polyester substrate using low cost screen-printing (thick-film) technology. These electrodes coupled with constant current stripping chronopotentiometry (CCSCP), has provided a convenient screening tool for on-site detection of trace levels of copper. Modification of the graphite carbon surface based on in situ deposition of mercury film has been carried out. By appropriate choice of supporting medium and applied constant stripping current, well-resolved and reproducible response for copper was obtained. The stripping response for copper following 2 min deposition was linear over the concentration range examined (10–2000 ppb) with detection limit of 6 ppb using 2 M hydrochloric acid (HCl). Successful applications of the sensing device to acetic-acid bioavailable fraction of a certified reference material (CRM 601, a lake sediment) and soil samples are demonstrated.

Keywords: Copper; Constant current stripping chronopotentiometry; Screen-printed electrode; Mercury film; Acetic-acid bioavailable fraction

1. Introduction

The development of analytical methods capable of monitoring bioavailable metals in the environment is highly desirable. This is in response to chemical pollution in areas where mining operations were prevalent in the past, and where considerable proportion of various elements were mobilised by weathering and leaching from abandoned mining wastes [1]. Since these heavy metals are readily retained in the environment [2], and at levels that can have effect on human and other biological life [3–5], it is necessary to have field screening methods for rapid assessment and remediation of contaminated sites. Electrochemical methods satisfy this need, with their low power demands lending itself to the design of portable field-based instruments [6–11]. In addition to this, the advent of screen-printing (thick-film) technology has made it possible to fabricate inexpensive disposable electrodes in large volumes [6,11–14], which can be used with these electrochemical instruments.

The coupling of electrochemical techniques with disposable screen-printed electrodes (SPEs), have been reported for the detection of heavy metals [6,11–31]. Copper(II) which is one, has been electrochemically measured with SPEs using amperometry [17] and anodic stripping voltammetry (ASV) [6,13,17–19,21,24,31]. However, such measurements

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have been applied in cases where two-electrode screen-printed system (integrated screen-printed carbon-working and silver/silver chloride-reference electrodes) was used [6] or where the screen-printed working electrode is placed alongside conventional silver/silver chloride (Ag/AgCl) reference electrode and platinum counter electrode in a conventional electrochemical cell [17,18,21,31]. Although, ASV coupled to an integrated three-electrode system has been reported for the detection of copper [13,19,24], the same cannot be said as far as we know about the use of constant current stripping chronopotentiometry (CCSCP) coupled to an integrated screen-printed three-electrode system. CCSCP is an electroanalytical technique that is readily amendable for decentralised screening, providing accurate measurements of low concentrations with rapid analysis times. Compared to ASV, CCSCP gives a much wider linear dynamic range for metal ion determination [32,33] and also uses a much simpler instrumentation [34,35] because there is no need for a potential waveform generator [35].

Using disposable screen-printed three-electrode system with CCSCP provides a simple, easy to use portable analytical device capable of handling small volumes. In this paper, we report results obtained in the development of an electrochemically-based device for decentralised measurement of copper in extracted environmental samples using CCSCP. Despite the fact that the methodical principle and most of the experimental parameters are more or less an adaptation of already existing protocol, the emphasis in this work, is on dealing with the ways in reproducibly generating a stripping chronopotentiometric response for copper on SPEs coated in situ with 10 ppm ($\mathrm{mg\,l^{-1}}$) of mercury(II). Because the dynamic linear range obtainable with ASV for copper on mercury film SPE is narrow, CCSCP technique with its reasonably broad linear range provides an opportunity to develop a screening device which can be used over a broad concentration range for determining copper in soil samples using the standard addition method. Also with this, there no need to massively dilute the sample extract, so as to obtain a linear standard addition plot.

Just like ASV, CCSCP exploiting in situ formation of a mercury film comprises two-steps, a preconcentration and a measurement step [36]. The preconcentration step in ASV and CCSCP, involves metal ions in the solution being reduced and simultaneously amalgamated (cathodic deposition) on the working electrode at constant potential according to the reactions:

$$\mathrm{Hg^{2+}} + 2\mathrm{e^-} \rightarrow \mathrm{Hg} \tag{1}$$

$$\mathrm{M}^{n+} + n\mathrm{e}^- \rightarrow \mathrm{M(Hg)} \tag{2}$$

In the subsequent measurement step, unlike ASV where the deposit (reduced and amalgamated analyte) is stripped by application of a potential while recording the current–potential dependence, in CCSCP, the potentiostat is transformed into a galvanostat and the deposit re-oxidised electrochemically by means of an applied constant oxidative current according to the reaction:

$$\mathrm{M(Hg)} + \text{applied constant oxidative current} \rightarrow \mathrm{M}^{n+} \tag{3}$$

The change of potential of the working electrode with time during the stripping of the deposited analyte is recorded and processed. The response is digitically processed into $\mathrm{d}t/\mathrm{d}E$ (the inverse potential derivative with time) versus potential (E), with the amount of deposited analyte determined by integrating the corresponding stripping peak.

2. Experimental

2.1. Electrode fabrication

Screen-printed electrodes were mass-produced inhouse by a multi-stage screen-printing process using a DEK 248 machine (DEK, Weymouth, UK) and screens with appropriate stencil designs (100 per screen) fabricated by DEK Precision Screen Division. Fig. 1 shows the fabrication cycle for the integrated screen-printed three-electrode system. The basal tracks for the three-electrode system was printed with graphite-based ink E423-SS (Acheson Colloids, UK) onto 250 μm thick Melinex ST725 polyester sheet (Cadillac Plastic, Swindon, UK) and dried in an oven at 60 °C for 2 h. A silver paste (MCA Services, Cambridge, UK) containing 15% silver chloride was printed on one of the basal tracks and left to dry. This track serving as a reference electrode and the two other tracks (graphite carbon-working electrode with a 1.3 $\mathrm{mm^2}$ planar area and a graphite carbon-counter

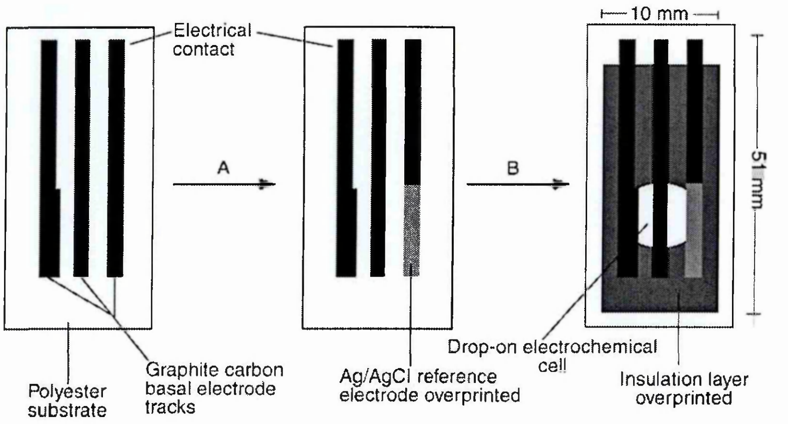

Fig. 1. Schematic representation of the SPE fabrication cycle.

electrode) were insulated using 242-SB epoxy-based protective coating ink (Agment ESL, Reading, UK), leaving a defined circular shaped area (electrochemical cell with an area of 3.2 mm^2) for measurements. The electrode strip produced was then heat treated at 120 °C for 2 h to cure the epoxy resin.

2.2. Reagents

All chemicals were of analytical reagent grade and used as received. Hydrochloric acid (HCl) and potassium chloride (KCl) were obtained from BDH Ltd. (Poole, England) while ammonium acetate was obtained from Merck (Darmastadt, Germany). The copper(II) and mercury(II) atomic absorption standard solutions (1000 ppm in 5 wt.% nitric acid) were obtained from Aldrich (Gillingham, Dorset, UK) and diluted with deionised water and supporting electrolyte to the appropriate concentration. The supporting electrolytes used in the experiments at various concentration levels were HCl, KCl and ammonium acetate containing HCl.

