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Speciation Analysis of Trace Metals in Natural Waters Using Vibrating Electrodes

Doctoral Dissertation

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Dedicated to my parents

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ABSTRACT

The speciation of trace metals plays a very important role in conditioning their biogeochemical cycles in the marine system. Their species are found to be strongly related to organic matters such as humic acids (HA) and fulvic acids (FA). This thesis aims to develop appropriate techniques to improve our understanding of the speciation of some trace metals, mainly in marine waters. Lead (Pb), cadmium (Cd) and chromium (Cr) which are widely used in industry were analyzed.

The concentration of lead in uncontaminated seawater is between 10 and 100 pM, whilst in coastal waters it is higher at up to low nanomolar levels. Little is known about the chemical speciation of lead since it is difficult to be determined at picomolar levels. The work in this dissertation was to develop a suitable electrode and procedure to determine lead in seawater, with the objective that the electrode could be applied for in-situ measurement (without reagents) and for speciation. Several electrodes and materials were tested, including a solid bismuth rod and different microwire materials. Vibration was used to enhance mass transport, instead of solution stirring, to decrease detection limits and facilitate in-situ monitoring.

The bismuth electrode was found to be suitable for monitoring lead in coastal waters: it is mercury-free and therefore environmentally friendly, but insufficiently sensitive for oceanic lead concentrations.

Comparison of microwires of carbon, gold and silver showed that all of these bare electrodes give a signal for lead, but they suffer variable interference from cadmium, although this is minor if the cadmium concentration is lower than lead.

A large improvement was obtained by coating the electrodes with mercury, which gave good peak resolution between cadmium and lead, and good sensitivity. The carbon and gold electrodes had good reproducibility and sensitivity when the mercury was renewed for each measurement. Once coated with mercury, the silver

electrode formed amalgam with the silver which could not be completely removed. This electrode was thus not suitable to be used as a renewable mercury film electrode. However, as a permanently amalgamated silver electrode, it was found to have good sensitivity for lead, good separation from cadmium, and was stable for long time usage. This electrode, the silver amalgam microwire (SAM) electrode, was selected for further speciation study of Pb in oceanic waters.

Optimum conditions for using the SAM electrode for trace lead detection in seawater involve the use of conditioning potentials. The limit of detection for lead was 4 pM lead in acidified seawater and 12 pM lead in seawater of pH 8. The higher limit of detection at pH 8 is due to the inorganic speciation at that pH.

The SAM electrode was successfully used for pseudopolarography of lead to determine its organic complexation. This method was calibrated using model compounds and applied for the first time to estuarine, coastal and oceanic samples at natural concentrations of Pb. It was found that lead occurs mostly as a labile (reactive, organic) species, with a smaller fraction as a strongly organically bound species.

The SAM electrode was also tested for chromium analysis using cathodic stripping voltammetry (CSV) in natural waters. Its detection limit is as good as the mercury drop electrode but reduces consumption of mercury.

Abbreviations and Symbols

Acronyms

AAS	Atomic Absorption Spectroscopy
AES	Atomic Emission Spectroscopy
ASV	Anodic Stripping Voltammetry
BBDE	Bismuth Bulk Disk Electrode
BFCPE	Bismuth Film Carbon Paste Electrode
BFGCE	Bismuth Film Glassy Carbon Electrode
CAE	Copper Amalgam Electrode
CLE-AdSV	Competing Ligand Exchange-Adsorptive Stripping Voltammetry
CE	Counter Electrode
CSV	Cathodic Stripping Voltammetry
CV	Cyclic Voltammetry
DO	Dissolved Oxygen
EDTA	Ethylenediaminetetraacetic Acid
GAME	Gold Amalgam Microwire Electrode
GME	Gold Microwire Electrode
HA	Humic Acid
HMDE	Hanging Mercury Drop Electrode
ICP-MS	Inductively Coupled Plasma Mass Spectroscopy
ISE	Ion-Selective Electrode
L	Ligand
LoD	Limit of Detection
LR	Linear Range
LSASV	Linear Sweep Anodic Stripping Voltammetry
M	Metal
MFCFE	Mercury Film Carbon Fibre Electrode
MFGCE	Mercury Film Glassy Carbon Electrode
MFIE	Mercury Film Iridium Electrode
ML	Metal Ligand Complex
MQ	Milli-Q Water
ORSE	Oscillating and Renewing Silver Electrode
PP	Pseudopolarography
RE	Reference Electrode
SAM	Silver Amalgam Microwire
SAMGE	Self-Assembled Monolayer-based Gold Electrode
SEM	Scanning Electron Microscope
SME	Silver Microwire Electrode
SWASV/SWCSV	Square Wave Anodic /Cathodic Stripping Voltammetry
DPASV/SWCSV	Differential Pulse Anodic /Cathodic Stripping Voltammetry
UVSW	Ultraviolet Digestion Sea Water
WE	Working Electrode

Symbols

k	Kinetic rate constant
δ	Diffusion layer thickness
D	Diffusion coefficient
α	Transfer coefficient
K	Thermodynamic stability constant

Experimental symbols

mM	millimolar
μM	micromolar
nM	nanomolar
pM	picomolar
ppb	part per billion
ppm	part per million
i_d	Diffusion limited current
i_{max}	Maximum peak current
t_{dep}	Deposition time
E_{con}	Conditioning potential
E_{dep}	Deposition potential
E_{des}	Desorption potential
E_{rest}	Resting potential
$E_{1/2}$	Half wave potential

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Papers resulting from the experiments carried out and described in this dissertation

Bi, Z., Chapman, C.S., Salaün, P. and van den Berg, C.M.G., 2010.

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Espada-Bellido, E., **Bi, Z.** and van den Berg, C.M.G., 2012. Determination of chromium in estuarine waters by catalytic cathodic stripping voltammetry using a vibrating silver amalgam microwire electrode. *Talanta*.(in press).

Thesis Structure

The methods and electrodes in this thesis were developed for metal speciation analysis in natural waters, including open sea, coastal and estuary. Metal concentrations are determined at acidified pH and speciation in natural pH.

Chapter 1. Introduction to the biogeochemistry of Pb, Cd and Cr in the marine system and methods to determine their speciation.

Chapter 2. A solid bismuth electrode was developed as a substitute for the more toxic mercury drop electrode. Lead and cadmium were measured in samples from Liverpool bay.

Chapter 3 and chapter 4. Mercury film electrodes were developed to improve on the bismuth electrode. Mercury was coated onto carbon fibre, gold and silver microwires. Chapter 3 is their comparison for low metals concentration analysis to select an agreeable one for open sea use. Chapter 4 shows the Pb and Cd analysis in open sea waters using silver amalgam electrode.

Chapter 5. The silver amalgam electrode is successful applied to analyze Pb speciation in natural waters, especially in the open sea which has not been reported using pseudopolarography yet.

Chapter 6. To test the possibility of CSV on the silver amalgam electrode, Cr was measured in samples from Mersey River shown in this chapter. The results are comparable to mercury drop electrode.

1

Chapter 1 Introduction

1. Introduction

This dissertation is on the topic of improved electroanalytical methods for the determination of Pb, Cd and Cr and their speciation in natural waters including seawater using voltammetry. It also aims to develop the methods that are suitable for use in the field, which means that the reagents need to be minimised. For these reasons mercury drop electrodes are unsuitable (they also have environmental drawbacks) and solid electrodes are pursued only. Therefore, a suitable solid electrode was developed for the determination of trace metals in seawater.

Study area of this dissertation: to set the scene for Pb, Cd and Cr.

Trace metals occur at picomolar to nanomolar level in natural waters including seawater (Riley and Skirrow, 1975). They are important for the study of the marine system because some of them, the biogenic elements, are essential to micro-organisms. The biogenic elements have a distribution in the oceanic water column similar to that of nutrients (phosphate and nitrate) and include Cu (Bruland, 1980), Zn (Bruland et al., 1978b), Ni (Westerlund and Ohman, 1991), and Cd (Boyle et al., 1976). Pb is not one of the biogenic elements and its oceanic profile is that of a scavenged elements like Mn and Co (Biller and Bruland, 2012; Jickells and Burton, 1988; Westerlund and Ohman, 1991): its concentration decreases with depth and it is strongly affected by anthropogenic effects (Flegal et al., 1993). Pb is important as it has been associated with environmental pollution, and its concentration in the ocean has been much increased because Pb is broadly used as a petrol additive (Johnson et al., 1995). The oceanic concentration of Pb is now decreasing again (Wu and Boyle, 1997b) (Lee et al., 2011) due to regulation policies (Migon and Nicolas, 1998; Nicolas et al., 1994). Because of its various industrial uses such as pigments and cosmetics (Casas Fernández et al., 2006), Pb is an excellent element to study anthropogenic effects on natural waters, and for this reason it is important to have a good method to monitor its concentration in the field. Furthermore, Information about its chemical

speciation in natural waters, including seawater, is sparse because it is difficult to determine natural concentrations at the low levels at which it occurs due to various interferences. For this reason it was decided to develop a better electrode for its determination.

The electrodes that were developed for Pb, were found to be also suitable for Cd using the same technique (Chapter 2 to Chapter 4). And, further experiments showed that one, a silver amalgam electrode, is also suitable for cathodic stripping, for which Cr was used as example (Chapter 6). For this reason Cd and Cr are included in the discussion below.

1.1. Distribution and speciation of Pb, Cd and Cr in natural waters

The geochemistry and bioavailability of metals is controlled by their chemical speciation (the distribution over various chemical species) as this determines their chemical charge and therefore the tendency to adsorb on particles (Bruland et al., 1994) (Sherrell and Boyle, 1992), and their availability for uptake by organisms (Ahner and Morel, 1995; Ahner et al., 1997) (Luoma, 1983) (Price and Morel, 1990). For this reason it is essential to determine the speciation as well as the concentration of metals in natural waters. Dissolved metal speciation is influenced by pH (Christensen and Christensen, 2000), temperature and pressure (Turner et al., 1981a) as well as the presence of redox reactions in the solution (Pohl and Hennings, 1999). Metals like Cu, Zn, Fe and Co have been shown to be mostly (>99%) complexed with organic matter in seawater (Ellwood and van den Berg, 2001; Gianguzza et al., 2002; Gledhill and Van Den Berg, 1994) (Lohan et al., 2002). Little information is available for Pb (Capodaglio et al., 1990) (Kozelka et al., 1997; Scarponi et al., 1995) and Cd (Bruland, 1992) (Wells et al., 1998). Table 1.1 briefly reviews basic information about the chemistry of Pb, Cd and Cr in the marine system.

1.1.1. Oceanic distributions of Cd, Pb and Cr

The oceanic profiles of Pb, Cd and Cr are summarized in Figure 1.1 Scavenged elements tend to decrease with depth (e.g. Pb), nutrient-like elements increase with

depth as they are re-cycled (e.g. Cd) and other elements can be mixed due to variations in oxidation states (Cr). The profiles for lead, cadmium and chromium in the oceans reflect this behaviour (Figure 1.1).

The oceanic profile of Pb is typical for that of a scavenged element: decreasing with depth. However, its distribution in the upper water column has recently been strongly affected by anthropogenic inputs, causing it to increase much above its natural concentration (Patterson and Settle, 1987) (Duce, 1991) (Ng and Patterson, 1981). For this reason, Pb is showing much higher concentrations in surface waters. Interestingly, the surface concentrations of Pb have been reported to decrease over the past 20ys as the use of Pb in petrol was phased out (Lee et al., 2011). The surface concentration of Pb can be clearly seen to decrease in Figure 1.1.

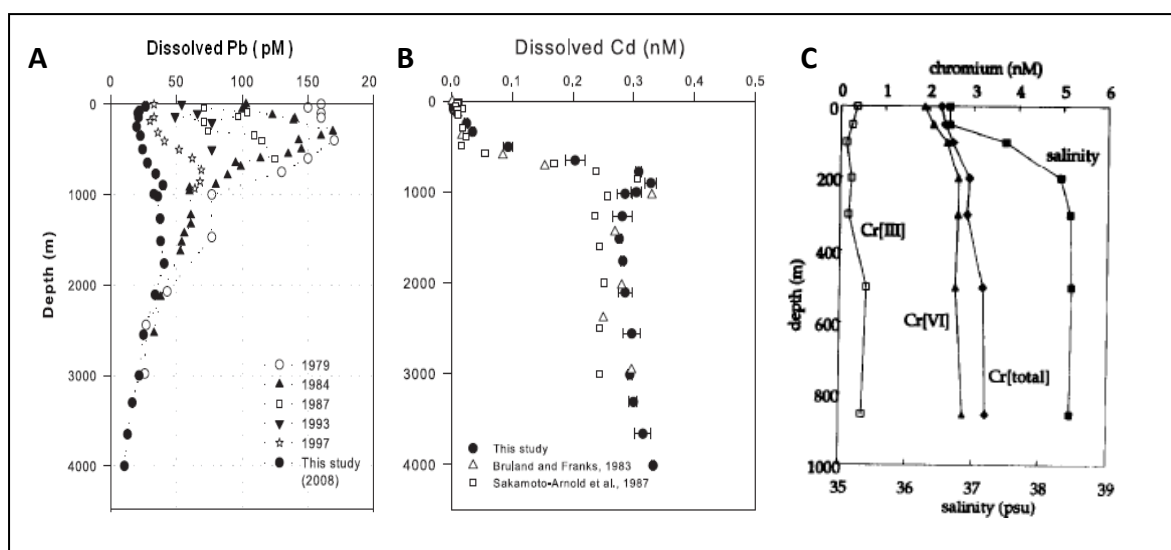


Figure 1.1. Typical profiles of Pb, Cd and Cr in the oceans. Profiles of Pb and Cd are from North Atlantic (Lee et al., 2011) ; Cr is in Mediterranean sea (Achterberg and Van Den Berg, 1994a).

The depth profiles of cadmium resemble those of phosphate (Debaar et al., 1994; Elderfield and Rickaby, 2000): It shows a surface depletion and deep release feature. A linear metal-nutrient relationship between Cd and Phosphate in oceans is observed (Bruland et al., 1978a; Danielsson, 1980; Hendry et al., 2008; Lee et al., 2011). Cd increases in deep water is due to a process of dead biota sinking and decomposing to release the metal (Westerlund and Ohman, 1991). Cadmium is

suggested to have much less input from atmospheric aerosols (0.4 pM) and is almost completely governed by its internal cycle (Bruland et al., 1994) (Hendry et al., 2008). Cd has been found to be a useful tracer of biogeochemical processes for recent: Cd has been used as a paleoproxy based on the observed relationship between Cd and CaCO_3 (Boyle, 1988) (Vangeen et al., 1992).

Chromium is known to occur in two oxidation states (Cr (III) and Cr (VI)). Cr (VI) is an anion tends to behave conservatively, whereas Cr (III) is strongly complexed with hydroxide species and occurs as the neutral $\text{Cr}(\text{OH})_3$. Cr (III) is readily adsorbed and scavenged by particles and this species therefore behaves as a scavenged element. The oceanic behaviour of Cr reflects these two processes: overall it was found to increase slightly with depth in the Mediterranean Sea and the North Atlantic Ocean (Achterberg and Van Den Berg, 1994a) as shown in Figure 1.1(C). Chromium has also been shown to have a nutrient like profile in Atlantic waters indicating it is involved in a biological cycle of surface uptake and regeneration in the deep sea (Sirinawin et al., 2000). Its concentration in the Atlantic sea was found to follow that of silicate (Campbell and Yeats, 1981).

1.1.2. Abundance of Pb, Cd and Cr in seawater

Figure 1.1 shows that the concentration of Pb in ocean waters is 20 to 50 pM, cadmium is up to 0.35 nM, and total Cr is 2.0 to 3.0 nM. The concentrations of these metals are greater in coastal waters where concentrations have been found of 0.1 to 8.1 nM Pb (Kozelka et al., 1997), 0.15 to 1.3 nM Cd (Vangeen et al., 1991) and 1.9 to 8.3 nM Cr (Abusaba and Flegal, 1995).

1.1.3. Metal Bioavailability and Speciation

Speciation, the distribution of an element amongst defined chemical species in a system (defined by IUPAC), is a very important concept to connect the interests of chemistry, biology and physics and others subjects together and to find new research fields. The chemical speciation of trace heavy metals has been shown to directly relate to their toxicity and availability in aqueous systems (Allen et al., 1980). The

speciation due to inorganic ligands is well known: The inorganic speciation of lead is dominated by carbonate species (Powell et al., 2009), with an α -coefficient of 28 for its inorganic species in pH 8.0 seawater. Cd is dominated by complexation with Cl^- and has a value for its α -coefficient of 35 in seawater (Turner and Whitfield, 1987) (More species are shown in Table 1.1). So both metals are predominantly complexed with inorganic ligands, but differing in tendency to bind with either Cl^- (Cd) or with OH^- (Pb) which probably explains the different geochemical behaviours of these elements. Cr (III) is completely complexed by OH^- (Turner et al., 1981a).

Organic complexation dominates the speciation of various biogenic metals, such as Cu (Hering and Morel, 1988), Zn (Wells et al., 1998) and Fe (Mikkelsen et al., 2006). These ligands are released by organisms (Morel and Price, 2003). Generally, the free metal species (free hydrated ions and weak inorganic complex ions) are more amenable to uptake by organisms and the bound metals are less toxic than the uncomplexed forms (Ahner et al., 1997). Their bioavailable and toxic effects are because of their ability to pass through the cell membranes of phytoplankton and macro-algae (Campbell et al., 2002) (Gledhill et al., 1997). In contrast, metals complexed by organic ligands (e.g. humic acid, fulvic acid, EDTA and NTA) can't pass the cell membranes, reducing the availability of metals to organisms (Sunda and Huntsman, 1998). Primary productivity in large parts of the ocean is limited by a lack of iron (Martin and Fitzwater, 1988) in which complexation reactions are likely to play a role.

Chromium is reported to have two stable valences: Cr (III) and Cr (VI). The transfer of Cr (VI) to Cr (III) occurs under both sub-oxic and anoxic conditions in situ by photolytically generated Fe^{2+} (Kieber and Helz, 1992; Pettine et al., 1998) (Abusaba and Flegal, 1995). It was reported that Cr (III) can be taken up by organisms (Tessier et al., 1995).