2.3. Procedure

A computer-controlled Autolab PGSTAT-10 (potentiostat/galvanostat) with general-purpose electrochemical software operating system GPES version 4.7 (Eco Chemie B.V., The Netherlands) was used for electrochemical measurements. The integrated three-electrode strips were connected to the Autolab PGSTAT-10 with a specially adapted electrical edge connector from Maplin (Milton Keynes, UK) and measurements carried out by placing a 100 μl sample drop on the electrochemical cell (Fig. 1). Each experiment was carried out with a new electrode strip in non-deaerated and unstirred solution. For comparison purposes, studies were carried with screen-printed carbon electrode (SPCE) or glassy carbon electrode (GCE) with conventional silver/silver chloride (Ag/AgCl) reference electrode and platinum counter electrode placed in 10 ml of solution. Stripping chronopotentiometric measurements were carried out by co-deposition of 10 ppm mercury(II) (for formation of mercury film on working electrode surface) with copper(II) at a potential of − 0.9 V (versus screen-printed Ag/AgCl) for 120 s. After the deposition period, a constant oxidative current was applied to strip the deposited copper with the stripping process terminated when the electrode had reached a potential of−0.05 V (versus screen-printed Ag/AgCl). The background signal resulting from the supporting medium was also measured in the same manner. For sample analysis, extract solutions (BCR acetic-acid bioavailable fraction) from reference material (CRM 601) and soil samples were analysed by spiking with appropriate concentrations of standard copper(II) solution. The first step of the BCR extraction procedure used in obtaining the extract solutions have been described elsewhere [37]. The results obtained are compared to the ones obtained independently with inductively coupled plasma-mass spectrometry (ICP-MS; model PQ EXCELL VG ELEMENTAL).

3. Results and discussion

3.1. CCSCP—effect of experimental variables

To find out the optimum experimental conditions for the detection of copper(II) on mercury film SPE (SPE plated in situ with 10 ppm mercury(II)), the effects of the supporting medium and the constant stripping current on the stripping response were studied.

3.1.1. Effect of supporting medium/composition of the medium

Hydrochloric acid and potassium chloride were examined as supporting electrolytes for the detection of copper(II) on screen-printed electrode plated in situ with 10 ppm mercury(II). Both chloride mediums were chosen due to the chloride concentration dependency [24] of the silver/silver chloride (Ag/AgCl) reference electrode on the three-electrode integrated strip. The screen-printed Ag/AgCl reference electrode on the strip is a solid-state electrode with no internal electrolyte solution [38]. Hence, chloride ions are needed in the supporting electrolyte medium at a constant level [13,24,38] in order to maintain the reference electrode potential.

Fig. 2a shows the stripping response for copper(II) obtained on the mercury film SPE using 0.1 M HCl as supporting medium. The stripping peak for copper was poorly defined and obscured by the large background signal for the blank supporting electrolyte solution in the potential range in which copper is detected. Similar response was obtained using screen-printed carbon-working electrode with conventional-sized Ag/AgCl (containing 3 M sodium chloride as the

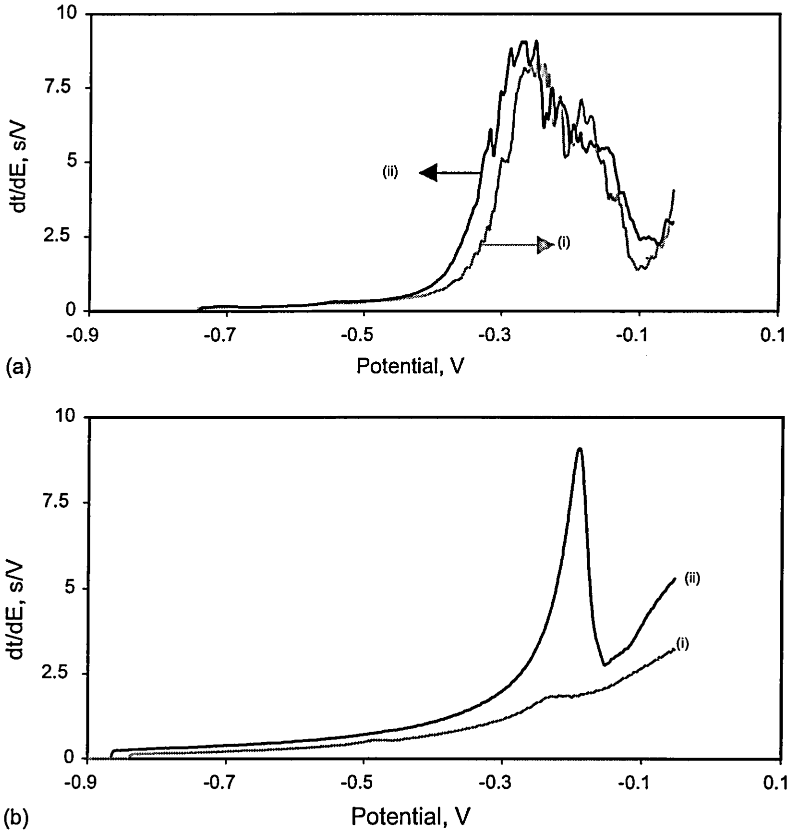

Fig. 2. Stripping chronopotentiograms for (i) blank and (ii) 50 ppb copper(II) on (a) mercury film SPE and (b) mercury film GCE. Supporting medium = 0.1 M HCl, concentration of mercury(II) plated in situ = 10 ppm, deposition potential = −0.9 V, deposition time = 120 s, constant current = 1 μA.

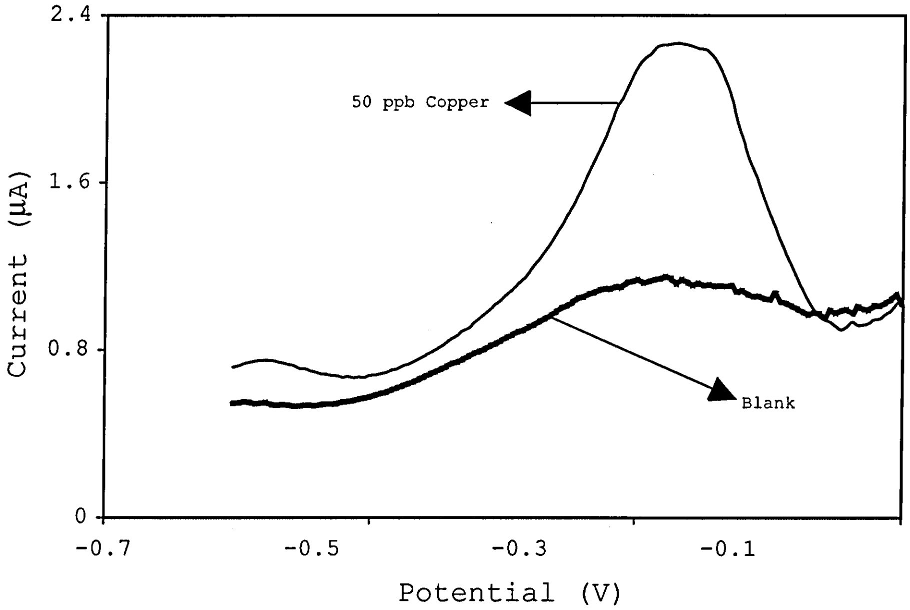

Fig. 3. Stripping voltammetric responses on SPE plated in situ with 10 ppm mercury(II). Supporting medium = 0.1 M HCl, deposition potential = −0.9 V, deposition time = 120 s, step potential = 5 mV, square-wave amplitude = 20 mV, frequency = 50 Hz.

internal electrolyte) reference electrode and platinum wire counter electrode (not shown). This large background signal obtained in this potential range (−0.4 to −0.1 V) is probably due to surface reactions when the electrode material is anodised or exposed to dissolved oxygen. With mercury film glassy carbon electrode (MFGCE), a well-defined and reproducible chronopotentiometric stripping signal was obtained for copper(II) and the blank supporting medium (Fig. 2b). Fig. 3 shows the response obtained using mercury film SPE coupled to ASV. The stripping voltammetric signal for the blank supporting medium did not obscure the copper stripping peak in the same way as it did using the mercury film SPE coupled to CCSCP (Fig. 2a). It seems the insufficient coverage of mercury film on the screen-printed working electrode surface and the inherent sensitivity of the CCSCP technique to the surface reactions on the working electrode are more or less the likely reason for this.

To solve the problem of the large background signal and the ill-defined copper stripping peak obtained on the mercury film SPE (10 ppm mercury plated in situ), the effect of increasing concentrations of HCl and KCl on the copper stripping chronopotentiometric response was investigated instead of increasing the concentration of mercury(II) plated in situ. The overlaid stripping chronopotentiograms obtained in Fig. 4 show that the copper stripping peak becomes more defined as the concentration of the chloride ion mediums exceed 1 M even though the magnitude of the stripping signal decreases as well. The well-defined peak obtained with both 2 M HCl and 2 M KCl (Fig. 4) in this work, shows that both of the highly concentrated chloride medium performs a role in reducing the amount of oxidants like residual dissolved oxygen exposed to the SPE working surface. High concentration of electrolyte is known [39] to increase the viscosity of solution and this allows for the reduction of the dissolved oxygen concentration. The high concentrated chloride also ensures that all copper measured is stripped as copper(I) chloride and not as a mixture of copper(II) and copper(I), as obtained with lower chloride concentrations [39,40]. Hence, a chloride concentration of 2 M was used for subsequent experiments with HCl as the choice, because it is normally free from metal ion impurities.

Other medium compositions were also examined because it would be desirable to be able to carry out the simultaneous detection of copper with other metals (cadmium and lead) on mercury film SPE (10 ppm

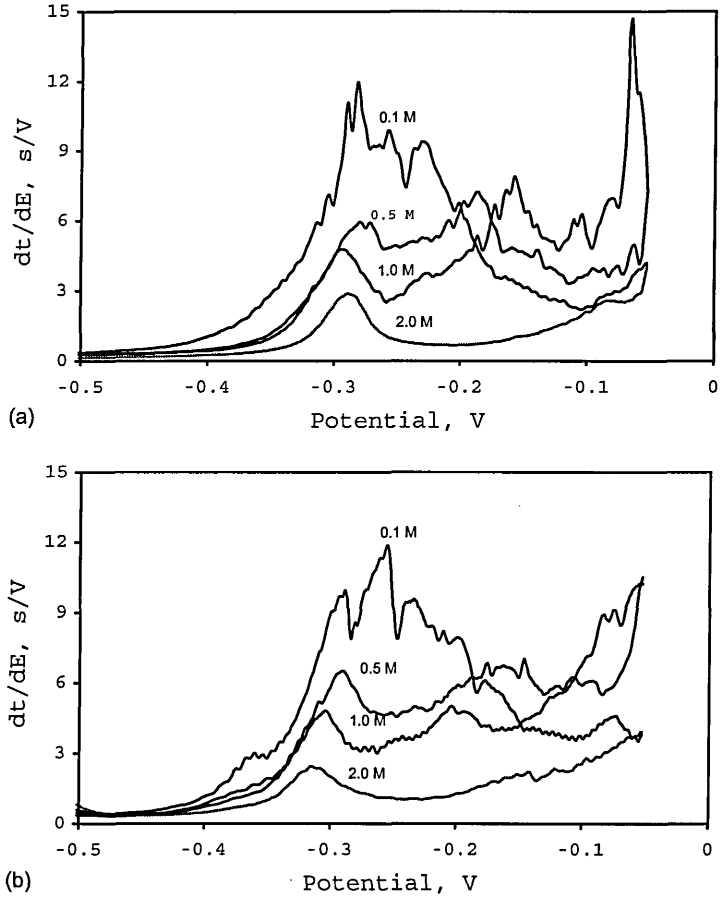

Fig. 4. Stripping chronopotentiogram of 50 ppb copper(II) in solutions containing (a) HCl and (b) KCl, at different concentrations using SPE plated in situ with 10 ppm mercury(II). Other conditions are as for Fig. 2.

mercury(II) plated in situ) in a less concentrated chloride-based medium and in a medium with higher pH (pH range 3–6). Hence, studies were carried out with 0.5 M ammonium acetate containing 0.1 M HCl because the pH of the medium composition (pH 5) is well-suited to the simultaneous detection of copper, lead and cadmium. The chronopotentiometric stripping response of copper obtained using this medium (not shown) was not as well-defined or reproducible as one generated with 2 M HCl and 2 M KCl (Fig. 4). Hence, the concentrations of HCl and ammonium acetate in the medium were increased to 0.5 and 1 M, respectively. The increase in viscosity and reduction in concentration of dissolved oxygen, assist in decreasing the chemical stripping (oxidation) rate [39]. The stripping peak obtained for copper using 1 M ammonium acetate containing 0.5 M HCl was much more stable and well-defined (not shown) compared to one generated at a lower concentration.

3.1.2. *Effect of applied constant stripping current*

The applied stripping current has significant influence on the sensitivity and reproducibility of response obtained using CCSCP. The profound effect of the applied stripping current upon copper stripping response and peak potential (E_{p}) is illustrated in Table 1. The stripping response decreased when the applied stripping current was high, because of the fast stripping of the deposited copper and increased when the stripping current was low due to the increased stripping time of

Table 1
Effect of applied stripping current on the stripping response of 50 ppb copper

	Stripping current (μA)					
	0.1	1.0	3.0	5.0	8.0	10.0
Peak potential (mV)	−0.315	−0.295	−0.250	−0.235	−0.200	−0.170
Mean peak area[a] (ms)	233	115	63	44	31	31
R.S.D. (%)	20.0	7.7	6.5	7.7	7.1	7.4

[a] Mean for six measurements.

the stripping step. The E_p also shifts to more positive values as the stripping current increases. This shift has been assigned [32,41,42] to an ohmic potential (iR) drop between the working and reference electrode.

For CCSCP measurements, inaccurate results can be obtained [32,42] if proper attention is not paid in applying the appropriate stripping current. The only way to circumvent this is, by careful optimisation of the stripping current applied. From studies carried out with 2 M HCl medium, a stripping current of +1 μA was chosen as a compromise between signal stability and sensitivity even though a higher response was obtained with a lower stripping current, 0.1 μA (Table 1). Also, the background baseline response obtained using an applied stripping current of 1 μA was much better compared to one obtained with 0.1 μA (not shown). The reproducibility data for stripping response of 50 ppb copper (shown in Table 1) was highest when using an applied stripping current of 0.1 μA due to the heavy stripping step and this is the case when too low a stripping current is applied. For 1 M ammonium acetate containing 0.5 M HCl, the application of a stripping current between +3 and +5 μA was considered to be the appropriate choice because the background baseline response improved considerably in contrast to response obtained with stripping current less than 2 μA.

3.2. Calibration plot, detection limit and precision

The stripping chronopotentiograms for increasing concentration of copper(II) are displayed in Fig. 5 and are generated on SPE coated in situ with 10 ppm mercury(II) using 2 M HCl as supporting medium.

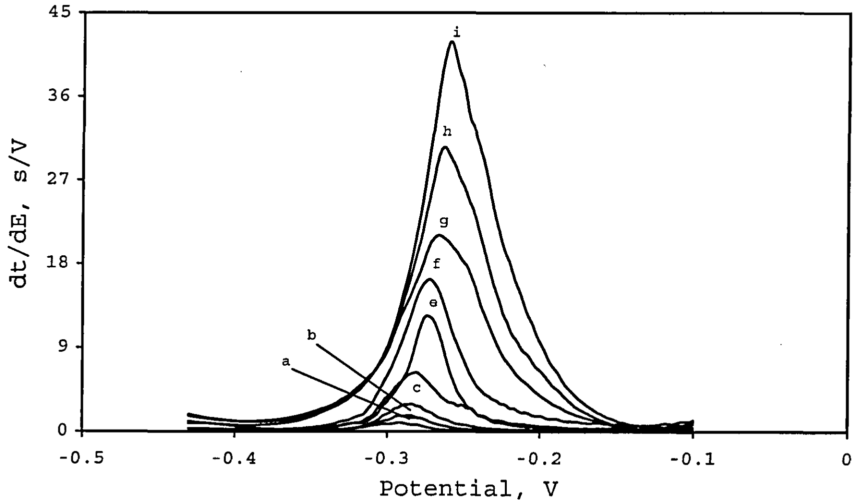

Fig. 5. Stripping chronopotentiograms for increasing concentration of copper(II). (a) 25 ppb; (b) 50 ppb; (c) 100 ppb; (d) 200 ppb; (e) 300 ppb; (f) 500 ppb; (g) 1000 ppb; (h) 1500 ppb and (i) 2000 ppb using 2 M HCl as supporting medium. Other conditions are as for Fig. 2.