1.1.4. Speciation classification

The speciation of trace metals in natural environment can be classified according to the analytical technique applied (Florence, 1982). The category and

classifying strategy have been widely reviewed (Davison, 1978) (Florence and Batley, 1980) (Turner et al., 1981a). Examples about Pb, Cd and Cr species are listed in Table 1.1.

Table 1.1. Chemical Speciation of Pb, Cd and Cr in Seawater. Speciation is classified according to the techniques applied including Separation methods, voltammetrical approaches and theoretical calculation.

Metal	Species	Pb	Cd	Cr	Reference
[M] /(nM)		0.005 - 0.10	0.001 - 1.0	2.0 - 3.0	(Biller and Bruland, 2012) (Achterberg and Van Den Berg, 1994a)
Valency State		Pb(II)	Cd(II)	Cr(III); Cr(VI)	(Elderfie.H, 1970)
Speciation Classification by Separation Technique	Particle or Colloidal	Pb-(IL/OL) _n · ·PIM/POM	Cd-(IL/OL) _n · ·PIM/POM	Cr(OH) _x /CrO ₄ ²⁻ ····PIM/POM	(Riley and Chester, 1971)
	Dissolved	Pb ²⁺ , Pb-(Cl ⁻) _n , Pb-OH ⁺ Pb-(CO ₃ ²⁻) _n , Pb-DOM	Cd ²⁺ , Cd-(Cl ⁻) _m Cd-DOM	Cr(OH) ₂ ⁺ , Cr(OH) ₃ CrO ₄ ²⁻ , NaCrO ₄ ⁻	
Speciation Classification by Voltammetry	Labile	Pb ²⁺ , Pb-(Cl ⁻) _n , Pb-(CO ₃ ²⁻) _n , Pb-(OL) _n	Cd ²⁺ , Cd-(Cl ⁻) _m	NA	(Bruland, 1992) (Wells et al., 1998)
	Inert	Pb-DOM (e. g. HA. FA)	Cd-DOM (e. g. HA. FA)	NA	
Separation Classification by Model	Free Metal	Pb ²⁺	Cd ²⁺	NA	(Millero and Hawke, 1992)
	Complex Metal	PbCl ⁺ , PbCl ₂ , PbCl ₃ ⁻ , Pb(OH) ⁺ , PbCO ₃ , Pb(CO ₃) ₂ ²⁻	CdCl ⁺ , CdCl ₂ , CdCl ₃ ⁻	Cr(OH) ₂ ⁺ , Cr(OH) ₃	(Turner et al., 1981a)
Note: IL: Inorganic Ligand; OL:Organic Ligand (e.g.HA, FA); DOM: Dissolved Organic Matter; PIM:Particle Inorganic Matter (e.g. MnO ₂ , Fe ₂ O ₃ , Al ₂ O ₃ ..); POM:Particle Organic Matter (e.g.Bacteria, Phytoplankton..) ; NA: Not Available					

According to the analysis techniques, metal speciation can be divided into different groups. Theoretically, metal speciation can be calculated using known thermodynamic constants such as log K. Many models have been proposed to explain the metal species in natural environment. Metal ions can adsorb on particles

(organic and inorganic) forming non-dissolved species. The dissolved metals include free hydrated ions, inorganic complexes and organic complexes which can be determined by various techniques. The most popular speciation analysis methods are based on the electrochemical techniques such as stripping voltammetry. Extensive research works can be found for Cu (Vandenberg, 1984; Wells et al., 1998) and Fe (Croot and Johansson, 2000; Mikkelsen et al., 2006) speciation study in seawater. Depending on the response to this technique, metal complexation can be grouped into labile and non-labile (or inert or irreversible species) (Florence, 1986). Labile metal is considered to be the more bioavailable fraction (Florence et al., 1983) (Young et al., 1979) (Muse et al., 2006) which includes free metal and inorganic complexes and some weak organic complexes. Free metals could be the reason that labile species are related to the bioavailability or toxicity (Allen et al., 1980). The inert metal contains the fraction that is stable and not bioavailable. Inorganic labile metal forms complexes and reaches equilibrium quickly compared to non-labile organic complexes (Duinker and Kramer, 1977) (Hering and Morel, 1989). It indicates that the natural organics in marine system could detoxify the input metals from industry or natural release gradually.

Measurements of speciation in Antarctic waters have shown that the complexed Pb and Cd with organics represent a significant portion of the total (46% and 48% of labile Pb and labile Cd) (Scarponi et al., 1995). Similarly, it has been found that about 50% dissolved Pb is complexed with natural organic ligands in seawater (Capodaglio et al., 1990). The complexation possibility between Cr (III) and humic acid was confirmed (Fukushima et al., 1995) suggesting that the Cr (III) might also be organical complexed in the sea in addition to the OH^- (Turner et al., 1981a), though this has not been demonstrated.

Amenability of elements in the periodic system for detection by voltammetry

A large proportion of elements could be amenable for electrochemical determination: in principle, any change in oxidation state is associated with currents that could be determined electrochemically. This is summarised in Table 1.2, which

shows a copy of the periodic system of the elements, and brief information about conditions under which these elements could be determined.

This dissertation is aimed at Pb, Cd and Cr, so methods for these elements will be discussed below.

1.2. Analysis methods for speciation

Metal concentrations in natural waters can be determined by spectroscopic methods (AAS, ICP-MS) after a separation step in which the metals are separated from seawater salts (Bruland, 1980; Wu and Boyle, 1997c). Although some studies have been reported for metal speciation analysis, such as Ni (Lam et al., 1999), Cu and Pb (Procopio et al., 1997) (Alrashdan et al., 1991) by combining with separation techniques, because the speciation could be lost during the separation step so they are not suitable to analyze the natural speciation system. Spectroscopy methods are also widely reported for chromium speciation studies (Cranston and Murray, 1978). They might be fine to measure Cr because chromium has simple chemical forms in natural waters: CrO_4^{2-} and Cr(OH)_x . However, Cr may be also involved the complexation systems, for example it can bind with biological ligand released from organisms.

Electrochemical methods are the method of choice to determine the speciation of Pb, Cd and Cr in seawater, as they directly determine the reactive species in the water. A number of their applications are summarized in Table 1.2.

Voltammetry, a kinetic electrochemical technique based on the relationship between potential and current, has become greatly popular for element analysis particularly for trace metals. Speciation procedures are either based on anodic stripping voltammetry (ASV) (Mikkelsen et al., 2006) or cathodic stripping voltammetry (CSV) (Vandenberg, 1984). The concentration and speciation of lead and cadmium in natural waters have been measured by both ASV (Fischer and van den Berg, 1999b) and CSV (Vandenberg, 1986). Chromium speciation has been mainly determined by CSV (Boussemart et al., 1992). More specific examples are given in Table 1.3.

Periodic Table of the Elements

H 1																	He 2
Li 3	Be 4											B 5	C 6	N 7	O 8	F 9	Ne 10
Na 11	Mg 12											Al 13	Si 14	P 15	S 16	Cl 17	Ar 18
K 19	Ca 20	Sc 21	Ti 22	V 23	Cr 24	Mn 25	Fe 26	Co 27	Ni 28	Cu 29	Zn 30	Ga 31	Ge 32	As 33	Se 34	Br 35	Kr 36
Rb 37	Sr 38	Y 39	Zr 40	Nb 41	Mo 42	Tc 43	Ru 44	Rh 45	Pd 46	Ag 47	Cd 48	In 49	Sn 50	Sb 51	Te 52	I 53	Xe 54
Cs 55	Ba 56	57-71	Hf 72	Ta 73	W 74	Re 75	Os 76	Ir 77	Pt 78	Au 79	Hg 80	Tl 81	Pb 82	Bi 83	Po 84	At 85	Rn 86
Fr 87	Ra 88	89-103	Rf 104	Db 105	Sg 106	Bh 107	Hs 108	Mt 109	Ds 110	Rg 111	Cn 112	Uut 113	Fl 114	Uup 115	Lv 116	Uus 117	Uuo 118
Lanthanides			La 57	Ce 58	Pr 59	Nd 60	Pm 61	Sm 62	Eu 63	Gd 64	Tb 65	Dy 66	Ho 67	Er 68	Tm 69	Yb 70	Lu 71
Actinides			Ac 89	Th 90	Pa 91	U 92	Np 93	Pu 94	Am 95	Cm 96	Bk 97	Cf 98	Es 99	Fm 100	Md 101	No 102	Lr 103

Unmeasurable by Electrochemical Techniques	Electrochemical Techniques	Electrochemical Techniques- Voltammetry	Electrochemical Techniques in Seawater	Electrochemical Technique – Voltammetry in Seawater
Period Table	Period Table	Period Table	Period Table	Period Table

Table 1.2. Application of electrochemical techniques for the determination of elements in environment.

Table 1.3. Pb, Cd, and Cr detection by voltammetry using different electrodes

Metal	Electrode	Method	t_{dep} (s)	LoD _{rep} (nM/L)	pH	Application	Reference
Pb	HMDE	SWCSV	180	0.029	5.0	Pacific	(Wu and Batley, 1995)
Pb	GME	SWASV	300	0.070	8.3	Mersey Estuary	(Billon and van den Berg, 2004)
Pb	SME	SWASV	300	0.20	2.0	Mersey Estuary	(Billon and van den Berg, 2004)
Pb	CFE	LSASV	300	3.9	3.0	NA	(da Silva, 1998)
Pb	DAE	PSA	120	15	1.0	Wine	(Mikkelsen et al., 2004)
Pb	BBDE	SWASV	120	0.50	4.5	Irish Sea	This work
Pb	MFIE	SWAV	240	1.0	4.5	Drinking water	(Kounaves and Deng, 1993)
Pb/Cd	HMDE	DPCSV	60	0.30/0.12	7.7	English Channel	(Vandenberg, 1986)
Pb/Cd	MFGCE	SWASV	300	1.6/0.56	5.6	Sea water	(Fischer and van den Berg, 1999b)
Pb/Cd	ORSE	DPASV	120	0.97/8.9	2.0	Tap water	(Mikkelsen and Schroder, 2001)
Pb/Cd	CAE	DPASV	60	0.0091/0.017	4.8	NA	(Yosypchuk and Novotny, 2003)
Pb/Cd	MFCFE	DPASV	600	0.15/0.22	4.5	NA	(Wang et al., 1987)
Pb/Cd	BFCPE	SWASV	120	3.8 / 8.9	4.5	Sea water	(Svancara et al., 2006)
Pb/Cd	SAME	SWASV	60	0.0076/0.079	2.0	Atlantic, Pacific	This work
Cr	HMDE	DPCSV	120	0.10	5.2	Mediterranean	(Boussemart et al., 1992)
Cr	MFIE	DPCSV	600	9.6	5.6	Ground water	(Wang et al., 1997)
Cr	SAFE	CCSCP	15	0.48	6.2	River	(Bobrowski et al., 2011)
Cr	BFGCE	SWCSV	60	0.34	4.6	River	(Jorge et al., 2010)
Cr	SAMGE	SWCSV	300	0.023	7.8	NA	(Turyan and Mandler, 1997)
Cr	SAME	DPCSV	30	0.23	5.5	Mersey Estuary	This work

Note: Electrode name refers to the Abbreviations and Symbols List in the front of this thesis

1.2.1. Stripping voltammetry

Anodic stripping voltammetry (ASV) is the most widely used form of stripping analysis. Its basic analysis process together with CSV is shown in Figure 1.2. In ASV measurement, the analyte metal is firstly accumulated onto the electrode surface followed by a positive potential scan to strip the metal back into the solution. The metal oxidation peak height obtained from the voltammograms is proportional to the metal concentration under a diffusion controlled condition according to equation (1); During the measurement of CSV, the electrode surface is firstly covered by adsorbed metal complex M-L and a metal ion reduction peak i'_d is measured to calculate the metal concentration from the negative potential scan.

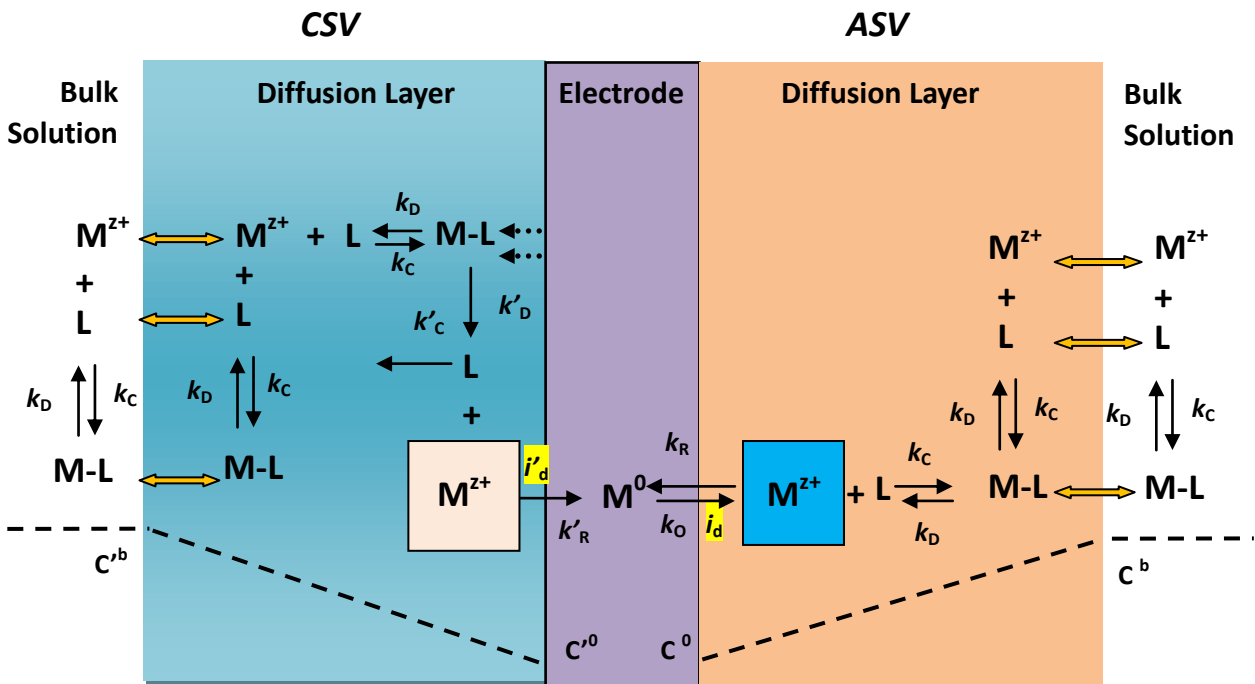


Figure 1.2. Electrode Mechanism using ASV and CSV for Free Metal and Complexed Metal

k_R / k_O : rate constant for oxidation M^{z+} to M^0 and for reduction M^0 to M^{z+}

k_C / k_D : rate constant for complexation $M^{z+} + L$ to $M-L$ and for dissociation $M-L$ to M^{z+} and L

δ : diffusion layer thickness

C^b / C^0 : metal concentration in the bulk solution and on the electrode surface

$$i_d = n F A J = \frac{n F A D (C^b - C^0)}{\delta} \quad (1)$$

However, CSV suffers a poor sensitivity for metal analysis due to the low

adsorption efficiency by natural organic complexes (Achterberg and Van Den Berg, 1994b). Usually, adsorptive complexing ligands are added into the solution to improve its sensitivity (called AdCSV). Their analysis procedures are shown in Figure 1.2 in the presence of ligand L. Interestingly, the electrode reaction mechanism is quite similar to some metal uptake modes by organisms (Tessier et al., 1995) (Refer to Annex III) indicating the possibility of using voltammetric techniques to study biological processes.

1.2.2. Electrode Materials for Voltammetric Analysis

The working electrode (WE) is where the metal is preconcentrated prior to the scan. The WE should be conductive, but its stability and its reaction with water, determines the potential range that is available for metal detection. On the negative potential side, the potential range is restricted by hydrogen evolution (Buffle and Horvai, 2000) or water reduction. H^+ can adsorb onto electrode surface full of negative charges which distribute variably on different materials. The easier two protons encounter, the easier H_2 forms, resulting in interference to metal analysis. So for this reason, it is important to have a WE with a high over-potential for hydrogen evolution. Such electrodes can consist of mercury, but alloy electrodes have also been developed specifically for this purpose (Mikkelsen et al., 2004; Mikkelsen and Schroder, 2001, 2003; Mikkelsen et al., 2008; Mikkelsen et al., 2006).

Mercury Electrode

Historically, mercury is the best choice for metal analysis due to its good performance, such as excellent reproducibility, and high hydrogen evolution over-potential (Ruetschi and Delahay, 1955). The hanging mercury drop electrode (HMDE) was introduced for the determination of Cd and Pb by Ross (Ross et al., 1956) and Demars (Demars and Shain, 1957). However, mercury use is now restricted for health reasons because of its high toxicity (Vyskocil and Barek, 2009; Wang et al., 2000b). There is a demand to find a replacement for mercury for analytical purpose for detection in the field as well as in the scientific laboratory. Mercury film

electrodes fabricated by plating a layer of mercury onto various solid substrates, have been introduced to Cd, Zn and Co analysis as early as the development of mercury drop electrode (Marple and Rogers, 1953). Compared to the mercury drop, mercury film electrodes exhibit higher sensitivity and are less toxic to the environment. A comparison list of their advantages and drawbacks is made in Table 1.4.

Table 1.4. Comparison of mercury and mercury film electrodes for metal analysis.

Electrode Comparison	Mercury Electrode	Mercury Film Electrode	Reference
Sensitivity	Low	High	(Batley and Florence, 1974)
Resolution	Poor	Good	(Batley and Florence, 1974; Stojek and Kublik, 1979)
Poisoning	High Toxic	Less Toxic	(Vyskocil and Barek, 2009)
In-situ analysis	Not Suitable	Suitable	(Vyskocil and Barek, 2009)
Mechanical Stability	Poor	Very Good	(Vyskocil and Barek, 2009)
Modified Coating	Not Suitable	Suitable	
Organic Interference	Serious	Less Serious	(Batley and Florence, 1974)
Oxygen Interference	Serious	Less Serious	
Reproducibility	Very Good	Poor	(Lund and Onshus, 1976; Stojek et al., 1976)
Intermetallic Interference	Less Serious	Serious	(Davison and Whitfield, 1977)
Electrolyte Influence	Less Serious	Serious	(Davison and Whitfield, 1977)
Memory Effect	Less Serious	Serious	(Abdullah et al., 1976)

Solid Electrode- Bi, C, Au, Ag, Pt, Ir

Solid electrodes as alternatives to mercury have been applied to trace metals and organics analysis based on a number of advantages such as being convenient to handle in field, having no toxic concern and being suitable for in-situ or on-site measurements. This has led to the development of mercury film electrodes and electrodes completely free of mercury.