Table 2
Analytical results for the determination of copper in reference sample

Reference sample (ppm)	CRM 601
ICP-MS[a]	9.8 ± 0.3
CCSCP[b]	10.38 ± 0.90
CCSCP[c]	11.11 ± 1.05

[a] Experiment carried out with ICP-MS (independent analysis).

[b] Experiment carried out with 2 M HCl, 10 ppm Hg(II) in situ plated, 120 s, 1 μA.

[c] Experiment carried out with 1 M ammonium acetate +0.5 M HCl, 10 ppm Hg(II) in situ plated, 120 s, 3 μA.

The calibration plot was linear ($y(\text{ms}) = 1.4035 \pm 0.015$ (ms ppb^{-1}) $+ 24.674 \pm 11.88$ ms) in the range examined (10–2000 ppb, correlation coefficient $r^2 = 0.9988$). For 1 M ammonium acetate containing 0.5 M HCl, a linear calibration plot ($y(\text{ms}) = 2.3529 \pm 0.043$ (ms ppb^{-1}) $- 20.935 \pm 18.13$ ms, $r^2 = 0.9980$) was obtained in the range examined (25–1000 ppb). Each calibration point was an average of three readings. The detection limits based on the use of $S/N = 3$ criterion were 6 ppb for 2 M HCl as supporting medium (following 2 min deposition) and 20.5 ppb for 1 M ammonium acetate containing 0.5 M HCl as supporting medium (following 2 min deposition). The stripping response was also reproducible with a series of eight repetitive measurements of 50 ppb copper(II) with 2 M HCl as supporting medium yielding relative standard deviation (R.S.D.) of 6.9% (following 2 min deposition) and for 1 M ammonium acetate containing 0.5 M HCl, a R.S.D. of 7.9% (following 2 min deposition).

3.3. Application to reference and soil samples

The adequacy of the CCSCP method coupled to the screen-printed three-electrode system for the determination of copper in environmental samples was verified by the use of extracted reference material and soil samples. Concentrations of copper in the acetic-acid bioavailable fraction of the reference and soil samples were determined by standard addition method. Results for the CRM 601 certified reference sample are given in Table 2. The result obtained for 1 M ammonium acetate containing 0.5 M HCl was also included, so as to provide an alternative to the use of 2 M HCl as supporting medium. A 1 M ammonium acetate containing 0.5 M HCl can be used if a medium with a higher pH is needed for simultaneous determination of copper(II), lead(II) and cadmium(II). The concentration value obtained for the acetic-acid bioavailable copper fraction using both the CCSCP methods was close to the ICP-MS analysis result as well as the indicative value (10.5 ± 0.8 ppm) cited in published literature [43] as the concentration for copper in CRM 601 reference material extracted with acetic-acid. A correlation plot between the results obtained for different soil samples using CCSCP coupled to the integrated screen-printed three-electrode device and ICP-MS is shown in Fig. 6. A relative high correlation coefficient value for results obtained between CCSCP and ICP-MS ($r^2 = 0.9712$, $n = 9$) shows good agreement between the methods. Also, the slope of the correlation curve, 0.9586, is close to 1 and this confirms the validity of the proposed method (CCSCP incorporating

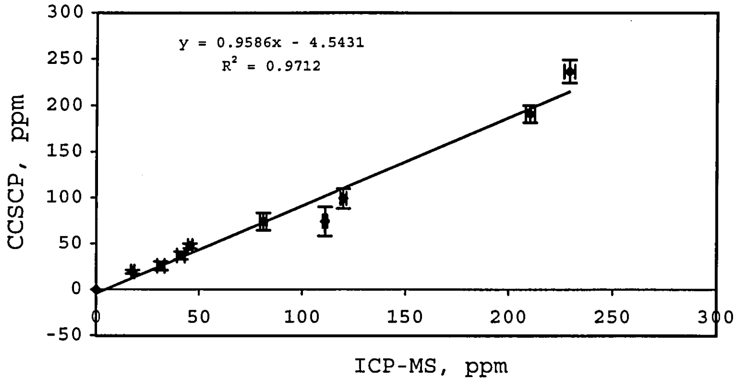

Fig. 6. Correlation between CCSCP and ICP-MS for the determination of copper in soil samples.

in situ plated mercury film SPE) for copper detection in environmental samples using 2 M HCl medium.

4. Conclusion

The difficulty in obtaining a stable and well-defined stripping chronopotentiometric peak for copper on the screen-printed three-electrode system coated in situ with mercury film was resolved by using an increased concentration of supporting electrolyte in the test solution. The use of either 2 M HCl or 1 M ammonium acetate containing 0.5 M HCl as the supporting medium ensured that the background signal obscuring the copper stripping response on the SPE (plated in situ with 10 ppm mercury(II)) was suppressed and this allowed copper detection to be carried out.

The results obtained within this investigation, has gone a long way in providing a screening device for copper determination in extracted soil samples using the first step of the BCR acetic-acid extraction procedure. The electrode configuration and it being a single-use electrode, makes it in combination with the detection technique, CCSCP, a convenient screening tool for copper at contaminated sites. Also, the analytical device allows for the transfer of the traditional three-electrode arrangement onto a plastic strip and this alleviates the need to manipulate the electrodes into position as experienced most times with the use of conventional electrodes. Results obtained correlated well with ones obtained with ICP-MS as well as the indicative value, quoted for the reference material. Also, the huge dynamic range inherent to the CCSCP technique makes it possible to analyse samples on the mercury film SPE over a wide linear concentration range especially as copper concentration in contaminated soils are in the ppm level.

Acknowledgements

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References

[1] B. Passariello, V. Giuliano, S. Quaresima, M. Barbaro, S. Caroli, G. Forte, G. Carelli, I. Iavicoli, Microchem. J. 73 (2002) 245.
[2] I. Palchetti, G. Marrazza, M. Mascini, Anal. Lett. 34 (6) (2001) 813.
[3] D.A. Blake, R.C. Blake II, M. Khosraviani, A.R. Pavlov, Anal. Chim. Acta 376 (1998) 13.
[4] A.L. Kukla, N.I. Kanjuk, N.F. Starodub, Y.M. Shirshov, Sens. Actuators B 57 (1999) 213.
[5] A. Vaisanen, R. Suontamo, J. Silvonen, J. Rintala, Anal. Bioanal. Chem. 373 (2002) 93.
[6] J. Wang, J. Lu, B. Tian, C. Yarnitzky, J. Electroanal. Chem. 361 (1993) 77.
[7] J. Wang, Analyst 119 (1994) 763.
[8] J. Wang, Trends Anal. Chem. 16 (1997) 85.
[9] J. Wang, B. Tian, V.B. do Nascimento, L. Angnes, Electrochim. Acta 43 (23) (1998) 3459.
[10] J. Wang, B. Tian, J. Wang, J. Lu, C. Olsen, C. Yarnitzky, K. Olsen, D. Hammerstrom, W. Bennett, Anal. Chim. Acta 385 (1999) 429.
[11] C. Yarnitzky, J. Wang, B. Tian, Talanta 51 (2000) 333.
[12] P. Ugo, L.M. Moretto, P. Bertoncello, J. Wang, Electroanalysis 10 (15) (1998) 1017.
[13] D. Desmond, B. Lane, J. Alderman, D.W.M. Arrigan, J.D. Glennon, M. Hill, Sens. Actuators B 48 (1998) 409.
[14] M. Jasinski, P. Grundler, G.-U. Flechsig, J. Wang, Electroanalysis 13 (1) (2001) 34.
[15] J. Wang, B. Tian, Anal. Chem. 64 (1992) 1706.
[16] J. Wang, B. Tian, Anal. Chem. 65 (1993) 1529.
[17] M. Somasundrum, J.V. Bannister, Sens. Actuators B 15–16 (1993) 203.
[18] C.G. Neuhold, J. Wang, V.B. do Nascimento, K. Kalcher, Talanta 42 (1995) 1791.
[19] D. Desmond, B. Lane, J. Alderman, G. Hall, M. Alvarez-Icaza, A. Garde, J. Ryan, L. Barry, G. Svehla, D.W.M. Arrigan, L. Schniffner, Sens. Actuators B 34 (1996) 466.
[20] G.S. Reeder, W.R. Heineman, Sens. Actuators B 52 (1998) 58.
[21] I. Palchetti, A. Cagnini, M. Mascini, A.P.F. Turner, Mikrochim. Acta 131 (1999) 65.
[22] I. Palchetti, C. Upjohn, A.P.F. Turner, M. Mascini, Anal. Lett. 33 (7) (2000) 1231.
[23] K.C. Honeychurch, J.P. Hart, D.C. Cowell, Electroanalysis 12 (3) (2000) 171.
[24] J.-C. Choi, K. Seo, S.-R. Cho, J.-R. Oh, S.-H. Kahng, J. Park, Anal. Chim. Acta 443 (2001) 241.
[25] J.-M. Zen, C.-C. Yang, A.S. Kumar, Electrochim. Acta 47 (2001) 899.
[26] K.C. Honeychurch, J.P. Hart, D.C. Cowell, Anal. Chim. Acta 431 (2001) 89.
[27] K.C. Honeychurch, J.P. Hart, D.C. Cowell, D.W.M. Arrigan, Sens. Actuators B 77 (2001) 642.
[28] J. Wang, J. Lu, S.B. Hocevar, B. Ogorevc, Electroanalysis 13 (1) (2001) 13.
[29] J.-M. Zen, C.-C. Yang, A.S. Kumar, Anal. Chim. Acta 464 (2002) 229.