Among the pure solid electrodes, only gold and silver are suitable materials for metal determination. The gold microwire electrode has been successfully used for Hg and Cu (Salaun and van den Berg, 2006), As (Salaun et al., 2012), Zn (Gibbon-Walsh et al., 2011) and Pb (Alves et al., 2011) determinations in natural waters. The silver

electrode has been reported for Pb (Billon and van den Berg, 2004) and Cd (Mikkelsen and Schroder, 2001) determination. Alloy electrodes such as bismuth film electrodes and various amalgam electrodes have been developed and used for the determination of Pb (Li et al., 2009), Zn (Mikkelsen et al., 2004), Cd (de Souza et al., 2011; Mikkelsen et al., 2001) and Fe (Mikkelsen et al., 2006) in natural waters.

Micro-wire electrodes contain more or less 'micro-properties' to minimize stirring and reagents compared to the macro-disc electrodes. Microwire electrodes behave like microelectrodes because they are micro-size in two dimensions even though they are macro-size in the third dimension (Salaun et al., 2006). The vibrator I used can increase analyte flux to approach micro-electrode characters.

Several solid electrodes therefore exist already and can be used as the basis for a new electrode with better properties. This dissertation explored the use of bismuth and mercury coated electrode materials to determine Pb and Cd in seawater. It also explored the possibility of making the electrode as a microelectrode.

1.2.3. Speciation by voltammetry

Conventionally, the chemical speciation of metals has been determined by complexometric titrations where the metal concentration was increased whilst measuring the labile metal concentration (Van Den Berg, 1995; Wells et al., 1998) (Pinheiro et al., 1994). However, this requires additions of metal to the water. Equilibrium models are also applied to calculate the free metal ions and inorganic complex metals to obtain the dominant forms and their percentages (Turner et al., 1981a). However, their accuracy for real natural solutions are seriously distorted due to lack of reliable data, and they are particularly limited for calculating the organic complexes (Batley and Gardner, 1978). Some techniques such as ultrafiltration (Wells et al., 1998), separation by chelex resin (Batley and Gardner, 1978), oxide adsorption (Van Den Berg and Kramer, 1979), ion exchange (Procopio et al., 1997), ligand competition (Black et al., 2007; Bruland, 1992) and UV digestion (Achterberg and Van Den Berg, 1994b) have been introduced to combine with the detection techniques, but they all bring disturbance to the natural equilibriums.

Potentiometry, a thermodynamic technique, is theoretically suitable for metal speciation analysis, such as Ion-selective electrode (ISE) which has been applied to Cu (Soares and Vasconcelos, 1994), Pb (Buffle et al., 1977), and Fe (De Marco and Martizano, 2008) determination. But their applications are limited by the low sensitivity and analyte metals sensitive to the electrode materials.

Metal speciation causes a shift in the half-wave potential of their reduction which can be determined by polarography (Heyrovsky and Kuta, 1966; Lingane, 1941). Pseudopolarography has been developed to build up a polarographic wave in a step-wise manner, which is suitable for trace levels of metals if a sufficiently sensitive voltammetric procedure exists. By pseudopolarography this detection can be lowered to nanomolar, or picomolar concentrations.

Pseudopolarography, consists of a series of accumulation potentials scans, and is appropriate to distinguish the labile from the inert complexes of dissolved trace metals (Pizeta et al., 2005). The position and shape of the polarogram depends on the thermodynamic stability constant, and reversibility of the chemical reaction and electron transfer process (Lewis et al., 1995) as well as the metal and ligand concentrations (Omanovic, 2006).

The stability constant $\log K$ can be estimated from the half-wave potential ($E_{1/2}$) value which is measured on a pseudopolarogram using logarithmic analysis proposed by Lingane (Lingane, 1941).

$$\log \frac{i_{max} - i}{i} = \frac{\alpha n}{0.059} (E_d - E_{1/2}) \quad (2)$$

i_{max} : peak height of metal at diffusion-limited condition

α : transfer coefficient

n : number of exchanged electrons

E_d : deposition potential

$E_{1/2}$: half wave potential

In a reversible condition, half wave potential $E_{1/2}$ shifts negatively with accumulation time t_{dep} but independent of metal concentration (Omanovic and Branica, 2003), while for irreversible complex, $E_{1/2}$ is independent of t_{dep} but depends

on standard redox reaction rate constant and transfer coefficient (Lovric, 1998). The limiting current i_{\max} can be used to calculate how much of the metal-ligand percentage occupies the total metal, showing the competition of complexation between each ligand.

Because no additions are made, pseudopolarography causes no change to the natural equilibrium and distribution of species (Tsang et al., 2006). It has been successfully used to study metal complexation with a variety of ligands in model solutions or natural samples. (Refer to pseudopolarography Table 1.5).

Practically, a chelate scale, like a working curve generated from measuring $E_{1/2}$ of model ligands, provides the chance to estimate the thermodynamic stability constant of the unknown complexes (Branica and Lovric, 1997) (Pizeta et al., 2005), diffusion coefficient α (Omanovic and Branica, 2003) (Chakraborty et al., 2007) and kinetic rate constant k (Pizeta et al., 2005). In chapter 5, a chelate scale is developed to determine the chemical speciation of Pb in seawater.

1.2.4. Electrodes for pseudopolarography study

Various electrodes have been tested in pseudopolarography studies (Table 1.5), among which the mercury drop electrode is the one most used. Previous work has generally been in model solutions rather than natural sea water due to the low metal concentrations (more than 10 minutes deposition time usually required for ASV of Pb using the HMDE (Gillain et al., 1979)).

Mercury film electrodes have been used successfully for pseudopolarography of Zn in open sea (Baars and Croot, 2011). However, there are no data of Pb available in such media mainly because of the lack of appropriate electrode for pM level lead analysis in the short history of use of this technique. The most sensitive mercury film electrode makes use of in-situ mercury plating in which mercury is added to the solution. However, this is thought to affect the speciation of other metals by competition (Kramer et al., 1984) which means that it is not suitable for pseudopolarography.

Other electrodes with high hydrogen over-potentials can consist of bismuth

(Wang et al., 2000b), or alloys (Mikkelsen et al., 2001). Bi film electrodes have good sensitivity, but are not suitable for in-situ Pb detection due to the need for re-coating with Bi. For this reason a solid Bi electrode (Bi et al., 2010) was developed as part of the research for this dissertation.

Table 1.5. Metal Complexation study by pseudopolarography

Metal	Electrode	Tech	t_{dep} (s)	Solution	pH	$[M]_{\tau}$ (nM)	Reference
Pb	SAFE	SWASV	60	Atlantic	7.7	0.03	This work
Pb	HMDE	SWASV	1800	Seawater	8.0	0.1-0.4	(Rozañ et al., 2003)
Pb	MFIE	SWASV	60	Sediment	2.0	6300	(Pizeta et al., 2005)
Pb	SMDE	SWASV	180	NaCl	7.8	400	(Branica and Lovric, 1997)
Pb /Cd	MFGCE	DPASV	150	KCl/NaCO ₃	4.7	10 /18	(Brown and Kowalski, 1979)
Pb/Cd	SMDE	DPASV	40	Folic Acid	5.0	500/500	(Chakraborty et al., 2007)
Cd	MFIE	SWASV	20	KNO ₃	5.4	500	(Kounaves, 1992)
Cd	HMDE	SWASV	600	Seawater	7.6	3.0	(Tsang et al., 2006)
Cu	VGME	SWASV	30	Seawater	7.8	17	(Gibbon-Walsh et al., 2012)
Cu	MFGCE	DPASV	120	Estuarine	7.7	37	(Hurst and Bruland, 2005)
Cu	HMDE	SWASV	300	Seawater	8.0	100	(Crooť et al., 1999)
Cu/Zn	SMDE	DPASV	360	Seawater	4.0	15/390	(Nicolau et al., 2008)
Zn	HMDE	SWASV	300	Seawater	8.0	10	(Lewis et al., 1995)
Zn	MFGCE	LSASV	180	NaNO ₃ /NaCl	3.0	10	(Komorskylovric et al., 1986)
Zn	MFGCE	SWASV	180	Southern Sea	8.0	1.5	(Baars and Crooť, 2011)

Specific aim of this dissertation:

- A nontoxic electrode for trace metal analysis in situ is necessary. Bismuth known as an environment friendly material, was tested for determination of Pb and Cd in natural waters. (Chapter 2)
- A sensitive electrode is needed to measure extremely low concentration of Pb and Cd in seawater. Mercury coated microwire electrodes, such as carbon fibre, gold and silver were investigated for Pb determination at pM level. (Chapter 3 and 4)
- By using the highly sensitive and stable electrode, Pb was chosen as a target metal for speciation analysis using pseudopolarography based on ASV measurement. (Chapter 5)
- To broaden the electrode applications, Cr was tested on the solid wire electrode using CSV which is widely applied for metal speciation analysis on mercury drop electrode. (Chapter 6)

Contributions to this dissertation:

Fundings and experimental supports are stated in the 'Acknowledgement' of each chapter (Chapter 2 to 6). In chapter 2 to 5, I prepared all data and graphs as well as the drafts for international journals. In chapter 6, I collaborated with Estralla. I did part of this work and made comments on this paper. My supervisors checked and made comments on all of them.

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2

Chapter 2

**Determination of lead and cadmium in sea and
freshwater by anodic stripping voltammetry
with a vibrating bismuth electrode**

2. Determination of lead and cadmium in sea and freshwater by anodic stripping voltammetry with a vibrating bismuth electrode

Abstract

A solid, bismuth (Bi), disk, electrode has been used to determine lead (Pb) in natural waters including seawater. The diffusion layer thickness was lowered from 93 to 29 μm by stirring and to 18 μm by using the vibrated version of the Bi electrode. The Bi electrode does not require removal of dissolved oxygen, which thus facilitates in-situ detection. The electrode was tested for the determination of Pb in coastal seawater samples. The detection limit for Pb was 0.15 nM in acetate buffer and 0.5 nM in seawater using a 2 min deposition time. The Bi electrode compares unfavourably to a mercury electrode in terms of sensitivity and ease of use.

2.1. Introduction

Voltammetry is a convenient method to determine trace metals in natural waters including seawater because it has high sensitivity and gives information about metal speciation. Voltammetric apparatus is portable allowing analysis in the field. Voltammetry involves “green” technology because of a low requirement for energy and consumables compared to a high-energy technique such as ICP-MS. Two main modes exist: anodic stripping voltammetry (ASV), which is normally based on pre-concentration of the metal by plating the metallic state on an electrode, and cathodic stripping voltammetry (CSV), which typically requires adsorptive deposition of a metal complex with an added ligand on the electrode. ASV has advantages over CSV for in-situ detection as there is no need for reagents if a prefabricated solid electrode is used. Mercury electrodes have been much used in the past, but because of environmental concerns, alternative electrodes are being advocated, although the mercury used in the laboratory environment is readily recycled. In particular, it has been suggested that bismuth may be a good alternative (Wang et al., 2000) (Wang,

2005) because of much lower volatility of the metal whilst retaining some of the electrochemical properties of mercury. Gold microwire electrodes have high sensitivity for copper, arsenic and mercury (Salaün and van den Berg, 2006) (Salaün et al., 2007) but gold is not suitable for detection of cadmium (Cd) and lead (Pb) due to overlapping peaks (Bonfil et al., 2002) and relatively poor detection limits. Bismuth (Bi) film electrodes are reportedly suitable for detection of Cd and Pb (Pauliukaite and Brett, 2005), but the requirement of replating of the film after each scan (Baldo and Daniele, 2004) is incompatible with in-situ measurement. Bi electrodes have also been modified using nanoparticle or nanostructure methods (Hwang et al., 2009) which has led to improved sensitivity for Pb and Cd (Toghill et al., 2008) but the need for in-situ deposition of the nanoparticles and a low pH are again incompatible with in-situ detection. Carbon paste electrodes have also been modified with Bi particles, imparting improved sensitivity for Pb and Cd (Hocevar et al., 2005). Bi can therefore be expected to be a suitable electrode material for detection of Cd and Pb.

A solid Bi electrode was optimised for detection of Cd and Pb, with the aim to enable in-situ detection without the need for reagents or deaeration. The sensitivity and analytical accuracy of the solid Bi electrode is compared here to that of a mercury drop electrode which it is aimed to replace. Only few studies have reported on the solid Bi electrode (Adamovski et al., 2006; Buckova et al., 2005; El Tall et al., 2010; Pauliukaite et al., 2004). One of these used a disk-shaped Bi electrode for Pb and Cd determination (Pauliukaite et al., 2004) achieving a limit of detection of 15 nM Pb. Previous work on the Bi film electrode suggested that the solid Bi electrode is less sensitive than the Bi film electrode. In this work, the experimental parameters for detection of Pb and Cd in natural waters are optimized with the aim of stabilizing the Bi electrode and achieve best sensitivity. It was found that the sensitivity of the optimised solid Bi electrode is significantly better than previous work suggests.

2.2. Experimental

2.2.1. Chemicals and reagents

Water used to prepare reagents and preliminary working solutions had a resistivity of $18 \text{ M}\Omega \text{ cm}^{-1}$ and was purified by a Millipore system. Metal stock solutions were prepared by dilution of metal atomic absorption standard solutions (BDH, UK). The working standard solutions contained 10^{-4} M , 10^{-5} M and 10^{-6} M of the metals. Triton X-100, $\text{K}_3\text{Fe}(\text{CN})_6$ (Analar), KCl (Analar), $\text{NaAc}\cdot 3\text{H}_2\text{O}$ (Analar) and Na_2CO_3 (Aristar grade) were from BDH, England. Hydrochloric acid, acetic acid and ammonia were purified by sub-boiling distillation on a quartz condenser.

Acetate buffer solution (3 M, pH 4.5) contained 1.5 M acetic acid and 1.5 M sodium acetate 3-hydrate. An aqueous solution of 10 mM $\text{K}_3\text{Fe}(\text{CN})_6$ (ferricyanide) in 0.5 M KCl was used to determine the diffusion layer thickness on the Bi electrode. The pH of water samples was buffered at pH 4.5 by addition of 100 μL acetate buffer stock solution (3 M) into 10 mL MQ water. Seawater used for the optimisation and other experiments was collected in Liverpool Bay (Irish Sea), during cruises with the research vessel Prince Madog in 2008 and 2009. Samples were collected using a Niskin bottle modified for water collection without trace metal contamination. Samples were 0.2 μm filtered through a Sartobran 300 cartridge. Some seawater samples were acidified to pH 2.2 by addition of 10 μL 50 % HCl /10 mL seawater and other samples were stored frozen. Two lake water samples were also tested: one from a small lake at the base of Snowdon (North Wales) and of lake Redo (Spain) which has been studied before (Ploger et al., 2005). To vary the pH, the solution pH was adjusted using sodium carbonate, hydrochloric acid and ammonia.

2.2.2. Apparatus

Voltammetric instrumentation was a 663 VA electrode stand from Metrohm, Switzerland, interfaced to a computer-controlled potentiostat ($\mu\text{Autolab-III}$, Eco Chemie, Netherlands). Data were processed with GPES 4.9 software. A 50 mL, glass voltammetric cell was used containing the Bi working electrode (WE), an iridium counter electrode (CE) (2cm length, 0.2mm diameter) and an Ag/AgCl/KCl (3M) double junction reference electrode. All the potentials measured in this work were versus that reference electrode. The seawater was UV-digested before measurement

for 45 min using a home-made UV system (125 W high-pressure mercury vapour lamp). The pH was measured using a Metrohm 605 pH-Meter. The effect of polishing of the electrode surface was inspected using a Philips XL30 scanning electron microscope.

2.2.3. *Bi electrode fabrication*

The bismuth vibrating electrode was prepared using a bismuth needle (2 cm long, 1 – 2 mm thick, 99.998% purity, from Alfa Aesar), which was polished with sandpaper and then placed in a 1 mL pipette tip. To obtain a vibrating electrode, a vibrating motor (1.5 V, 1.5 cm length, 0.5 cm diameter, Jinlong Machinery, China) was placed in the back of the tip (Figure 2.1). The wires were connected with soldering tin and sealed using PVC tape. The end of the tip was heated to ~120 °C using a heat gun sealing the Bi needle in place and backfilled with silver-loaded epoxy to connect the bismuth to a 4-core cable: one core connected the working electrode to the electrode output on the 663 VA stand, 2 cores connected the vibrator to an interface (made by Ecochemie, Netherlands) to the potentiostat (IME663), which provided power (1.5 V) and on/off control via the stirrer statement in the GPES software, and one connection went to earth. The working electrode was polished manually using 0.3 µm and 0.05 µm Al₂O₃ sandpaper for about a minute followed by rinsing with ethanol and water before measurement. Polishing was repeated using 0.05 µm Al₂O₃ paper when the sensitivity of the electrode showed deterioration, or when the electrode became visibly oxidised. The electrode was stable for extended periods, not requiring polishing during a working week, when the electrode was used continuously and the potential was controlled to prevent oxidation. However, the used electrode had to be polished when left in air overnight unless dried carefully using tissue paper.

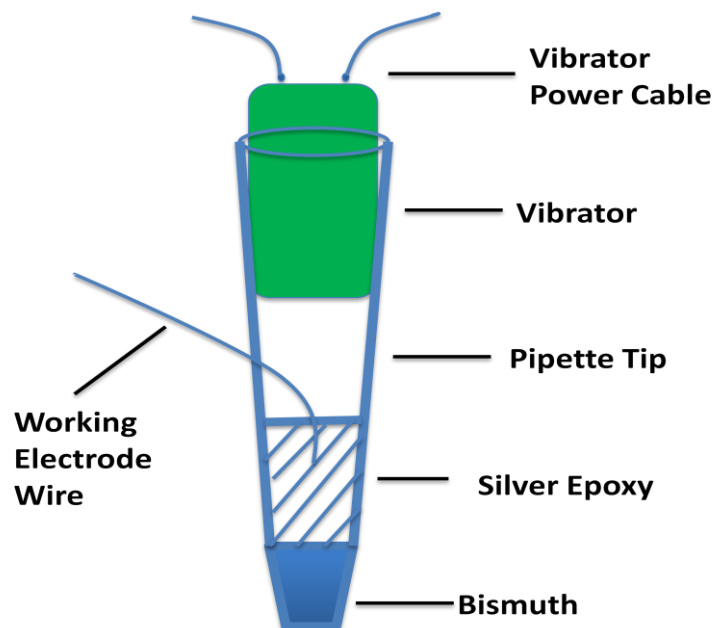


Figure 2.1. Diagram of the vibrating bismuth electrode

2.2.4. Optimised settings to determine Pb and Cd in water

The optimized voltammetric conditions to determine Pb and Cd in acetate buffer were as follows: deposition potential (E_{dep}) = -1.2 V whilst stirring or vibrating for 60 s, then an 8 s quiescence time, and a voltammetric scan from -0.8 to -0.35 V. The scan mode used the square-wave modulation, frequency 50 Hz, step height 2.5 mV, pulse height 25 mV. Each scan was preceded by a 10 s conditioning step (E_{con}) at -0.3 V to remove any deposited metals; a standby potential (E_{rest}) of -0.4 V was used between measurements to prevent oxidation of the electrode surface. Settings were modified for acidified seawater (pH 2.2) to the following: scan range -1.0 to -0.4 V, E_{con} = -0.4 V and E_{rest} = -0.45 V. The deposition time was increased up to 10 min for determination of low concentrations of Pb (< 1 nM) and Cd (< 5 nM) in seawater.

2.3. Results and Discussion

2.3.1. Usable potential range of the Bi electrode

Preliminary scans by ASV using a Bi electrode showed that peaks for Pb and Cd were well separated and could be individually quantified. The stability of the Bi electrode in buffer solution and in seawater was established by cyclic voltammetry

(CV), and the sensitivity of the method to determine Pb and Cd was optimized. The potential range that can be accessed using the Bi electrode is restricted by current related to the reduction of hydrogen ions at negative potentials and by the oxidation current of Bi at potentials greater than its oxidation potential. Bi is strongly hydrolysed at neutral pH values with a value of 14 for $\log \alpha_{\text{Bi(OH)}_3}$ in pH 8 seawater (calculated using an ion-pairing model using salinity-corrected constants (Turner et al., 1981)). At pH values < 4.7 chloride species become more important than hydroxide species but the Bi(OH)_3 hydroxide species has low solubility and can precipitate on the electrode prior to conversion to Bi_2O_3 in spite of the chloride species being the dominant dissolved species.

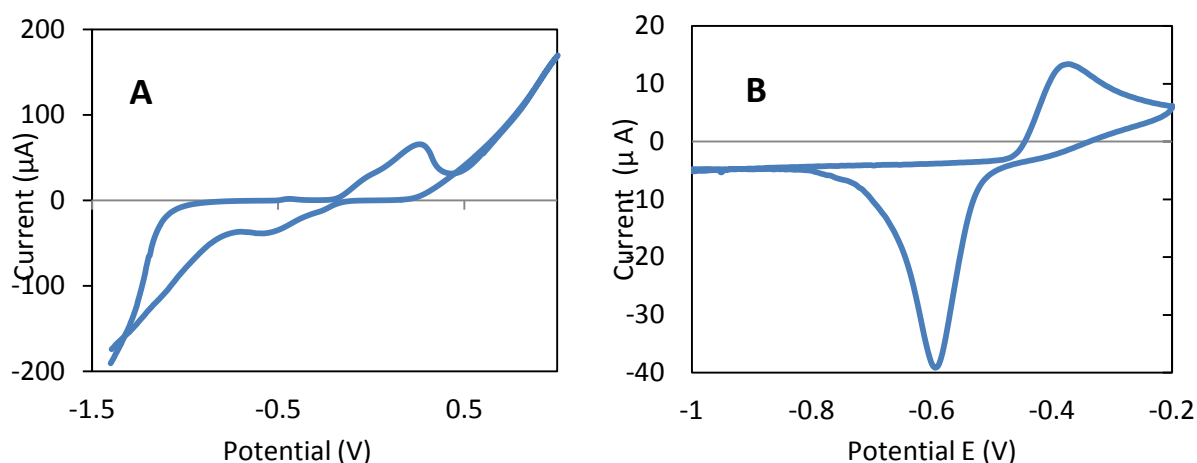


Figure 2.2. Cyclic voltammetry of the Bi electrode (A) in 0.03M Acetate Buffer, pH 4.5. (B) in 10 mM $\text{K}_3\text{Fe(CN)}_6$ / 0.5 M KCl, from -1 to -0.2 V, 150 mV s^{-1} , in pH 6 buffer solution.

The usable potential window in acetate buffer (0.03 M, pH 4.5) was investigated by cyclic voltammetry. The positive-going potential scan showed an oxidation peak of Bi starting at -0.2 V and a second peak at +0.3 V (Figure 2.2.A). The first peak was thought to be where the surface of the electrode becomes oxidised to Bi(III)-hydroxide, the second peak is either due to a second oxidised layer, or due to the formation of Bi_2O_3 . The negative-going return-scan showed a small reduction wave at -0.3 V and a second wave at -0.6 V for the reduction of oxidised Bi. The large difference between the oxidation and reduction peak potentials indicates a poor electrochemical reversibility of the Bi redox reaction. In figure 2.2A, a small peak

around -0.5V was observed probably due to the contamination of Pb in the cell.

The background current of the negative going scan initially stabilised at potentials negative of the Bi(III) reduction wave at -0.6 V, but the reduction current increased again at potentials <-0.9 V. The difference between the current of the positive going scan and the negative going scan at potentials < -0.9 V suggests that residual Bi₂O₃ on the electrode catalysed the generation of hydrogen whilst the residual Bi₂O₃ was being reduced. At potentials < -1.1 V, the hydrogen ion reduction response at the partially oxidised Bi electrode was similar to that at the reduced Bi electrode. The positive-going scan, which started from a surface without Bi₂O₃ at -1.4 V (as no Bi₂O₃ had yet been produced) returned more quickly to a near-zero current giving a usable range for ASV scans between -1.1 and -0.2 V similar to that found previously (Pauliukaite et al., 2004). The oxidation of the electrode surface was prevented by stopping the voltammetric scans in pH 4.5 acetate buffer at -0.3 V, before the first oxidation wave at -0.2 V. The potential window was shortened in acidified seawater due to oxidation of bismuth at a lower potential than in acetate buffer due to chloride complexation. Therefore voltammetric scans were halted at -0.4 V in acidified seawater. In between measurements the Bi electrode was held at -0.45 V to prevent its oxidation as the potential drifted to ~ 0 V under open circuit conditions. The voltammetric settings required to retain a stable Bi electrode are summarised in Table 2.1.

Table 2.1. Determination of the limit of detection for lead in pH 4.5 seawater containing 0.03 M acetate buffer. Conditioning potential: -0.3 V for 10 s; standby potential: -0.35 V; frequency: 50 Hz, potential step: 5 mV, amplitude: 25 mV; equilibration time: 8 s

System	Solution	[Pb] added	E_{dep}	t_{dep}	RSD(%)	LoD(nM)
Vibrated	Acetate	1 nM	-1.2V	120s	4.8	0.14
Stirred	Acetate	2 nM	-1.2V	120s	22	1.3

The reduction of surficial Bi₂O₃ at potentials between -0.6 and -1 V indicates

that the oxidised Bi electrode proposed for voltammetry of Pb and Cd (Pauliukaite et al., 2004) loses its oxidised coating during deposition at a negative potential making it identical to the non-oxidised Bi electrode.

2.3.2. Diffusion layer thickness of the stationary and vibrating bismuth electrode

A vibrator was fitted in an attempt to increase the sensitivity over that achieved by other means of stirring. Previous work using microwire electrodes found that the sensitivity was much improved by using electrode vibration instead of stirring of the solution (Chapman and Van Den Berg, 2007; Gibbon-Walsh et al., 2010). This electrode was made by fitting a vibrating motor in the polypropylene pipette tip used for the Bi electrode (Figure 2.1).

The sensitivity of the electrode was quantified by measuring the diffusion current in conditions similar to that used in stripping voltammetry. The diffusion current was used to calculate the diffusion layer thickness, with the aim of minimizing this as it increases the diffusion flux to the electrode surface during the deposition step. The diffusion layer thickness was determined by measuring the reduction current of 10 mM ferricyanide ($K_3Fe(CN)_6$) in 0.5 M KCl at pH 8 (Salaün and van den Berg, 2006). CV showed that the reduction peak of the ferricyanide complex was at -0.6 V on the Bi electrode (Figure 2.2.B), much more negative than that (at -0.2 V) found previously on an “oxidised” Bi electrode (Pauliukaite et al., 2004): the previous work used a different pre-treatment of the electrode suggesting that the Bi-surface remained oxidised, occurring as Bi_2O_3 . However, the CV experiments (Figure. 2.2B) indicate that the bismuth oxide layer is reduced back to metallic Bi at negative potentials, suggesting that there should be no difference between the Bi electrode and the oxidised Bi electrode. The peak separation of the CV scans on the Bi electrode ($\Delta E = 200$ mV) is similar to that found previously at the “oxidised” Bi electrode (Pauliukaite et al., 2004), suggesting that the electrochemical irreversibility of the ferricyanide reduction is similar. Comparative CV using a gold microwire electrode showed a reversible redox reaction for the ferricyanide with a

half-wave potential of -0.2 V, much more positive than at the Bi electrode, where it has a large peak separation.

The following equations are valid for diffusion transport at a macro electrode (Bockris, et al., 2000):

$$I = n F D A C_{\text{bulk}} (\pi D t)^{-1/2} = \kappa \cdot t^{-1/2} \quad (1)$$

$$\kappa = n F D A C_{\text{bulk}} (\pi D)^{-1/2} \quad (2)$$

$$A = \kappa (\pi D)^{1/2} (n F D C_{\text{bulk}})^{-1/2} \quad (3)$$

$$\delta = n F D A C_{\text{bulk}} (I_{\text{lim}})^{-1/2} \quad (4)$$

where I_{lim} is the limiting current (amps), n is the number of transferred electrons, F is the Faraday constant, A is the electrode surface area (cm^2), C_{bulk} is the bulk concentration (mol cm^{-3}), δ = diffusion layer thickness (cm), and D is the diffusion coefficient ($\text{cm}^2 \text{s}^{-1}$). In this condition, $n = 1$, $F = 96,500 \text{ C mol}^{-1}$, $D = 7.17 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$ (Baur and Wightman, 1991), $C = 10^{-5} \text{ mol cm}^{-3}$. Equation (1) is valid for unstirred, diffusion limited conditions, and equation (4) for diffusion limited current in stirred solution and for the vibrating electrode.

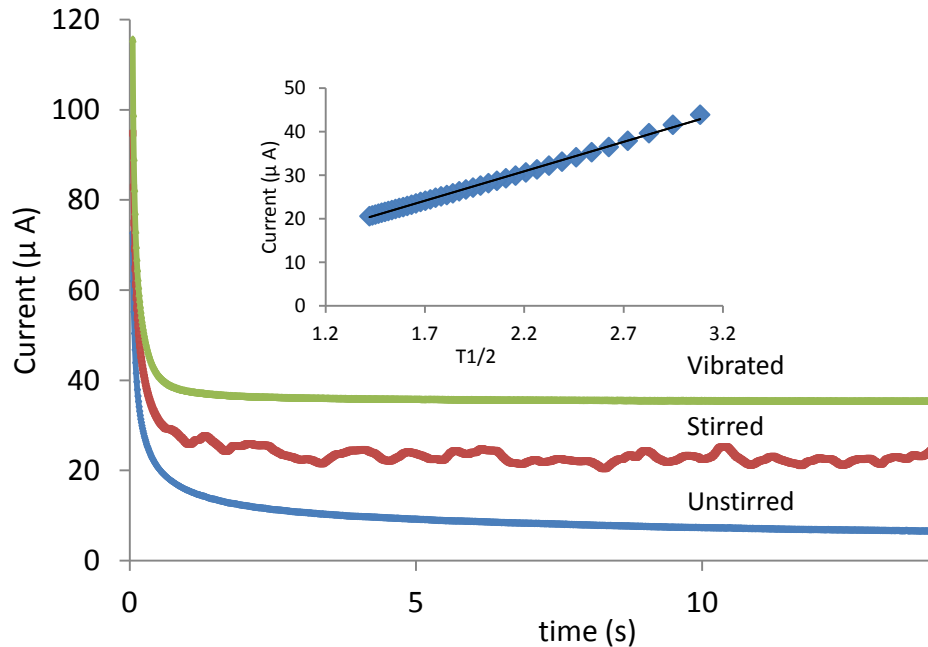


Figure 2.3. Comparison of the diffusion current at the Bi electrode in unstirred, stirred and vibrated conditions by chronoamperometry in 10 mM $\text{K}_3\text{Fe}(\text{CN})_6$ with 0.5M KCl, Working Potential: -0.9V for 15s, sample time/s : 0.01, Conditioning potential: -0.35V for 300s. Equilibration time: 5s. Standby potential: -0.4V. Inset: current as function of $(\text{time})^{0.5}$ for the vibrated Bi electrode.

The diffusion layer thickness and electrode surface area were calculated using equations (3) and (4). The diffusion current of ferricyanide was monitored by amperometry at -0.9 V (negative of the reduction potential of ferricyanide), in unstirred condition, using stirring, and using vibration (Figure 2.3). The electrode surface area was calculated by plotting current at -0.9 V as a function of (time)^{-0.5} over a period of up to 15 s (Figure 2.3, inset) using the data from the first few seconds of the amperometric current (1 < t < 5 s) for the calculation. Between experiments, the potential was set to -0.35 V for 300 s and a standby potential of -0.4V was used to maintain a constant ratio of Fe³⁺/Fe²⁺ in the solution. The slope, κ , was 1.35×10^{-5} and $r^2 = 0.998$. According to Equation (4), the surface area of the Bi electrode was 0.93 mm² which was close to the value measured manually using a micrometer (approximately 1 mm²).

In agreement with Equation (1), the chronoamperometric current continuously decreased with time, reflecting the expansion of the diffusion layer within the solution. However, the current tends towards a limiting current (due to natural convection) with a corresponding diffusion layer thickness (δ) of $93 \pm 4 \mu\text{m}$ in un-stirred condition (Equation (4)). The δ decreased to $29 \pm 2 \mu\text{m}$ with stirring, and $17.6 \pm 0.1 \mu\text{m}$ with vibration. In spite of the large size of the disk Bi electrode (diameter $\sim 1.2 \text{ mm}$), the diffusion current of the vibrated electrode was nearly half that of the fixed electrode with stirred solution, suggesting that the sensitivity of the vibrating electrode can be expected to be nearly twice that of the stirred system. The diffusion current of the vibrating electrode was much more stable than the stirred version which was found to strongly improve the reproducibility of measurements (from $\sim 5\%$ down to 2%).

2.3.3. Parameter optimization for Pb measurement

The frequency, step potential and amplitude potential were varied to optimise the ASV sensitivity and the data were fitted to an equation to minimise subjectivity in the evaluation to maximise the response for each parameter:

$$P_O = P_H (N_I P_W)^{-1} \quad (5)$$

Where P_0 is a measure of the maximised response, P_H is the peak height, N_i is the “noise” in the current (magnitude of the current variations around the baseline) and P_w is the peak width. The voltammetric response is improved when P_0 increases, which is assisted by a high peak height, low current noise and a narrow peak width, as used in the equation (5). Values for P_0 for different settings of the parameters are shown in Table 2.2. Preliminary measurements showed that the square-wave modulation improved the response of the linear sweep mode. The peak height for lead was found to increase with the pulse frequency, step potential and amplitude potential, but the peak broadened due to a fast scan rate at high values for these parameters indicating poor electrochemical reversibility. A pulse frequency of 50 Hz, pulse height of 25 mV and a step size of 5 mV, were found to be optimal for lead detection.

Table 2.2. Parameter optimisation in acetate buffer with 100 nM Pb, $E_{con} = -0.3$ V for 30 s, $E_{dep} = -1.2$ V for 60 s. P_0 values were calculated using: $P_0 = P_H (N_i P_w)^{-1}$, where P_H = peak height (nA), N_i = noise in the current and P_w is peak width (mV). The sensitivity is optimal for the highest values of P_0 of each parameter, indicated in bold.

Parameter	Value	P_H (nA)	P_w (mV)	N_i (nA)	P_0
Frequency (Hz)	10	656	63	0.361	28.8
	25	1960	63	0.606	51.4
	50	4620	63	1.13	65.1
	100	1150	59	5.17	37.6
Step Potential (mV)	2	3900	63	0.669	92.6
	4	5000	62	0.855	94.3
	5	5290	65	0.667	122
	8	4680	63	0.860	86.5
Amplitude (mV)	10	3070	54	0.685	83.2
	20	4840	63	0.802	95.8
	25	5180	68	0.718	106
	40	5270	78	1.05	64.5

Purging to remove dissolved oxygen (DO) was found to have no discernible effect on the response for Pb confirming previous work (Pauliukaite et al., 2004) that the Bi electrode is insensitive to DO presumably due to a large irreversibility of its redox reaction at the Bi electrode, similar to the irreversibility of the ferricyanide

reaction. Subsequent measurements of Pb and Cd were therefore carried out without deaeration.