[30] K.C. Honeychurch, J.P. Hart, D.C. Cowell, D.W.M. Arrigan, Electroanalysis 14 (3) (2002) 177.
[31] K.C. Honeychurch, D.M. Hawkins, J.P. Hart, D.C. Cowell, Talanta 57 (2002) 565.
[32] E. Beinrohr, M. Cakrt, J. Dzurov, L. Jurica, J.A.C. Broekaert, Electroanalysis 11 (1999) 1137.
[33] R.M. Town, H.P. van Leeuwen, J. Electroanal. Chem. 509 (2001) 58 (Corrigendum, J. Electroanal. Chem. 515 (2001) 129).
[34] M.M. Wilson, H.D. Dewald, Microchem. J. 69 (2001) 13.
[35] D. Jagner, Y. Wang, Electroanalysis 7 (1995) 614.
[36] E. Beinrohr, Accred. Qual. Assur. 6 (2001) 321.
[37] P. Quevauviller, Trends Anal. Chem. 17 (5) (1998) 289.
[38] D. Desmond, B. Lane, J. Alderman, J.D. Glennon, D. Diamond, D.W.M. Arrigan, Sens. Actuators B 44 (1997) 389.
[39] D. Jagner, E. Sahlin, L. Renman, Talanta 42 (1995) 1447.
[40] A.M. Green, A.C. Clark, G.R. Scollary, Fresenius J. Anal. Chem. 358 (1997) 711.
[41] E. Beinrohr, P. Csemi, A. Manova, J. Dzurov, Fresenius J. Anal. Chem. 349 (1994) 625.
[42] E. Beinrohr, M. Cakrt, J. Dzurov, P. Kottas, E. Kozakova, Fresenius J. Anal. Chem. 356 (1996) 253.
[43] F. Mossop, M. Davidson, Anal. Chim. Acta 478 (2003) 111.

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ORIGINAL PAPER

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Stripping chronopotentiometric measurements of lead(II) and cadmium(II) in soils extracts and wastewaters using a bismuth film screen-printed electrode assembly

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Abstract The key to remediative processes is the ability to measure toxic contaminants on-site using simple and cheap sensing devices, which are field-portable and can facilitate more rapid decision-making. A three-electrode configuration system has been fabricated using low-cost screen-printing (thick-film) technology and this coupled with a portable electrochemical instrument has provided a a relatively inexpensive on-site detector for trace levels of toxic metals. The carbon surface of the screen-printed working electrode is used as a substrate for in situ deposition of a metallic film of bismuth, which allows the electrochemical preconcentration of metal ions. Lead and cadmium were simultaneously detected using stripping chronopotentiometry at the bismuth film electrode. Detection limits of 8 and 10 ppb were obtained for cadmium(II) and lead(II), respectively, for a deposition time of 120 s. The developed method was applied to the determination of lead and cadmium in soils extracts and wastewaters obtained from polluted sites. For comparison purposes, a mercury film electrode and ICP-MS were also used for validation.

Keywords Cadmium · Lead · Stripping chronopotentiometry · Screen-printed electrode · Bismuth film electrode · Soils extracts · Wastewaters

Introduction

In recent years there has been an increasing need to assess impairment made by heavy metal pollution in the environment. This pollution, which arises from former industrial areas, contaminated agricultural lands and others sources, has led to a variety of problems for land-use, groundwater, terrestrial and aquatic ecosystems. Due to the risk to human health and the environment [1, 2, 3, 4], the extent of heavy metal pollution needs to be assessed as it occurs, so that it can be remedied before it becomes hazardous. To carry out this function, there is a need to develop a simple and cheap sensing device, which is field-portable and can highlight "hotspots" and facilitate more rapid decision-making for remediation of polluted sites.

Environmental measurements of heavy metals are normally carried out with the use of conventional analytical techniques like atomic absorption spectroscopy (AAS), inductively coupled plasma–atomic emission spectroscopy (ICP-AES) and inductively coupled plasma–mass spectrometry (ICP-MS). These techniques are impractical for on-site screening or as a quantification decision tool owing to their size and vast labour and analytical costs. Hence, there are needs for portable analytical systems. Electrochemical techniques, especially stripping analysis, satisfy many of the requirements for on-site screening of heavy metal ions [4, 5, 6, 7, 8] because of the low cost, low-power requirements, speed of analysis, sensitivity and instrument compatibility. The remarkable sensitivity obtainable with stripping analysis is attributed to the deposition step in which the target analyte(s) is(are) preconcentrated onto the working electrode [4, 5]. With the advent of portable, battery-powered computers and potentiostats/galvanostats, as well as the increasing application of microfabrication technologies like screen-printing to produce electrodes, the on-site measurement of heavy metals has been made much more accessible. Recently, a hand-held (miniaturized) portable electrochemical analyser relying on the coupling of potentiometric stripping analysis (PSA) and disposable screen-printed sensors was reported by Yarnitzky et al. [7] for the detection of lead (II). The performance and portability show that the system holds great promise for environmental or industrial applications. There are already other portable electrochemical stripping analysers presently on the market that can be used in conjunction with screen-printed electrodes for quantitative measurements of heavy metal ions. These are the Scanning Analyser 5000 (Palintenst Ltd, UK), HSA-1000 (Hach Company, USA), PG580 Hand-held Potentiostat/ Galvanostat (Princeton Applied Research (AMETEK), USA) and PalmSens bat-

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Although, there has been a lot of published work on the detection of metals with screen-printed electrodes using stripping analysis techniques [6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22], apart from Akhtar et al. [6] who described one for aluminium in soil solutions and Kadara et al. [8] for copper in soil samples, there have not been any other reports on the application of screen-printed electrodes in connection with stripping analysis to complex matrices like polluted soils and sediments. The analysis of samples that have been carried out are exclusively for simple matrices like drinking [7, 9, 10, 11, 12, 13], tap [13, 14], river [15, 16, 17], channel [18], lake [19], pond [20, 21], ground [21] and potable water [22]. Here, we report the simultaneous determination of lead and cadmium in soils extracts and wastewaters by using constant current stripping chronopotentiometry (CCSCP) in conjunction with a disposable screen-printed bismuth film electrode (SPBFE). This is the first reported use of a bismuth film electrode for the determination of heavy metals in extracted soil samples. The bismuth film electrode is an attractive alternative to the mercury electrode for electrochemical detection of heavy metals due to its low toxicity [4, 12]. We have also used a screen-printed mercury film electrode (SPMFE) to access the accuracy of the results obtained with the bismuth film electrode because mercury film electrodes are still considered the electrode of choice for stripping analysis [23] due to their outstanding electrochemical characteristics. For verification of the analysis results, ICP-MS was used.

Polluted soils especially from mining and smelting areas exhibit high concentrations of heavy metals. Hence, their availability in soils and surrounding waters is a crucial factor in estimating the potential hazardous effect on the environment. To access the availability of lead(II) and cadmium(II) from soils, the solid–liquid extraction with 0.11 M acetic acid according to the first step of the BCR sequential procedure [24] was applied. The available content obtained from this extraction procedure constitutes the mobile fraction of heavy metals in soil (ion exchangeable and bound to carbonates).