2.3.4. Effect of variation of the pH

The effect of the solution pH on the voltammetric response of the Bi electrode was investigated by varying the pH in a solution which was partially buffered using acetate buffer. Sodium carbonate was added to increase the pH to 8.5, and the pH was gradually lowered by adding hydrochloric acid until a pH of 2.2. Lead was added to a concentration of 20 nM. Because of changes in the stability range of the Bi electrode as a function of the pH, the conditioning potential and standby potential were adjusted with pH to retain optimal conditions. The response for lead was found to reach a maximum at a pH value of 5.5, giving lower response at lower and higher pH values (Figure 2.4). The peak potential of lead was found to shift continuously towards more negative potential with increasing pH, by ~100 mV from pH 4 to 8. This pH effect is similar to that obtained using the Bi film electrode (Lee et al., 2007). Best sensitivity for lead is obtained at pH values between 4 and 6, which is readily stabilised using an acetate pH buffer. The data shows that lead can also be detected in water acidified to pH 2.3, which is convenient for analysis of acidified samples. The decrease at pH values greater than ~5.4 could be due to increased hydrolysis of lead.

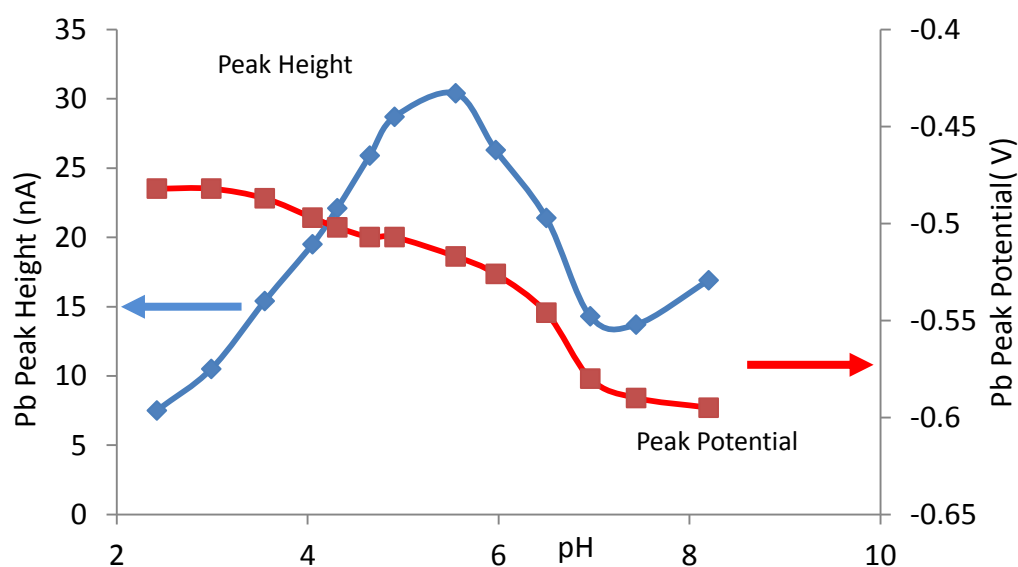


Figure 2.4. Effect of varying the pH on the response of the Bi electrode for 20 nM Pb. Conditioning potential: -0.3V for 10s; Deposition potential: -1.2V for 60s; Standby potential: -0.35V

2.3.5. *Stability of the Bi electrode over time*

The stability of the Bi electrode was tested by repeated measurement of Pb over a period of ~3 h in acetate buffer (pH 4.5) containing 10 nM Pb. A freshly polished electrode was used. The data showed that the response for Pb increased by ~30 % during the first 5 scans (6 min) after which the response stabilised for a further uninterrupted 2 h of measurements (Figure 2.5) during which 80 measurements were carried out. The reason for the initial increase in the response, over the first 5 scans, may lie in a slight roughening of the electrode surface, but evidence for this was not obtained. The gradual decrease was probably due to adsorption on the glass voltammetric cell which is known to adsorb significant amounts of Pb (Cuculic and Branica, 1996). In a separate experiment, the electrode surface was stabilized (it did not show discoloration due to Bi₂O₃ formation) using a standby potential of -0.35 V between scans over a period of 14 days. The response for 10 nM Pb in acetate buffer was monitored on a daily basis using the same electrode whilst under continuous potentiostat control by setting a standby potential at -0.35 V in between measurements. In this condition the response was found to be stable (SD 16 %) for a period of 2 weeks, indicating that the Bi electrode is stabilised for long term use by preventing oxidation of the Bi electrode by ensuring that the electrode potential remains at values less than -0.3 V. Measurements showed that a freshly polished, un-wetted, electrode was not oxidised after several days dry storage. On the other hand, storage of a wet electrode in air caused it to be oxidised in ~1 h. These observations showed that the Bi electrode is stable under potentiostatic control in water, became oxidised when stored without drying in air for longer than 1 h, or within a few minutes when without potentiostatic potential control in water.

2.3.6. *Reproducibility of the Bi electrode*

The Bi electrode requires polishing when oxidised (for instance after overnight storage). 0.5 µm Al₂O₃ paper was used for manual polishing, but others' work has even used moist filter paper for the purpose of a basic regeneration of the Bi electrode surface (Buckova et al., 2005). Scanning electrode microscopy of a freshly

polished Bi electrode showed that the surface was smooth apart from fine parallel scratches (thickness < 0.3 μm) caused by the polishing procedure.

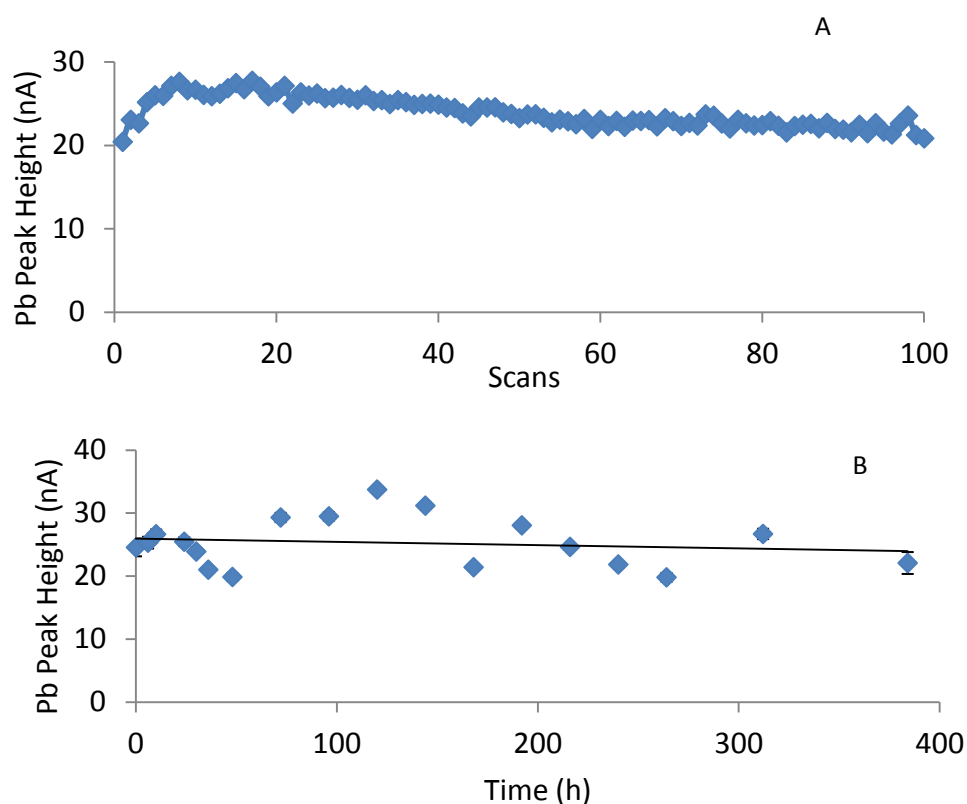


Figure 2.5. Stability test of the Bi electrode. Acetate (0.03M) with Pb (10nM) added. E_{con} : -0.3 V for 10 s, E_{dep} : -1.2 V for 60 s, E_i : -0.35 V. t_{dep} : 8 s. A) 100 repeated scans; B) repeated scans using the same electrode over a period of 2 weeks and data were collected at different frequency. For example, data were collected after a short time wait at the beginning and the wait time became longer in the end of experiment.

The reproducibility of the electrode regeneration by polishing was tested by repeated measurement (10 scans) of 10 nM Pb in acetate buffer, and this process was repeated 7 times. The response typically showed an increase of between 20 and 100 % in the response for the first 3 scans after polishing, which were therefore subsequently used for electrode conditioning and not for the stability measurement. The peak height for Pb in the remaining 7 scans for each experiment had a relative standard deviation (RSD) of between 1.6 and 4.6 %. The overall RSD of all the scans of the repeatedly polished electrode was 6 %, indicating that there was no systematic variation (apart from the initial rise during the first 3 scans) for the electrode

response before and after different polishes. In further work 5 scans would be used to condition a newly polished electrode prior to initiating measurements after which the response was stable as long as the electrode was kept under potentiostatic control.

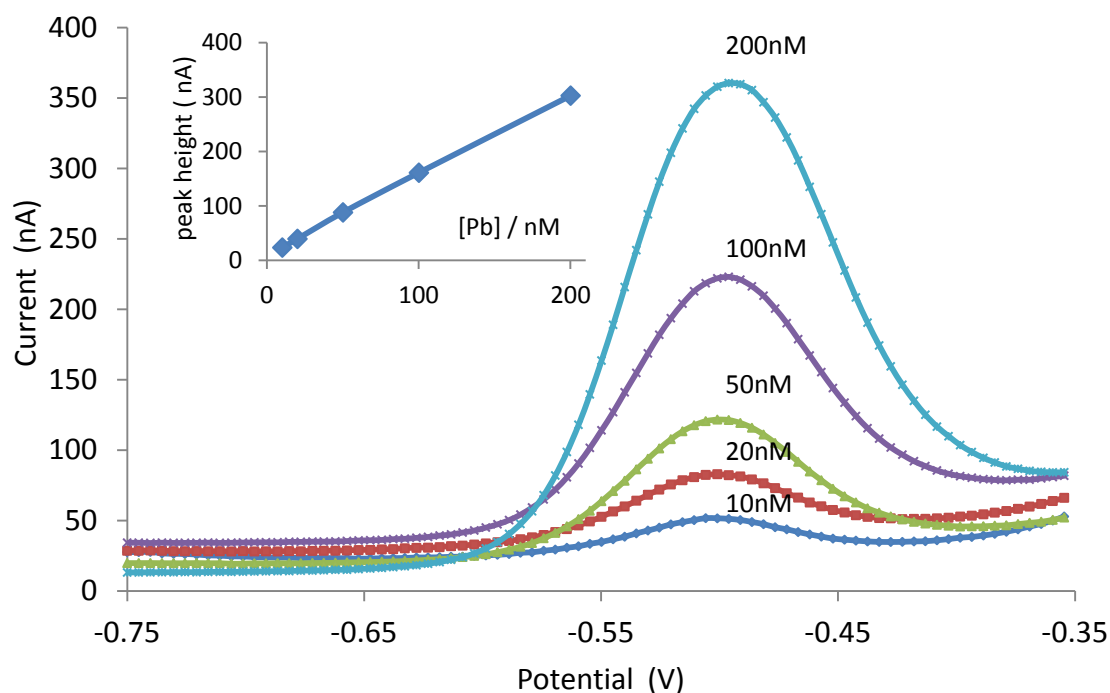


Figure 2.6. Voltammetric scans for lead additions to acetate buffer. Each scan was preceded by 60 s deposition at -1 V. The inset shows the response as a function of the Pb concentration.

2.3.7. Linear range and limit of detection

The linear range of the Bi electrode for detection of Pb was determined in vibrated and stirred conditions, in acetate buffer using a deposition time of 60 s. The response was found to increase linearly with the concentration of lead up to at least 1.5 μM Pb ($r^2=0.997$) (shown to 200 nM in Figure 2.6). The sensitivity was 2.3 nA/nM using the vibrated electrode and 0.7 nA/nM using the stirred system. The wide linear range is convenient for monitoring of lead or other metals using the Bi electrode in contaminated natural waters.

The limit of detection limit (LoD) for lead using the Bi electrode was determined in acetate buffer using the stirred and vibrated electrode systems on

basis of the SD ($\text{LoD} = 3 \times \text{SD}$) of repeated analysis of a solution containing a low concentration of Pb (1 nM for the vibrated electrode in stationary solution, and 2 nM for the stationary electrode in stirred solution). A deposition time of 120 s at -1.2 V was used. Background subtraction was used to correct the scans for curvature in the baseline using the GPES software of the instrumentation. The background scan was obtained using a deposition time of 1 s. The SD using the vibrated Bi electrode was 4.8 %, equivalent to a LoD of 0.14 nM Pb, whilst using the stirred system the SD was 22 %, leading to a LoD of 1.3 nM Pb. The LoD values are both lower than those obtained previously with solid Bi electrodes; the best previously obtained LoD for Pb using either a solid Bi or Bi-film electrodes is ~ 0.5 nM Pb, obtained with a BiFE (de Carvalho et al., 2007). The LoD using the vibrated Bi electrode was nearly 10x lower than that of the conventional stirred system, due to a combination of better sensitivity and a lower SD. Comparison of conventional, stationary electrodes in stirred condition suggests that the LoD for Pb using the solid Bi electrode is ~ 2 x higher than that using the BiFE, but it is lower by a factor of ~ 3 using the vibrated solid Bi electrode. The sensitivity of the vibrated Bi electrode is sufficient to determine Pb in coastal waters contaminated with continental lead but not in uncontaminated ocean waters which can have lead concentrations from the low pM (Stukas et al., 1999) to the ~ 100 pM (Wu and Boyle, 1997) level.

Interferences

Voltammetric stripping is known to be affected by surfactant and intermetallic effects. Adsorption of surface-active constituents leads to lower or broader peaks and shifts in the peak potential (Wang, 2005). Intermetallic effects are more likely than on mercury electrodes due to pre-concentration of the metals on the surface of the electrode. Formation of intermetallic compounds would result in suppression of one or both of the constituents. It has been reported that Pb, Cd and Zn can be measured simultaneously using stripping voltammetry on the Bi film electrode (Pauliukaite and Brett, 2005) so intermetallic effects are not expected between these elements.

Experiments were carried out to establish interference from metals and organic

substances with the determination of lead and cadmium using the Bi electrode. The ASV response for 20 nM Pb in acetate buffer (60 s deposition, detection by SWASV) was found to decrease by 16 % upon addition of 100 nM cadmium. Addition of 100 nM zinc caused the response for 20 nM Pb to increase by 20%. The response for Pb did not change further at higher additions for Cd and Zn. The Cd additions produced a peak at -0.75 V, well separated from that for Pb at -0.45 V. The solid Bi electrode is therefore suitable for the combined determination of Cd and Pb in contaminated waters. The sensitivity for Pb was 5x greater than for Cd indicating a LoD of ~1 nM Cd using the vibrated Bi electrode, whereas Cd concentrations in uncontaminated waters, including coastal waters, are normally less than those for Pb and would then be below the LoD of the Bi electrode.

The ASV response for Pb was strongly affected by additions of Cu, which caused the response for Pb to decrease: addition of 50 nM Cu caused the Pb peak to decrease by 30 %, 100 nM Cu by 60 %, and 200 nM Cu by 80 %. It is likely that the Cu deposition coats the surface of the Bi electrode with a layer of elemental copper which is apparently not suitable for Pb detection. Though not always mentioned, Cu interferes with metal detection with all Bi-based electrodes. It is difficult to alleviate this interference as the Cu peak is more positive than that for Pb. The Bi electrode cannot be used to determine Cu because the Cu peak is located on the shoulder of the Bi oxidation wave. One possible way to alleviate the Cu interference might be to add a Cu-specific binding ligand to prevent the deposition of Cu. This has been done with some apparent success to eliminate the Cu interference with Pb detection in 0.1 M HCl with a Bi film electrode by adding 0.1 mM ferricyanide (Kadara and Tothill, 2005). Preliminary experiments in the laboratory involving addition of 0.1 mM ferricyanide to a pH 4.5 acetate solution did not improve the response of the Bi electrode for Pb in the presence of copper: instead the lead peak was shifted 0.1 V more negative and the baseline was caused to curve upward towards the Bi oxidation wave, starting underneath the Pb peak, suggesting a strong negative shift of the Bi oxidation wave. Natural waters contain nanomolar levels of Cu, usually about 10x higher than that of Pb which might be expected to interfere. However, the Cu in

natural waters tends to be complexed with organic matter forming relatively inert species, which may explain why the measurements in seawater were not affected by Cu interference. However, it is an interference which could build up on the electrode unless the Cu is removed periodically, either by scanning across the Cu peak using the solid Bi electrode, or by replacing the Bi film on the BiFE (Kokkinos et al., 2008).

2.3.8. Interference by surfactants

Triton X-100, which is a non-ionic surfactant, was used as a model surface-active compound to investigate the effect of surfactants on ASV of Pb using the Bi electrode. Natural waters are reported to contain organic matter with a surfactant effect similar to that of 0.05 to 0.5 mg L⁻¹ Triton-X-100 (Cosovic and Vojvodic, 1982). Additions of Triton X-100 were found to decrease the lead peak: 0.1 mg L⁻¹ Triton caused a 15 % decrease, 0.5 mg L⁻¹ a 30 % decrease, and 1 mg L⁻¹ a 70 % decrease in the peak height for 10 nM Pb. This decrease is similar to that caused to the Bi film electrode (Hocevar et al., 2002).

It was attempted to alleviate this interference by coating the electrode with a Nafion film, similar to that done using a Bi-film electrode (Gouveia-Caridade et al., 2006), but this was found to cause the baseline to curve up from potentials greater than -0.65 V, largely masking the Pb peak. The disappearance of Pb peak was probably due to the surface block effect by too much nafion coated.

2.3.9. Influence of Cl⁻

The influence of chloride ion was investigated by additions of NaCl to an acetate buffer solution containing 10 nM Pb. The deposition potential was -1.2 V for 60s, and the conditioning potential was -0.3V. The lead peak was found to decrease with increasing Cl⁻, initially quickly (a 60 % decrease by addition of 0.05 M Cl⁻) followed by a much more gradual decrease at higher Cl⁻, decreasing by 80 % at 0.5 M Cl⁻. The decrease in the peak current may be due to inactivation of the bismuth surface by chloride ions, due to BiCl₃ formation, or less likely to chloride complexation of Pb. The chloride effect caused the sensitivity in seawater to be much reduced (by up to 80 %) compared to that in freshwater or buffer solutions. This

means that the LoD (2 min deposition) using the vibrating Bi electrode for Pb in seawater is around 0.5 nM.

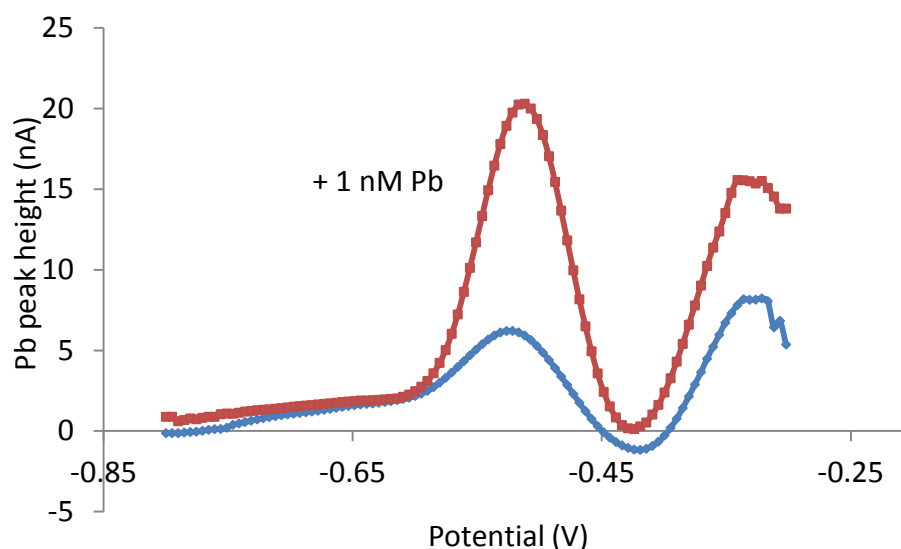


Figure 2.7. Voltammetric scan for Pb in UV-digested seawater containing 0.4 nM Pb, and a standard addition of 1 nM Pb. t_{dep} : 12 min, E_{dep} : -1 V.

2.3.10. Application to lake water samples and seawater samples from the Irish Sea

The bismuth bulk electrode was used for the determination of lead in natural water samples by SWASV. Seawater samples from cruise “Cobs No 62” in Liverpool Bay in June 2009, were stored acidified with distilled HCl to pH 2. Prior to analysis, the samples were UV-digested for 45 min, and subsequently the pH was adjusted to 4.5 using acetate buffer. Samples from lake Redo and a lake in North Wales (at the base of Snowdon) were treated similarly. Lead was determined by ASV using the Bi electrode as described, using a deposition time which was extended to 12 min to improve the limit of detection, whilst comparative measurements were carried out using a hanging mercury drop electrode (HMDE). Background corrected scans for Pb and a 1nM Pb standard addition to one of the seawater samples are shown in Figure 2.7. Differences in the calculated concentrations obtained using the HMDE and the Bi electrode (Table 2.3) were within the standard deviation and there was no evidence for a systematic error of the results using the Bi electrode. The comparative

measurements indicate that correct results are obtained for Pb at the 0.5 nM level using the Bi electrode. However, the standard deviation of individual determinations was larger for the Bi electrode than the HMDE in all samples. This is due to the low reproducibility on bismuth compared to HMDE. It was found to be impossible to detect Cd in the same samples using the Bi electrode due to the lower sensitivity for Cd than for Pb.

Table 2.3. Comparison of lead concentrations found using the Bi and hanging mercury drop electrodes in seawater samples from Liverpool Bay. Salinity values were: Station 10: 31.2; Station 13: 33.1; and Station 22: 31.8.

Electrode	Method	Irish Station 10	Irish Station 13	Irish Station 22
VBBE	SWASV	0.69±0.07 nM	0.41±0.04 nM	0.18±0.04 nM
HMDE	DPASV	0.60±0.02 nM	0.48±0.03 nM	0.16±0.02 nM
Electrode	Method	Lake in Wales	Lake Redo (1 m)	Lake Redo (10 m)
VBBE	SWASV	0.51±0.06 nM	0.62±0.03 nM	0.46±0.05 nM
HMDE	DPASV	0.57±0.04 nM	0.54±0.02 nM	0.43±0.03 nM

2.3.11. Points of merit of a Bi versus a mercury electrodes

The results suggest that a carefully prepared solid Bi electrode is sufficiently stable for long term analysis if its potential is kept under potentiostatic control at all times. Because of a lower sensitivity in seawater than in freshwater, lead detection in seawater requires a deposition time of 10 min, which is then suitable for lead detection at concentrations > 0.5 nM, as occurring in lightly contaminated coastal waters. The sensitivity of the Bi electrode is similar to that of a Bi film on a glassy carbon rotating disk electrode (de Carvalho et al., 2007), and considerably better for instance than a boron-doped diamond electrode coated with a Bi film which has a limit of detection of ~10 nM Pb (Toghill et al., 2008). The BiFE utilises in-situ Bi deposition whereas the Bi electrode can be used without any reagents. The Bi film

electrode and Bi electrode electrodes are both insensitive to dissolved oxygen which is convenient for analyses in the field, whereas the HMDE and mercury film electrodes require deaeration.

The Bi electrode requires careful potential control at all times, and requires polishing after extended exposure to air. The HMDE was more reproducible and more sensitive than the Bi electrode. The HMDE is not the most sensitive mercury electrode as much better sensitivity, leading to a 10 x better limit of detection for lead, can be obtained for instance using a mercury-coated glassy carbon electrode (Fischer and van den Berg, 1999). This means that the sensitivity using a Bi electrode, or a BiFE, is less good than the HMDE, and about 10 x less sensitive than a mercury film electrode. The Bi electrode is therefore not a good replacement for the mercury based electrode for trace levels of Pb and Cd.

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3

Chapter 3

Study of bare and mercury-coated vibrated carbon, gold and silver microwire electrodes for the determination of lead and cadmium in seawater by anodic stripping voltammetry

3. Study of bare and mercury-coated vibrated carbon, gold and silver microwire electrodes for the determination of lead and cadmium in seawater by anodic stripping voltammetry

Abstract

Carbon, gold and silver microwires are revisited under vibrated conditions for detection of trace lead and cadmium in seawater. The Pb and Cd peaks fully overlapped on the bare gold and carbon electrodes and partially on the silver electrode. The sensitivity of all three was insufficient for detection in uncontaminated waters. Peak separation was obtained after coating with mercury (Hg). Only the Hg-coated silver electrode is suitable when electrodes are pre-plated. Limits of detection for Pb using the Hg/C and Hg/Ag electrodes (20 - 40 pM), and Cd (70 pM), is sufficiently low for Pb and Cd detection in seawater.

3.1. Introduction

Lead and cadmium occur at similar concentrations in seawater: 10 – 200 pM Pb and 10 – 1200 pM Cd (Aparicio-Gonzalez et al., 2012). Their electrochemistry is similar (divalent metal ions, readily plated in the metallic state and soluble in mercury) which means that they can be determined simultaneously at low concentration by anodic stripping voltammetry (ASV) using mercury (Hg) electrodes (Batley and Florence, 1976; de Carvalho et al., 2007). Mercury film electrodes give better sensitivity than mercury drop electrodes due to the greater Hg surface / volume ratio. Potentially the sensitivity could be better on solid electrodes than on mercury as there would be no diffusion during the scan after the deposition step. However, deposition on solid electrodes leads to other problems related to intermetallic compounds, multi-layered deposits, interaction with the electrode material (Herzog and Arrigan, 2005) and accumulation of interfering organic matter, causing shifts in peak potentials and lower sensitivity. These problems need to be resolved, or at least the

electrode behavior needs to be stabilized, to facilitate these electrodes further development.

No electrode material has been found yet that improves on mercury electrodes for the measurement of Pb and Cd, with respect to sensitivity and resolution. The review here is largely aimed at detection of Pb and Cd in natural waters including seawater. Many papers have been written on the testing of electrode materials including gold, silver (Bonfil et al., 2002), carbon, graphite, boron-doped diamond (Toghill et al., 2009), silver-amalgam (Mikkelsen and Schroder, 2003), bismuth (Bi et al., 2010; Saturno et al., 2011) and modified electrode materials (Lu et al., 2011; Toghill et al., 2008) including metal alloys (Skogvold et al., 2006), bismuth nanostructures (Saturno et al., 2011) and renewal of the silver counter electrode by oxidation of the silver reference electrode (Bonfil and Kirowa-Eisner, 2002). Several of these electrode materials have been shown to be suitable to nanomolar levels of Cd and Pb, which is insufficient for their detection in uncontaminated seawater which contains these metals at picomolar levels. On the other hand, mercury-film electrodes have been known for a long time to be suitable for picomolar levels of these metals in seawater (Mart et al., 1984).

Excitation of the water at the electrode surface by ultra-sound (Compton et al., 1994) and low-frequency sound (Mikkelsen and Schroder, 2000) has been successfully used to improve the sensitivity of voltammetry by lowering the diffusion layer thickness. Electrode vibration can be achieved using simple means with a low energy requirement and is suitable for in-situ application (Chapman et al., 2012; Chapman and Van Den Berg, 2007). Much smaller diffusion layer thicknesses ($\sim 1\text{-}2\ \mu\text{m}$) and better reproducibility are achieved in vibrated conditions compared to standard stirring at macroelectrodes (Chapman and Van Den Berg, 2007; Salaün et al., 2012). Gold is a particularly good electrode material to determine copper and mercury in seawater (Gustavsson, 1986). Using a gold microwire electrode copper is determined with much better limit of detection than using a mercury based electrode (Salaün and van den Berg, 2006). A thin diffusion layer alone is not essential for detection of low metal concentrations but it has the advantage of reducing the analysis time. The wire electrodes have other advantages related to their insensitivity to hydrogen generation as the bubbles stream off the electrode, which means that extreme deposition

potentials can be used (Salaün et al., 2011).

The microwire electrodes are relatively easy to fabricate and to condition, without a requirement for polishing which is difficult to achieve at micro-disk-electrode, and impossible at microwire electrodes. Preliminary results obtained at the gold electrodes have highlighted the long term stability, reproducibility, relative sensitivity and ease of use of the vibrating microwire electrodes (Chapman and Van Den Berg, 2007; Salaün and van den Berg, 2006). Here various electrode materials were tested as substrate for vibrating microwire electrodes. The same materials have been used as electrodes in other studies, but they have been rarely compared in a single study, with standardized conditions across the three electrode materials, and never as vibrated microwire electrodes for trace levels in seawater. It is possible that these electrodes have a unique response characteristic, especially when a mercury coating is used due to diffusion into a microwire which has a limited capacity for mercury dissolution. The aim of this study is to establish the optimal material and procedure to determine Pb and Cd in seawater without the need of sample pre-treatment (reagent addition or separate pre-concentration). Because their peaks were found to overlap on the bare electrodes, mercury coating was subsequently investigated to improve the peak separation.

3.2. Experimental

3.2.1. Chemicals and Reagents

Milli-Q water (Millipore, $18 \text{ M}\Omega\text{cm}^{-1}$) was used for cleaning, dilutions and reagent preparation. Atomic absorption standards for Cd and Pb (BDH, UK, $1 \text{ g}\cdot\text{L}^{-1}$) were diluted appropriately and acidified to pH 2 with HNO_3 . Hg plating solution was prepared from $\text{Hg}(\text{NO}_3)_2$ salt (BDH,UK). Sodium acetate (Analar grade, BDH, UK) and acetic acid (purified by sub-boiling distillation on a quartz condenser) were used to produce a trace metal clean acetate buffer solution of 4 M (pH 4.5). HCl (sub-boiling distilled), KCl (BDH, UK), HNO_3 and H_2SO_4 were all Analar grade from BDH, UK.

Seawater, for method development, was collected from Liverpool Bay during a cruise with research vessel Prince Madog using a metal-free Niskin bottle (5 litres), filtered on-board through a Sartobran cartridge ($0.45\mu\text{m}$ and $0.2 \mu\text{m}$), and stored at room

temperature in an acid rinsed 50 L Nalgene container.

3.2.2. Apparatus

Voltammetric experiments were performed using a PGSTAT-101 (Metrohm, UK) potentiostat connected to an IME663 interface and a VA696 electrode stand (Metrohm, Switzerland), controlled using NOVA software (Version 1.6). The mercury electrode was removed from the electrode stand and replaced with lab-built, microwire, working electrodes. Microwires were variously composed of gold (10 μm diameter, 99.99%, hard, Goodfellow UK), carbon (7 μm diameter) or silver (12.5 μm diameter, Goodfellow, UK). The counter electrode (CE) consisted of an iridium wire (2cm length, 0.15mm diameter). The reference electrode (RE) was Ag/AgCl/KCl (3M) with a double salt bridge (KCl, 3 M).

3.2.3. Electrode fabrication and conditioning

WEs were fabricated similar to before (Salaün et al., 2011; Salaün and van den Berg, 2006) : a Cu wire (100 μm diameter and about 4 cm long, Goodfellow, UK) was inserted in a 10-100 μL polypropylene pipette tip and the end outside the tip dipped in a conductive and adhesive silver solution (Leitsilber L100, UK) to connect either the gold, carbon or silver wires of which lengths of <1 cm were used. The wire was then pulled back into the tip leaving ~ 1 mm exposed. Sealing was achieved by heating the polypropylene using a home-made tubular oven. The end of the tip was melted and sealed over several mm causing the two wires to be trapped. The temperature of this oven was set to 450 $^{\circ}\text{C}$ for Au and Ag electrodes and 200 $^{\circ}\text{C}$ for carbon electrodes, and the sealing process took about 8 s. It is possible to make the WEs without the intermediate copper wire (Billon and van den Berg, 2004), but then a greater length of the wires is required and the thicker copper wire facilitates the insertion of microwires with a diameter <25 μm into the plastic tip.

The microwire electrodes were attached to a vibrator using a procedure modified from published methods (Chapman and Van Den Berg, 2007) to facilitate electrode replacement. A vibrator (150 Hz, 1.5 V) was press-fitted within a 1 mL pipette tip, which was given a short (1 cm) collar of polypropylene tubing (1 mm thickness) to facilitate mounting in the top of the electrode stand (VA696, Metrohm) (instead of the mercury electrode) and to provide a

good seal to keep atmospheric oxygen out of the cell when necessary. An electrical wire was pulled through this pipette tip, connected on one end to the potentiostat and protruding on the other end to reach the bottom of the microwire-pipette tip when fitted. The microwire-pipette tip was press-fitted tip onto the end of the 1 mL vibrator tip (Figure 3.1). Friction was sufficient to hold the tip in place. The system is simple, allowing the replacement of an electrode within 30 s. Only the end of the electrode tip was dipped in the solution. The voltage (1.5 V) required for the vibrator was provided by a home-made interface connected to the IME663 and controlled by the NOVA software. The on/off command was taken from the stirrer command in the software which switched the vibrator power off via the IME663.

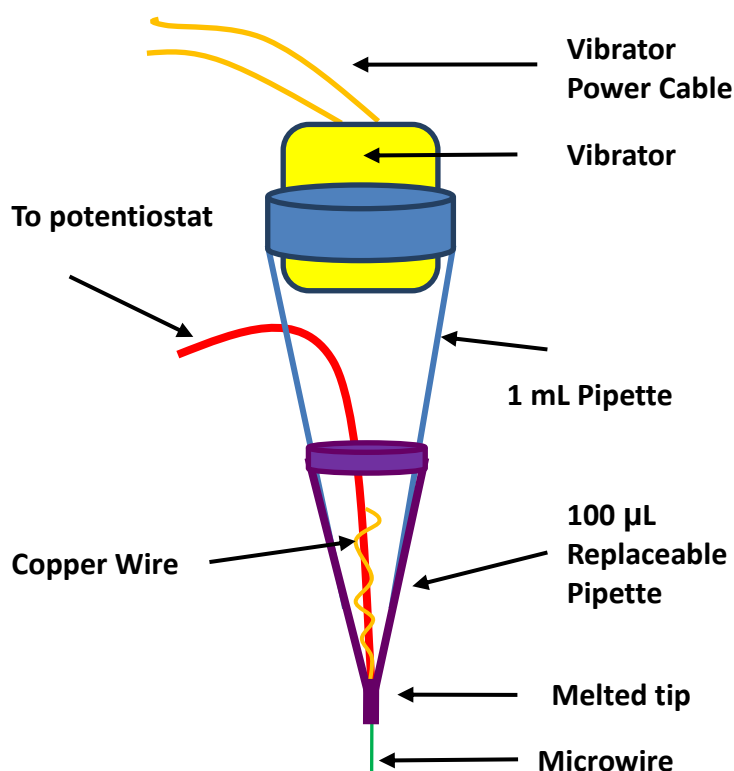


Figure 3.1. Schematic representation of the vibrated microwire electrode

Prior to use, and daily thereafter, the gold electrode was cleaned in 0.5 M H_2SO_4 by electrochemical hydrogen generation (-2 V for 60 s) followed by a cyclic voltammogram between 0 and 1.5 V (linear scan, $100 \text{ mV}\cdot\text{s}^{-1}$) to check for proper functioning of the electrode (Salaün and van den Berg, 2006). Silver and carbon electrodes were cleaned by electrochemical hydrogen generation at -2 V in 0.1 M HNO_3 followed by cyclic voltammetry between 0 and 0.35 V and between 0 and 0.5 V respectively.

3.2.4. Mercury deposition, removal and ASV measurements

Measurements used bare as well as mercury-coated microwires. Mercury deposition was carried out from 10 mM HNO₃ that had been purged to remove dissolved oxygen (DO). Mercury was added to the HNO₃ to various concentrations, depending on the electrode material. Deposition was either by setting the potential to a suitable value, or in combination with chronoamperometry at the selected deposition potential to enable calculation of the amount of Hg that was deposited. The deposition potential was -0.6, -0.4 and -0.4 V on C, Au and Ag respectively. The deposited mercury was removed electrochemically by reoxidation which was carried out in presence of thiocyanate (5-10 mM KSCN) by scanning the potential linearly from -0.4 V to 0.5 V at a scan rate of 1 mV s⁻¹. This slow rate of scanning was used to complete the mercury oxidation and obtain well separated peaks. SCN⁻ is known to be an effective complexant of Hg, prevents the problem of calomel formation when Hg is re-oxidised from an electrode and ensures a complete removal from the electrode (Fischer and van den Berg, 1999; Meyer et al., 1996).