Experimental

Chemicals and reagents

All chemicals were of analytical reagent grade and used as received. Hydrochloric acid (HCl) was obtained from BDH Ltd (Poole, England) while ammonium acetate was obtained from Merck (Darmastadt, Germany). The cadmium(II), lead(II), copper(II), mercury(II) and bismuth(III) standard stock solutions (1,000 ppm or mg L^{-1}) were obtained from Aldrich (Gillingham, Dorset, UK) and diluted with deionised water and supporting electrolyte. The supporting electrolyte used in the experiments was 0.1 M HCl, adjusted to the required pH with ammonium acetate. Wastewater samples and soil samples extracted with acetic acid were supplied by Professor Manuel Valiente of the Autonomous University of Barcelona, Spain. Mww1 and Mww2 are wastewater samples while TP1, PX, C20, C22, SA79b, SA80b, SA83b, SA84b and SA91b are soils extracts. The extraction procedure used in obtaining the soils extracts is described by Mossop and Davidson [24].

Fabrication of electrode device

Screen-printed electrodes (SPEs) were mass-produced in-house by a multi-stage screen-printing process using a DEK 248 machine (DEK, Weymouth, UK) and screens with appropriate stencil designs (100 per screen) fabricated by DEK Precision Screen Division. The basal tracks for the three-electrode system were printed with graphite-based ink E423-SS (Acheson Colloids, UK) onto 250-μm-thick Melinex ST725 polyester sheet (Cadillac Plastic, Swindon, UK) and dried in an oven at 60°C for 2 h. A silver paste (MCA Services, Cambridge, UK) containing 15% silver chloride was printed on one of the basal tracks and left to dry. This track served as a reference electrode and the two other tracks (graphite–carbon working electrode with a 1.3-mm^2 planar area and a graphite-carbon counter electrode) were insulated using 242-SB epoxy-based protective coating ink (Agment ESL, Reading, UK), leaving a defined circular shaped area (electrochemical cell with an area of 3.2 mm^2) for measurements. The electrode strip produced was then heat treated at 120°C for 2 h to cure the epoxy resin.

Procedure

A hand-held, battery-powered PalmSens potentiostat/galvanostat (Palm Instruments BV, The Netherlands) interfaced to a Compaq iPAQ pocket PC and a computer-controlled Autolab PGSTAT10 Electrochemical Analyser with general-purpose electrochemical software operating system GPES version 4.7 (Eco Chemie B.V., 3508 AD Utrecht, The Netherlands) were used for electrochemical measurements. The screen-printed electrode strip was connected to the potentiostat/galvanostat with a specially adapted electrical edge connector from Maplin (Milton Keynes, UK). Studies were carried out by placing a 100-μL sample drop on the three-electrode strip. Each electrochemical measurement was carried out in triplicate with a new electrode strip in non-de-aerated and unstirred solution. Stripping chronopotentiometric measurements were carried out by depositing a metallic film of bismuth or mercury in situ with the target metal ions (lead and cadmium). A deposition potential (versus screen-printed Ag/AgCl reference electrode) was applied to preconcentrate the analytes. After the deposition period, a constant current was applied to strip the preconcentrated analytes until a limit of –0.2 V. The background signal for the supporting electrolyte was also measured in the same manner.

The concentrations for lead(II) and cadmium(II) in the wastewater samples and acetic acid extracts of soils were quantified by the use of standard additions method. CCSCP measurements were conducted by diluting the water samples and soils extracts 10- to 4,000-fold in the supporting electrolyte. The diluted wastewater and acetic acid extracted soil samples were analysed by spiking with appropriate concentrations of standard cadmium(II) and lead(II) solutions. Inductively coupled plasma–mass spectrometry (ICP-MS-model PQ EXCELL VG ELEMENTAL) was used to verify the results obtained.

Results and discussion

Stripping chronopotentiometry: optimisation of experimental variables for lead and cadmium

In order to perform the simultaneous determination of lead(II) and cadmium(II) in minewater samples and acetic acid extracts of soils, the parameters which most influence the measurement process were optimised: these were the concentration of bismuth(III) co-deposited in situ with the target metals (lead and cadmium), deposition time (t_{dep}), deposition potential (E_{dep}), pH and linear range. The effect of interfering ions on stripping responses was also examined and a comparison made between the performance of

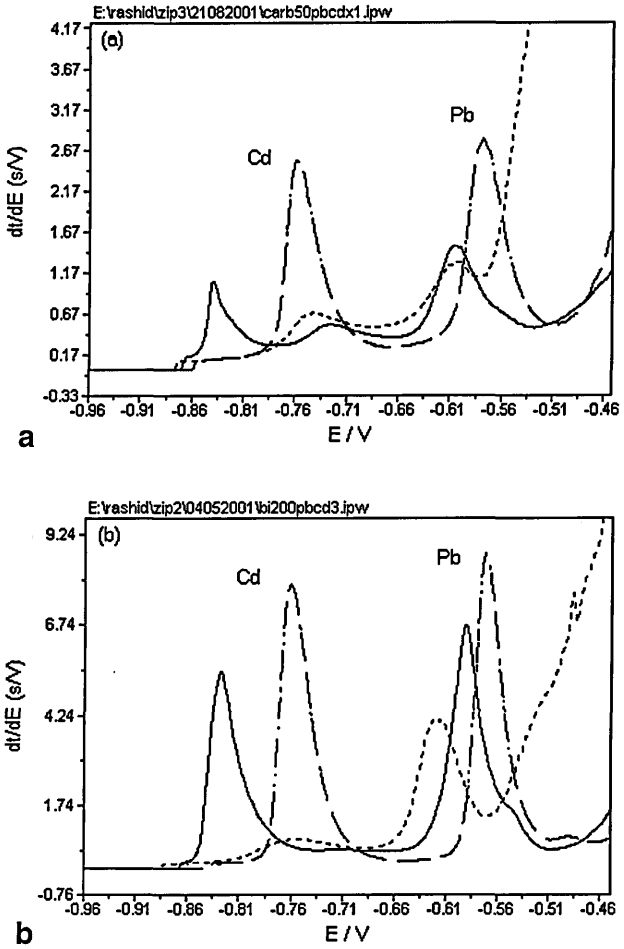

Fig. 1a,b Stripping chronopotentiograms for lead(II) and cadmium(II) at SPCE (············), SPBFE (———) and SPMFE (– – – –). Concentration: **a** 50 ppb and **b** 200 ppb; supporting electrolyte: 0.1 M HCl containing ammonium acetate (pH 4.6); deposition potential: –1.0 V; deposition time: 120 s; constant current: 1 μA; maximum time of measurement: 6 s

the bulk sized laboratory electrochemical analyser and the portable electrochemical analyser coupled to screen-printed electrodes.

For the co-deposition of bismuth(III) ions with the target analytes, the screen-printed carbon working electrode acted as the substrate on which a metallic film of bismuth was formed. A bismuth(III) concentration of 500 ppb was chosen for co-deposition onto the bare screen-printed carbon electrode (SPCE) because they offered a higher, more stable and reproducible response for lead(II) and cadmium(II) compared to electrodes without a metallic film. The effect of the coverage of the SPCE with bismuth film and mercury film can be seen from the increased stripping responses obtained in Fig. 1. The coverage makes the trace detection of lead and cadmium at low concentrations much more possible compared to detection on bare SPCE. The re-oxidation peaks for cadmium and lead on the SPBFE appear at potentials of approximately –0.84 V and –0.61 V (–0.76 V and –0.57 V for SPMFE and approximately –0.76 V and –0.63 V for SPCE), respectively (versus screen-printed Ag/AgCl reference). A time of 2 min was chosen for deposition, as this is a compromise between length of analysis and sensitivity. On the basis of sensitivity, resolution and reproducibility, an E_{dep} of –1.0 V was employed for lead and cadmium measurements. This is because at potentials more negative than –1.0 V, the stripping responses became very erratic probably due to hydrogen evolution and to interferences emanating from the electrode surface. From the studies carried out on the effect of pH, the sensitivity and the reproducibility of the lead and cadmium stripping response depend on the pH of the test solution in which they are analysed. The chronopotentiometric stripping response for lead is highly sensitive at low pH (<3.5), while cadmium chronopotentiometric stripping response is highly sensitive between pH 3.5 and 5. A compromised pH range of 4–5 was chosen for the simultaneous detection of lead and cadmium because of the stability of the stripping response and also the potential window in this intermediate pH range is wider than at a low acidic pH region (<3.5).