ASV measurements were made in filtered UV digested seawater (SW) buffered to pH 4.5 by addition of 40 mM acetate buffer and purged with N₂ for deoxygenation. Previously deposited metals were removed at a re-oxidation potential of -0.2 V prior to the deposition step of the subsequent scan. The Hg surface was periodically (2-3 times per day) re-activated by application of a cleaning potential at -3 V for 2 s in the SW. This treatment was repeated in case the Cd and Pb peaks were not displaying the expected shape.

ASV scans used the square-wave mode with a typical frequency of 50 Hz, amplitude of 25 mV and a step size of 5 mV, unless indicated differently.

3.3. Results and discussion

3.3.1. Bare electrodes of C, Au and Ag

Bare microwire electrodes were heat-sealed in polypropylene as described. The temperature setting (200 °C) for the sealing of the C fibers was lower than for the other wires (450 °C) to avoid oxidation of the C wire which are reported to be sensitive to high temperatures (Nyholm and Wikmark, 1992). However, this may have been unnecessary as

much higher temperatures (600 °C) have been used in the past to seal C fibers in glass (Baranski, 1987) although those were subsequently polished. C, Au and Ag electrodes were tested separately to compare their sensitivity and resolution for the detection of Pb and Cd in synthetic buffer solutions and in sea water. Stripping voltammograms of UV digested sea water with and without addition of 10 nM Pb followed by 50 nM Cd using the three bare vibrated micro-wire (Au, C and Ag) electrodes are shown in Figure 3.2. Peaks were produced by Pb and Cd using all electrodes, but peak separation was poor using the Au and C electrode. Peak separation was better at the Ag electrode (100 mV) (Pb at -420 mV and Cd at -520 mV), but also here the peaks overlapped.

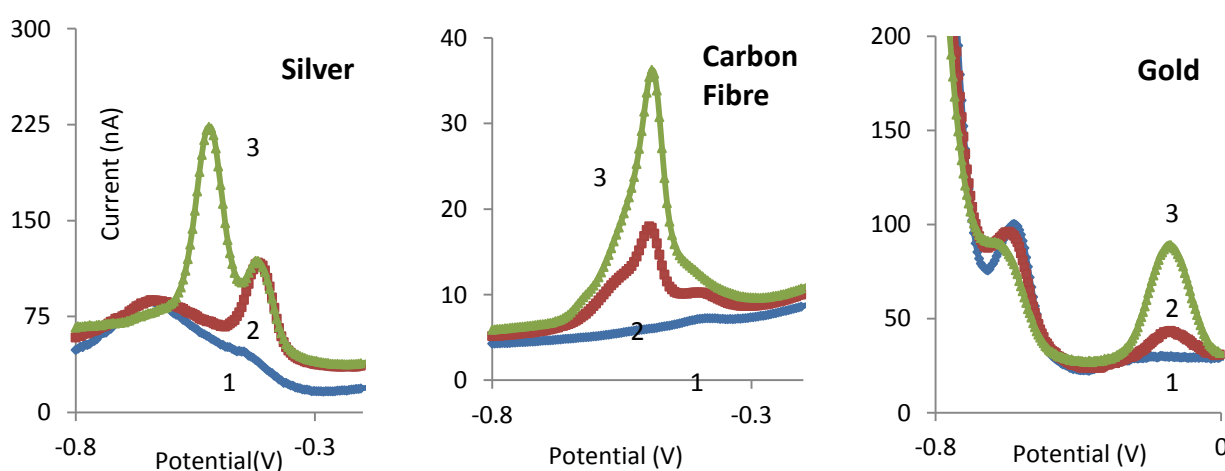


Figure 3.2. Comparison of ASV scans for Pb and Cd in UV-digested seawater ($E_{\text{dep}} = -1.2$ V for 60 s) using bare microwire electrodes. The electrodes were vibrated during the deposition step. 1: Blank; 2: +10 nM Pb; 3: +10 nM Pb and 50 nM Cd.

The sensitivity for Cd using Ag wire is considerably less (~ 0.25 x) than for Pb (the Cd additions in Figure 3.2 are 5x greater than of Pb) so it is likely that Pb can be determined with only minor interference by Cd. This lower sensitivity is also known to occur at Ag disk electrodes (Bonfil et al., 2002) and was then explained as a result of competition between deposition of Pb/Cd adatoms and anion adsorption on the Ag surface during the deposition step (Bonfil et al., 2002). It is also possible that these adsorbed anions play a role during the stripping process as observed for Cu on gold substrate (Salaün and van den Berg, 2006). The limit of detection for Pb using a 25- μM Ag microwire is ~ 0.2 nM Pb (5 min deposition) (Billon and van den Berg, 2004) which is insufficient for the levels typical for seawater.

Preliminary tests using a 12.5 μm wire under vibrated conditions improved the signal but not to a sufficient extent to enable Pb detection in uncontaminated seawater. On the

other hand, the bare electrodes, especially the silver wire, may be of use for Pb detection in contaminated waters with a relatively low concentration of Cd. These detection limits are high in spite of the very thin diffusion layer thickness of these electrodes. The overlapping peaks have also been obtained using rotating disk electrodes (Bonfil et al., 2002; Bonfil and Kirowa-Eisner, 2002), although a better limit of detection was obtained in that work in dilute acid solutions than here in seawater.

The peak potentials for Pb and Cd varied on the different electrode materials: Pb was at -0.42 V and Cd at -0.52 V on silver; both metals were at -0.49 V on carbon and at -0.14 V on gold (Figure 3.2). That means Cd and Pb cannot be resolved on bare carbon and gold electrodes. There is an opportunity to differentiate between Pb and Cd on silver, but there too the peaks show overlap. The large shift to more positive potential on gold is due to under-potential deposition (UPD). UPD is known to occur on metal electrodes and is caused by adsorption of the metal in metallic state on the substrate of the bare electrode, causing the first few monolayers of deposited metal to have a more positive potential than subsequent layers (Lovric and Scholz, 1997). A third peak on the gold electrode at -0.6 V (Figure 3.2) is due to zinc at ~20 nM in this water.

In order to improve further the limit of detection, the electrodes were modified by coating with mercury as mercury gives good separation of Pb and Cd. The mercury was deposited by plating, either prior to use from a Hg solution to produce an ex-situ Hg-coated electrode, or by adding some Hg to the analysed seawater and plating in-situ during the deposition step of Pb and Cd. The in-situ deposited Hg was removed electrochemically at the end of each scan. The ex-situ plated electrodes have advantages related to measurements where the electrode is lowered into the water body, thus enabling reagent-less monitoring of Pb and Cd. The analytical quality and stability of the corresponding Hg-coated electrodes was tested by ASV with the aim of detection of low concentrations of Cd and Pb in seawater.

3.3.2. *Hg-carbon electrodes:*

Ex-situ and in-situ Hg deposition were tested and optimized in terms of sensitivity and signal stability. Ex-situ Hg deposition from 20 μM Hg^{II} in 10 mM HNO_3 was evaluated by cyclic voltammetry. The cyclic voltammogram displayed two reduction peaks at -0.2 and -0.6

V corresponding to reduction of mercury and oxygen respectively. The oxidation peak of Hg was at +0.26 V, which is more negative than on gold where it is located between +0.35 and +0.5 V depending on the anion concentration (Salaün and van den Berg, 2006)). The more positive potential on gold can be ascribed to UPD which is not occurring on the carbon. In presence of thiocyanate (SCN^-), the Hg re-oxidation peak shifted negative to +0.15 V with a 5 times higher oxidation charge than without the SCN, in general agreement with Hg re-oxidation on iridium (Belmont-Hebert et al., 1998). The influence of the deposition potential on the amount of mercury deposited was assessed in 10 mM HNO_3 + 20 μM Hg^{2+} + 5 mM KSCN by measuring the charge of the oxidation peak using linear sweep voltammetry (LSV) at $1 \text{ mV}\cdot\text{s}^{-1}$, after 60 s deposition at various potentials between -1.2 V and +0.1 V, from quiescent solution. The plated Hg was removed by oxidation at +0.4 V in the same solution prior to each subsequent scan. A pseudopolarogram (PP) curve was obtained with a half-wave potential for Hg at -0.2 V. The response was stable between -0.4 V and -1.2 V (Figure 3.3A), which is characteristic of diffusion-limited conditions.

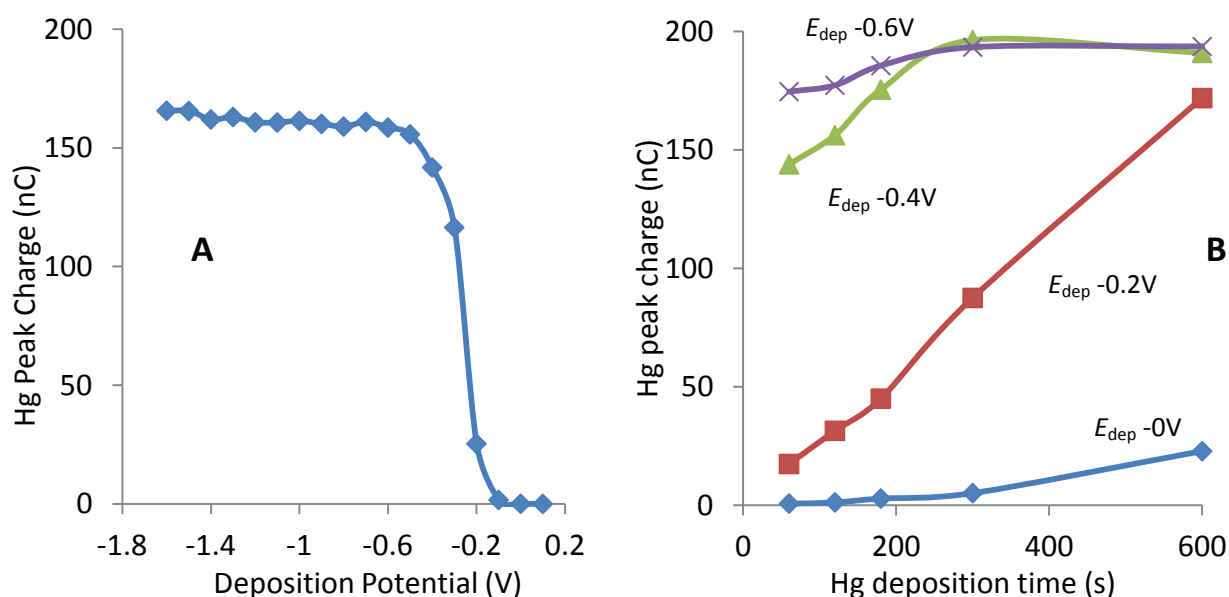


Figure 3.3. Effect of varying the deposition potential (A) and deposition time (B) on the amount of mercury (charge) oxidised from the electrode. The deposition time was 60 s. The plating solution contained $200 \mu\text{M}$ Hg^{2+} in 10 mM HNO_3 and 5 mM KSCN. Oxidation was by a slow linear sweep ($1 \text{ mV}\cdot\text{s}^{-1}$). A: effect of varying the Hg deposition potential; B: Effect of varying the deposition time using a deposition potential of 0 V (1), -0.2 V (2), -0.4 V (3) or -0.6 V (4).

At deposition potentials $< -0.4 \text{ V}$ the charge of Hg re-oxidised during the scan in thiocyanate did not increase with deposition time (Figure 3.3B), although the Hg-deposition charge continued to increase with time. The difference between the continuously increasing

deposition (reduction) charge and the stabilised oxidation charge suggests a mechanical instability of the Hg film which was worse at greater deposition times (greater mercury thickness). The Hg deposit on carbon is thought to be unstable because the carbon is not wetted by the Hg (Baranski, 1987).

The stability of the Hg film was assessed by taking the Hg coated electrode out of the water and exposing it to air for 1, 15, 30, 60, 120 and 300 s before dipping it again in the SCN solution (5 mM SCN) to oxidise the remaining mercury on the C surface. This procedure simulates the transferring of the electrode between plating and measuring solutions after ex-situ deposition. It was found that the Hg oxidation charge decreased rapidly with exposure time to air, losing about 30% Hg within 1 s and 85% within 15s.

The number of layers of mercury atoms deposited on the electrode was computed from the charge of oxidized mercury and the surface area of the electrode. The length of the carbon fiber exposed to the water was first fitted from the reduction current measured by chronoamperometry (during ~1 min) at -0.8 V of ferricyanide (10 mM in 0.5 M KCl) (Salaün and van den Berg, 2006). The surface S was then obtained using a diameter of 7 µm as given by the manufacturer. The charge of mercury (Q) was obtained from the Hg oxidation peak area recorded by LSV in 1 M KSCN. The thickness of the mercury layer (in number of deposited layers) was then calculated from the deposited charge as follows:

$$n = Q \pi R^2 / (2 S F)$$

where n= number of mercury layers. R: radius of mercury atom (1.51×10^{-10} m (Slater, 1964)). The amount of mercury (in number of layers assuming a fully packed structure of deposited Hg atoms) remaining at the electrode surface when the electrode was briefly pulled out of the solution is shown as function of exposure time in Figure 3.4. The decrease as a function of time was found to stabilize at a thickness of ~1 layer, whilst the accumulated layers on top of the first layer were rapidly lost upon lifting of the electrode out of the water. The relative stability of the first layer was ascribed to adsorption stabilization on the carbon surface.

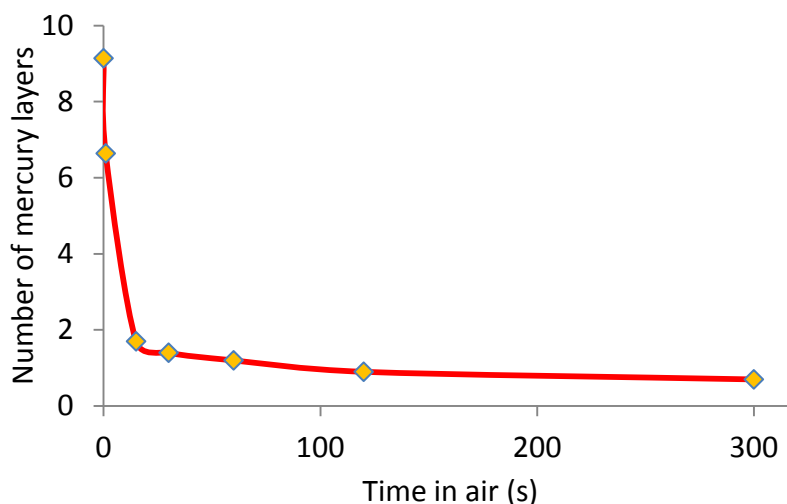


Figure 3.4. Loss of Hg plated on a carbon fiber electrode as a function of time when the electrode was drawn out of the solution and exposed to air. The residual Hg was determined from the charge obtained from Hg oxidation during a slow linear sweep scan in 1 M KSCN (1 mV s^{-1}).

Instability of Hg on platinum wires has been reported previously, whereas on C fibers the peak for Hg has been seen to increase upon transfer to a different solution (Nyholm and Bjorefors, 1996), conversely this work clearly shows a decrease (Figure 3.4). The decrease was ascribed to poor adhesion of the Hg to the C fiber. The instability of Hg on the C fiber means that most of the deposited Hg would be lost within seconds of moving the electrode between solutions. The sensitivity for Pb using the residual Hg layer is poor so the carbon fiber is not suitable to use as a pre-plated Hg-coated electrode for Pb. Flow-analysis procedures could be used to minimize this effect (Bjorefors and Nyholm, 1996), but this restriction makes this electrode inconvenient for in-situ detection.

In-situ Hg deposition can be used instead of the pre-plated Hg film, as then the Hg is continuously topped up during the measurement, or is replaced after each scan. This is readily done during the deposition step of a typical ASV measurement (cleaning at 0.4 V for 10 s, deposition for 120 s at -1.2 V) by adding a low concentration of Hg to the measured water. The following conditions were tested with good success: $20 \mu\text{M Hg}^{2+}$ and 5 mM KSCN in UV digested SW, buffered at pH 4.5 with 40 mM acetate buffer. Addition of KSCN directly in the solution was shown to strongly improve the stability and measurement reliability, especially at low concentrations. These conditions were slightly modified from previous studies using a rotating carbon disk electrode by lowering the concentration of Hg to $20 \mu\text{M}$ from $30 \mu\text{M}$ Hg used previously (Fischer and van den Berg, 1999) because of the thinner

diffusion layer of the wire electrode. The high sensitivity for Pb and Cd makes the in-situ Hg-plated C fiber electrode suitable for determination of Pb and Cd at trace level in seawater. However, the instability of the Hg coating makes it unsuitable for in-situ monitoring without in-situ Hg renewal.

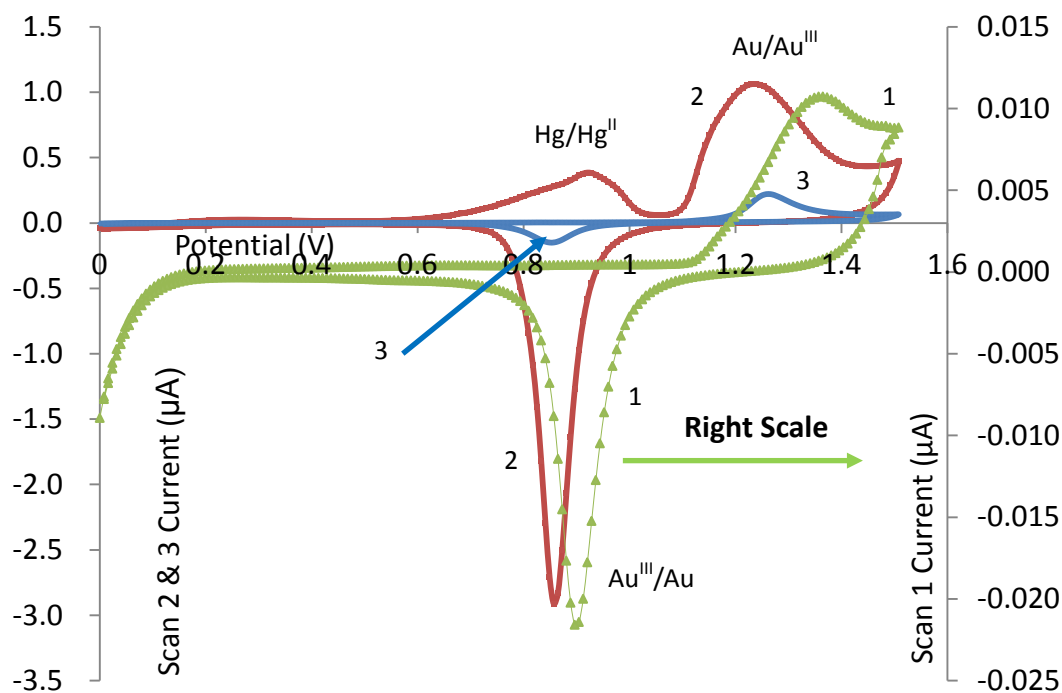


Figure 3.5. Cyclic voltammograms of the Au microwire electrode in 0.5 M H_2SO_4 (100 mV s^{-1}). Individual scans are indicated by numbers on the diagram. Scan 1: Before Hg deposition (scan 1 right hand scale); Scan 2: After Hg deposition 300 s from $200 \mu\text{M Hg}^{2+}$; Scan 3: After Hg removal in 1 M KSCN.