Table 1 Analytical features for the simultaneous detection of lead(II) and cadmium(II) by CCSCP[a]

Electrode type	Metal ion	Regression equation	Coefficient correlation (r^2)	Range (ppb)	Detection limit (ppb)
SPBFE	Pb(II)	Y=0.85x–6.42	0.9966	20–300	10
SPBFE	Cd(II)	Y=0.84x–8.82	0.9977	20–300	8

[a] E_{dep}=–1.0 V; t_{dep}=120 s; constant current=1 μA; maximum time of measurement=6 s; 500 ppb Bi (III) in 0.1 M HCl containing ammonium acetate

Under these conditions, the linear concentration range in which lead(II) and cadmium(II) could be detected on SPBFE was evaluated. The linear ranges of the calibration curves for both metal ions and the detection limits are summarised in Table 1. The linear ranges are wide enough to perform quantitative determinations of lead and cadmium after appropriate dilutions of samples. The linear range obtained for the stripping chronopotentiometric measurements of the metal ions at the SPBFE using a 120-s deposition time is much wider than reported by Wang et al. [4, 12] for simultaneous measurements of lead and cadmium at bismuth-coated glassy carbon electrodes (40–200 ppb, deposition time of 120 s) and for lead at bismuth-coated screen-printed carbon electrodes (10–100 ppb, deposition time of 120 s) using a measurement protocol that involves stirring. The detection limits were evaluated with the criterion $3\times S_b/m$, where S_b is the standard deviation of the intercept and m is the slope of the linear plot. The stripping response obtained with CCSCP at SPBFE was reproducible with a series of eight repetitive measurements of 50 ppb lead(II) and cadmium(II) yielding a relative standard deviation (RSD) of 4.5% and 5.1% (following a 2-min deposition), respectively.

As copper(II) is a common ion in a variety of analytical samples, its effect on the stripping responses of lead

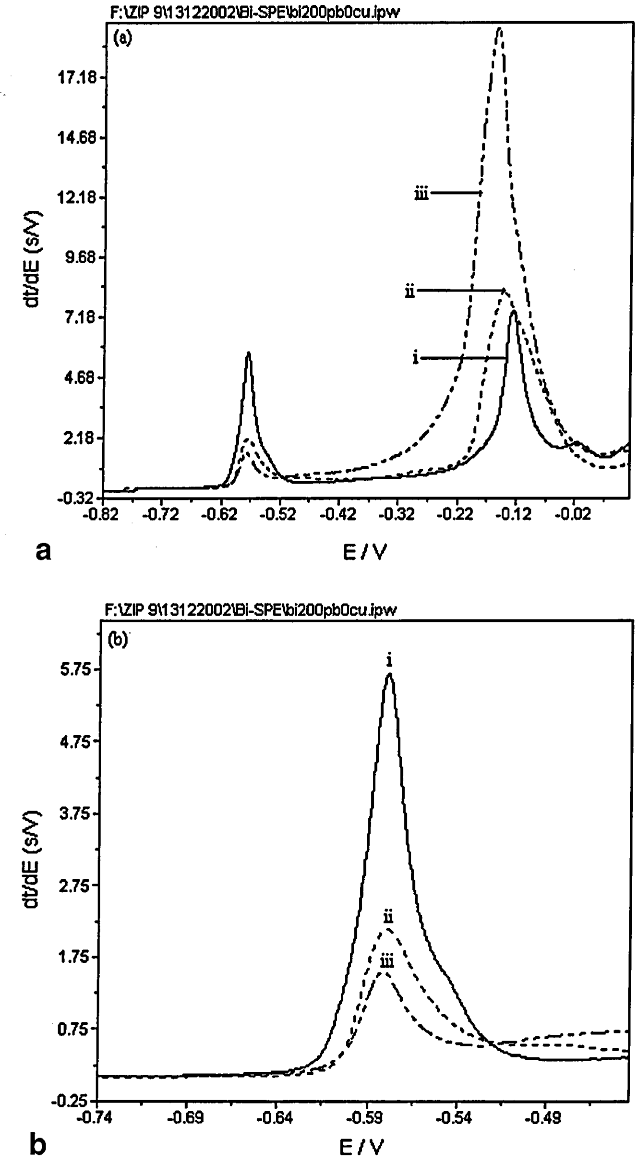

Fig. 2 **a** Stripping chronopotentiograms for lead(II) in the presence of copper(II) on SPBFE; **b** Close-up of the effect of copper on lead stripping response. *i* 200 ppb lead(II)+0 ppb copper(II); *ii* 200 ppb lead(II)+50 ppb copper(II); and *iii* 200 ppb lead(II)+500 ppb copper(II). Bi(III)=500 ppb, other conditions as in Fig. 1

and cadmium was studied. Copper(II) was found to inhibit the stripping response of lead(II) and cadmium(II) on the SPBFE. Figure 2 shows the effect of various concentrations of copper(II) on a fixed concentration of lead(II). The peak at –0.12 V (which represents the re-oxidation of bismuth, Fig. 2a, (i)) increased in size as the concentration of copper was increased because the copper(II) re-oxidation peak, which normally appears in the potential range –0.2 to –0.3 V, overlapped with the bismuth peak. The decrease in the lead(II) stripping response (at –0.58 V) obtained in the presence of copper(II) is due to copper altering the character of the electrode by formation of mixed layer of lead and copper during the deposition step. Hence, the enhancing effect of the bismuth film plated in situ on the electrode surface is negated.

Table 2 Simultaneous determination of lead(II) and cadmium(II) in wastewater samples

Sample	Metal ion	CCSCP (ppm)[a]		ICP-MS (ppm)[b]
		SPBFE[c]	SPMFE[d]	
Mww1	Pb(II)	0.905±0.050[e]	1.233±0.060	0.890±0.020
	Cd(II)	0.600±0.035	0.658±0.020	0.620±0.040
Mww2	Pb(II)	0.969±0.060	1.008±0.050	0.740±0.040
	Cd(II)	0.646±0.045	0.712±0.035	0.630±0.009

[a] *CCSCP* constant current stripping chronopotentiometry
[b] *ICP-MS* inductively coupled plasma–mass spectrometry
[c] *SPBFE* screen-printed bismuth film electrode
[d] *SPMFE* screen-printed mercury film electrode
[e] Value is the mean of triplicate determinations±standard deviation

Table 3 Determination of lead(II) and cadmium(II) in acetic acid extracted soil samples

Sample	Metal ion	CCSCP (ppm)		ICP-MS (ppm)
		SPBFE	SPMFE	
TP1	Pb(II)	203±11[a]	256±14	250±8
	Cd(II)	70±5	100±6	85±1
PX	Pb(II)	140±8	190±12	165±3
	Cd(II)	50±3	61±3	57±1
C20	Pb(II)	3.79±0.2	4.12±0.2	4.46±0.06
	Cd(II)	nd[b]	nd	nd
C22	Pb(II)	4.21±0.2	4.35±0.3	4.29±0.05
	Cd(II)	nd	nd	nd
SA79b	Pb(II)	0.33±0.02	1.28±0.08	1.27±0.04
	Cd(II)	nd	nd	nd
SA80b	Pb(II)	1.35±0.05	5.60±0.3	6.20±0.2
	Cd(II)	nd	nd	nd
SA83b	Pb(II)	2.10±0.13	2.80±0.2	3.15±0.04
	Cd(II)	nd	nd	nd
SA84b	Pb(II)	0.85±0.05	1.23±0.08	1.54±0.03
	Cd(II)	nd	nd	nd
SA91b	Pb(II)	1.35±0.1	2.03±0.2	2.85±0.04
	Cd(II)	nd	nd	nd

[a] Value is the mean of triplicate determinations±standard deviation
[b] *nd* not detected

The analytical outputs generated with the portable electrochemical analyser and the bulk-sized laboratory electrochemical analyser using the methodology first conceived with the latter, were compared. A relatively high correlation coefficient value (r^2=0.9965) was obtained for lead calibration in the range 20–300 ppb (not shown). Also, the slope value (0.9897), that is close to unity, indicates that there is good agreement in the output obtained for both instruments. Thus, the portable battery-powered potentiostat offers tremendous potential for obtaining analytical information of heavy metal pollutants at contaminated sites in a much simpler and cheaper manner.

Application to soil extract samples and wastewater samples

The SPBFE and SPMFE coupled to the portable battery-powered potentiostat were applied to the CCSCP determination of cadmium and lead in wastewater samples and acetic acid-extracted soil samples. The results obtained were compared with those given by ICP-MS and these results are tabulated in Tables 2 and 3. The concentrations of cadmium(II) and lead(II) in the samples were determined by the extrapolation of the standard addition plot (Fig. 3) constructed using the standard addition method. Standard addition is a well-known method for circumventing the problems associated with matrix effects common to many analytical methods.

As can be seen, there is a fairly close agreement between some of the results obtained with ICP-MS, stripping chronopotentiometric determinations at SPBFE and SPMFE. The non-detection of cadmium in some of the acetic acid extracts of the soil samples was because of the very low concentrations of cadmium in the extracts. As for the lower concentration of lead determined with CCSCP measurements at the SPBFE (compared to ICP-MS results and CCSCP measurements at the SPMFE) for some of the soil extract samples, the inhibiting effect of copper present in the soil extract samples is the reason. Copper(II) concentration at significant levels reduces the enhancing effect of the bismuth film on the electrode. The copper contents of all the soil samples analysed were 35 ppm (TP1), 12 ppm (PX), 0.80 ppm (C20), 0.85 ppm (C22), 5.2 ppm (SA79b), 38 ppm (SA80b), 1.24 ppm (SA83b), 0.26 ppm (SA84b) and 1.88 ppm (SA91b). These values were obtained with ICP-MS.

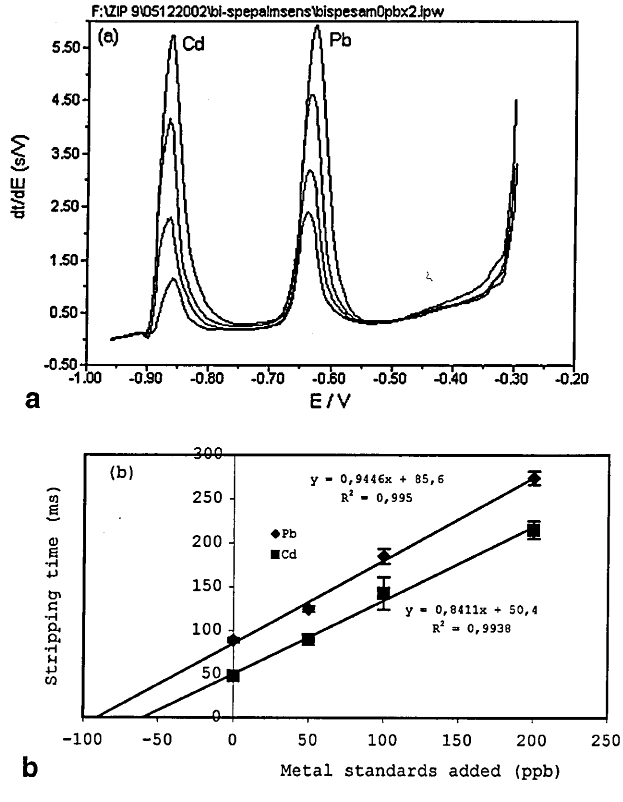

Fig. 3 **a** Typical stripping chronopotentiograms and **b** standard addition plot for the determination of lead and cadmium in wastewater sample on SPBFE. Standard solutions of lead(II) and cadmium(II) were added in 50, 100 and 200 ppb increments to the sample. Bi(III)=500 ppb, other conditions as in Fig. 1. The *error bars* represent standard deviation of 3 independent measurements

Apart from using the standard addition method for quantification, the linear equations derived from the calibration plots of lead and cadmium (Table 1) were also used for analysing the concentration of both metal ions in the wastewater samples. For stripping chronopotentiometric determination using SPBFE, the concentrations of lead(II) and cadmium(II) obtained were 0.993±0.007 ppm and 0.578±0.004 ppm, respectively, for Mww1 sample. For Mww2 sample, the concentrations of lead(II) and cadmium(II) were 1.12±0.01 ppm and 0.697±0.009 ppm, respectively. These results in comparison to results generated using the standard addition method (Table 2, CCSCP at SPBFE), indicate that direct analysis can be carried out for water samples using a preconstructed calibration plot.

Conclusions

The disposable screen-printed electrodes coupled to the portable electrochemical potentiostat used in this work offer some attractive features for field applications, including the use of microlitre (≤100 μL) sample solution, elimination of de-aeration or stirring steps, extremely low cost and role as a complementary screening or early tool indicating the presence of heavy metal contamination.

The coupling of the three-electrode configuration system and the portable electrochemical analyser using the CCSCP technique proved to be an excellent tool in the determination of the available lead and cadmium in soils as well as the concentration of both metals in water samples. Though the performance of the SPBFE used for stripping chronopotentiometric measurements measured up to those at SPMFE; the only downside is the undesirable effect of copper present in significant proportion in the samples. This needs to be minimised and work is ongoing to find a method of suppressing this effect, as this will allow for the replacement of mercury film electrodes with bismuth film electrodes for electrochemical stripping analysis of lead, cadmium and other metal ions like zinc.

Although the soils extracts were derived from soils extracted with a conventional extraction method (shaking to extract with 0.11 M acetic acid at room temperature) which takes too long, the use of alternative extraction procedures like microwave extraction and ultrasonic extraction would considerably reduce the extraction time. These two types of extraction methods can be deployed in the field and so enhance the applicability of the screening procedure for soil sample analysis at contaminated sites.

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References

1. Blake DA, Blake RC II, Khosraviani M, Pavlov AR (1998) Anal Chim Acta 376:13
2. Elik A (2002) Int J Environ Anal Chem 82:37
3. Vaisanen A, Suontamo R, Silvonen J, Rintala J (2002) Anal Bioanal Chem 373:93
4. Wang J, Lu J, Hocevar SB, Farias PAM (2000) Anal Chem 72: 3218
5. Wang J (2002) Trends Anal Chem 21:226
6. Akhtar P, Devereaux HA, Downard AJ, O'Sullivan B, Powell KJ (1999) Anal Chim Acta 381:49
7. Yarnitzky C, Wang J, Tian B (2000) Talanta 51:333
8. Kadara RO, Newman JD, Tothill IE (2003) Anal Chim Acta 493:95
9. Wang J, Tian B (1992) Anal Chem 64:1706
10. Wang J, Tian B (1993) Anal Chem 65:1529
11. Wang J, Lu J, Tian B, Yarnitzky C (1993) J Electroanal Chem 361:77
12. Wang J, Lu J, Hocevar SB, Ogorevc B (2001) Electroanalysis 13:13
13. Masawat P, Liawruangrath S, Slater JM (2003) Sens Actuators B 91:52
14. Palchetti I, Cagnini A, Mascini M, Turner APF (1999) Mikrochim Acta 131:65
15. Wang J, Tian B, Nascimento VB, Lu J, Park DS, Angnes L (1996) Electroanalysis 8:635
16. Honeychurch KC, Hart JP, Cowell DC, Arrigan DWM (2001) Sens Actuators B 77:642
17. Honeychurch KC, Hart JP, Cowell DC, Arrigan DWM (2002) Electroanalysis 14:177
18. Ugo P, Moretto LM, Bertoncello P, Wang J (1998) Electroanalysis 10:1017
19. Choi J-C, Seo K, Cho S-R, Oh J-R, Kahng S-H, Park J (2001) Anal Chim Acta 443:241
20. Honeychurch KC, Hart JP, Cowell DC (2000) Electroanalysis 12:171
21. Zen J-M, Yang C-C, Kumar AS (2002) Anal Chim Acta 464: 229
22. Honeychurch KC, Hart JP, Cowell DC (2001) Anal Chim Acta 431:89
23. Lee T, Chung K-C, Park J (2002) Electroanalysis 14:833
24. Mossop KF, Davidson CM (2003) Anal Chim Acta 478:111