3.3.3. Hg-Au electrodes

The Au wire electrode was ex-situ coated with Hg by electrolysis from $200 \mu\text{M Hg}^{2+}$ in 0.1 M HNO_3 , at -0.4 V for 300 s in quiescent solution. The plated Hg was removed, when necessary, by a slow LSV scan to +0.4 V in 1 M KSCN. Cyclic voltammetry in 0.5 M H_2SO_4 showed that the Au surface was changed by the plating and removal of the Hg (Figure 3.5). Without Hg-coating the CV scan produced one Au oxidation peak at $\sim 1.3 \text{ V}$, and one reduction peak at +0.8 V (scan 1, Figure 3.5, right-hand scale). With the Hg-coating, a large asymmetric anodic peak appeared (scan 2, Figure 3.5), starting at +0.6 V and reaching its maximum at +0.9 V corresponding to the irreversible oxidation of Hg^0 to Hg^{2+} followed by the formation of Au oxide at +1.2 V, much greater (120 x) than the same peak in scan 1.

However, this scan was found to gradually decrease until reaching a final state (scan 3, Figure 3.5) with the reduction peak for the Au-oxide still considerably higher than the original peak indicating an overall roughening of the electrode surface. The drastic increase of roughness after mercury oxidation is known to be associated with the appearance of pits and islands of 2 to 30 nm diameter (Levlin et al., 1996). This is due to the high solubility of gold into mercury (0.13%) with gold atoms diffusing into the mercury film. Subsequently, when the mercury film is oxidized, these gold atoms re-crystallize (Lovric and Scholz, 1997) the surface to be roughened. This surface is unstable as the gold atoms tend to rejoin their lattice (Inukai et al., 1996), explaining the gradual decrease of the roughness (scan 3, Figure 3.5) to a final steady state value which was still ~ 7 to 10 times higher than the original one.

Scan 3 (Figure 3.5) was made after removal of Hg in KSCN. Reoxidation of mercury on the gold electrode in 1 M KSCN using slow LSV gave two peaks at +0.46 V and +0.66 V (not shown), compared to only one peak on the carbon electrode. The more positive peak corresponds to reoxidation of Hg stabilised on the Au by the under-potential deposition (UPD) mechanism (Lovric and Scholz, 1997), whereas the more negative peak at 0.46 V is for the reoxidation of layers of Hg on top of the first layer of Hg.

The deposition potential and deposition time of Hg were varied to optimize the sensitivity for Pb and Cd, and the electrode stability. Deposited Hg was removed after each scan and the oxidation charge measured in 1 M KSCN. With more negative deposition potential (decreasing E_{dep}) and increasing deposition time (t_{dep}) more Hg was deposited on top of the first layer of Hg (Figure 3.6). Because the first layer gave a different peak from the subsequent, bulk Hg, deposition, these could be measured individually. The charge of the bulk deposited Hg (Hg on Hg) continued to increase with deposition time, corresponding to the on-going reduction of mercuric ions on Hg^0 at the electrode surface. In contrast, the peak height for Hg on Au remained relatively constant. This Hg-on-Au peak corresponds to the first few monolayers of Hg at the surface of the gold, stabilized by interaction with the gold surface. The fact that the Hg-Au peak remained constant after several electrolysis/oxidation cycles indicate that the gold surface was stable under such conditions, showing that the formation of Au-Hg amalgam reached a steady state after the large change observed after the first Hg electrolysis. This stability means that the gold wire can withstand

cycles of Hg deposition and re-oxidation.

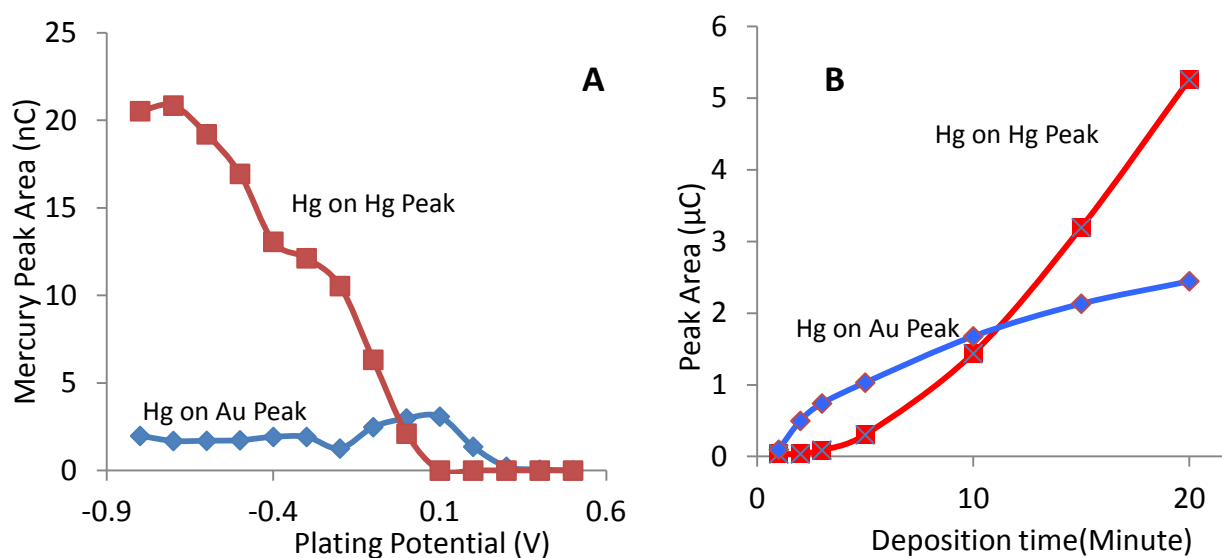


Figure 3.6. A: Influence of the deposition potential and time on the amount of Hg deposited. Two layers are formed: the first layer is Hg on Au, the second layer is Hg on Hg (bulk Hg). Values obtained by LSV (1 mV s^{-1}). The solution contained: $200 \mu\text{M Hg}^{2+}$ in 10 mM HNO_3 . A: Deposition potential ($t_{\text{dep}} = 60 \text{ s}$); B: Deposition time ($E_{\text{dep}} = -0.4 \text{ V}$).

The Hg coated Au microwire electrode was used for the detection of Cd and Pb in synthetic and marine waters. It was found that the amount of Hg deposited on the Au strongly affected the Cd response by ASV while the Pb peak remained largely unaffected by variation of Hg. Results were satisfactory with good separation between the two peaks and good detection limits (Table 3.1). However, with deposition of a thick mercury coating the mechanical stability was found to be poor with loss of the signal after only few days and the wires were found to break easily. It has been previously proposed to use the gold Hg-coated electrode for cathodic stripping voltammetry (CSV) (Dansby-Sparks et al., 2009; Gun et al., 2006). These experiments suggest that it is best to keep the Hg-coating on the gold microwires thin.

3.3.4. Hg-Ag electrodes

Hg plating on the silver microwire electrode was optimized by variation of the deposition potential and time from a solution containing $1\text{-}5 \text{ mM Hg}^{2+}$ solution acidified to pH 2 with HNO_3 . The Hg deposition was evaluated by comparison of scans for Cd and Pb in

seawater. Preliminary experiments showed that Hg continued to accumulate on the Ag microwire with increased Hg concentration, and therefore the Hg plating concentration used was about 100x that used for the Au and C wires. The cyclic voltammogram from 0 to 0.5 V contained one broad reduction peak at -0.12 V and one oxidation peak at +0.18 V corresponding to the reduction and oxidation of Hg^{2+} respectively. Slow LSV produced also only one re-oxidation peak when scanning positive in 1 M KSCN in an attempt to remove the plated Hg.

The amount of Hg on the silver wire was varied and its effect on the ASV peak height for Pb and Cd evaluated. Measurements were using a 1-min plating time of 2 nM Pb and 10 nM Cd in UV digested seawater (buffered at pH 4.5 with 40 mM acetate). The mercury plating time was varied between 1 and 10 min (Figure 3.7A) using a deposition potential of -0.4 V. Both Pb and Cd reached a plateau value at Hg-deposition time > 2 min which was selected as optimal for best analytical condition. Voltammograms using 1 min, 5 min and 10 min Hg-plating are shown in Figure 3.7B. At low Hg coverage (60 s deposition) the hydrogen wave at -1.1 V was similar to that at the bare silver electrode showing that the electrode was not fully covered with Hg. The hydrogen wave strongly decreased with increasing Hg-deposition and became similar to that expected for a Hg-electrode. The data suggest that a Hg-plating time of 2 min from 2 mM Hg^{2+} was optimal as then the hydrogen wave was shifted negative and the peaks for Pb and Cd were well separated and stable (Figure 3.7B). Anomalous Cu peaks were observed on these scans (Figure 3.7B), which may be due to contamination from the Cu wires. A conditioning step was introduced to clean the electrode prior to each subsequent measurement by applying an oxidation potential, E_{con} , to remove any residual plated Pb and Cd. Variation of the this potential within the range of -0.4 V to 0 V gave good result for Cd and Pb for E_{con} between -0.4 and -0.075 V. The peaks decreased at higher conditioning potentials due to Hg oxidation. A conditioning potential of -0.15 V was chosen, sufficiently low to avoid oxidation of Hg while preventing accumulation of Cu in the Hg film. Between measurements, a standby potential of -0.3 V was applied.

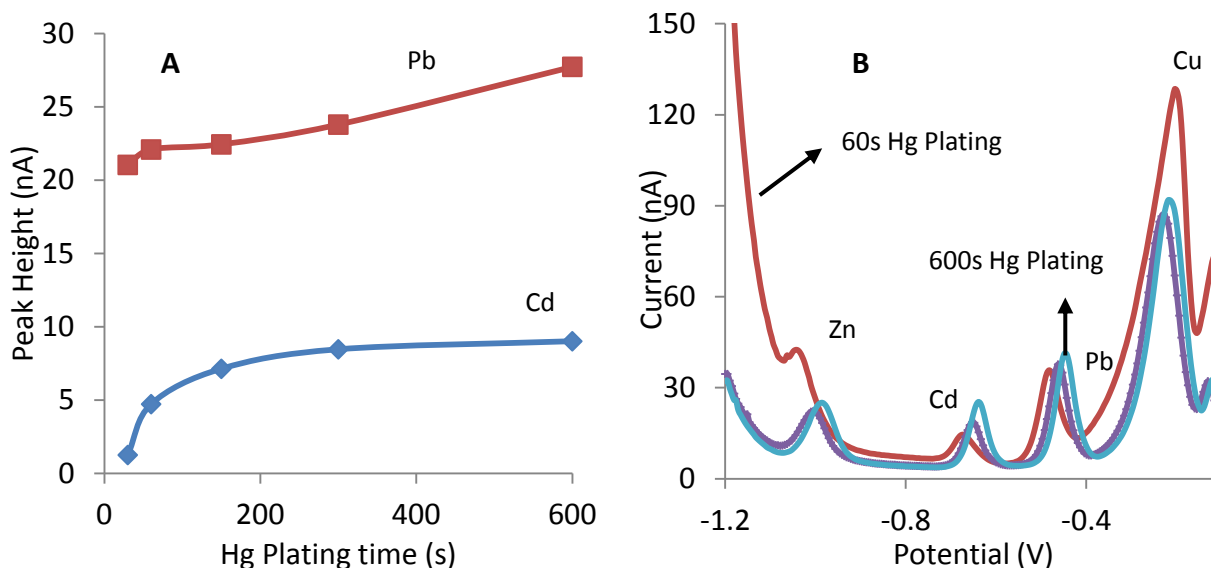


Figure 3.7. Influence of the mercury pre-plating time (deposition potential -0.4 V in 2 mM Hg^{2+} , 10 mM HNO_3) on ASV of Pb and Cd on the SAM electrode. The seawater was UV-digested and buffered to pH 4.5 using 40 mM acetate buffer, and 2 nM Pb and 10 nM Cd had been added. Voltammograms were preceded by 1 min deposition of Pb and Cd at -1.2 V. A) Peak height for Cd and Pb for different pre-plating times of mercury B) Voltammograms obtained for Pb and Cd after 60 s (top scan, red), 5 min (dark blue) and 10 min plating of Hg (scan with largest peak height for Pb, lighter blue).

3.3.5. Comparison of the Hg-coated electrodes for Pb and Cd detection in seawater

The three electrode materials (7 μm C wire, a 10 μm Au wire and a 12.5 μm Ag wire) were compared by measurement of 2 nM Pb and 10 nM Cd in seawater. The electrodes were Hg coated using the optimized conditions (Table 3.1). In-situ Hg deposition was used for the C electrode to avoid instability problem of the Hg film while ex-situ deposition was used for the Au and Ag electrodes. In all cases a deposition potential of -1.2 V was used for 60 s. Detection of Pb (2 nM) and Cd (10 nM), added to UV-digested seawater buffered with 40 mM acetate to pH 4.5, was by square-wave ASV: 50 Hz frequency, 5 mV step and 25 mV amplitude. The voltammograms (Figure 3.8) show reasonable peak separation for Cd and Pb on all three Hg-coated electrodes, showing that each electrode can be used for the detection of Cd and Pb in seawater. In spite of the coating with mercury, the three electrodes retain clear differences in that the peak potentials differ. The Pb peak is at -0.45 V on the Hg-Au and Hg-Ag electrodes, whilst it is at -0.48 V on the Hg-C electrode. The Cd peak has much greater variability between -0.56 V and -0.74 V. The peak separation between the Pb and Cd peaks is summarized in Table 3.1.

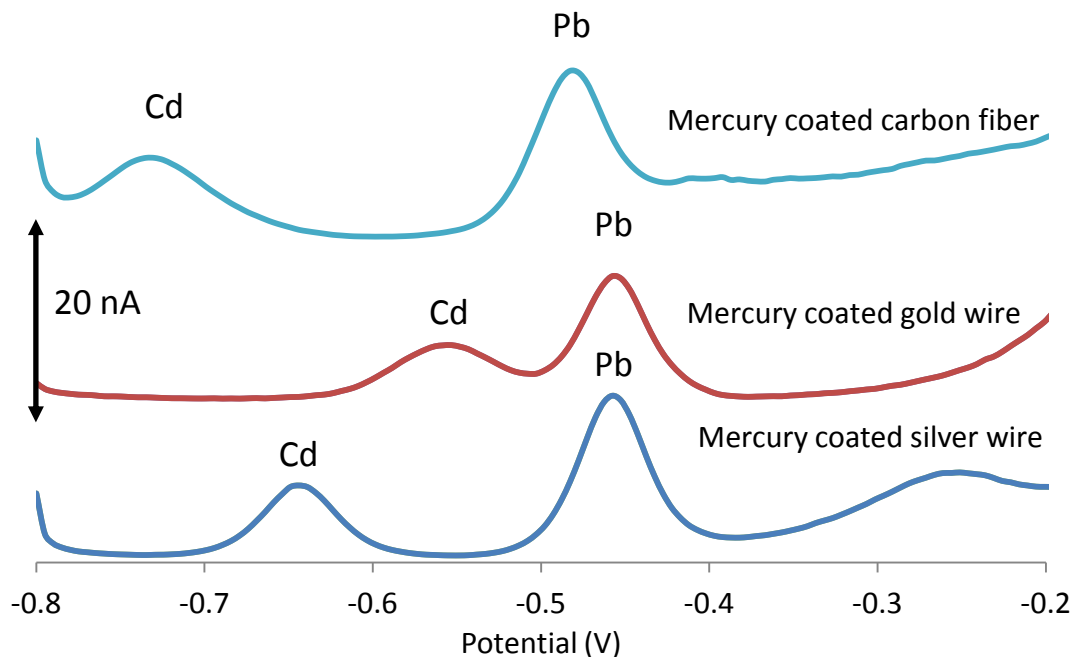


Figure 3.8. Voltammograms of 2 nM Pb and 10 nM Cd obtained at the Hg-C, Hg-Au and Hg-Ag electrodes. The seawater was purged, UV digested and buffered to pH 4.5 with 40 mM acetate buffer; ASV parameters: 60 s deposition time with vibration, deposition potential -1.2 V, square-wave modulation, 50 Hz frequency, 5 mV step, 25 mV amplitude.

The limit of detection (LoD) was determined for each electrode from 3x the standard deviation of 7 consecutive measurements at 0.2 nM Pb and 1 nM Cd in seawater (UV-digested). The LoD was highest on the Hg-Au electrode (Table 3.1), and lower on the other electrodes. The LoD was 70 pM Cd at the Hg-C and Hg-Ag electrodes. The LoD for Pb was lowest on the Hg-Ag electrode (Table 3.1). These LoD values were obtained using a deposition time of 1 min and can be decreased by extending the plating time.

The result with the vibrated Hg-C microwire electrode can be compared to the in-situ Hg-plated glassy carbon rotating disk electrode (GC-RDE): this electrode has been used for a long time (Florence, 1970) with very good result and LoDs of 2 pM Pb and 8 pM Cd, using 12 min plating in the presence of 10 μ M Hg (Mart et al., 1980). The LoD using the RDE (2 pM Pb at 12 min plating) is less than obtained here using the Hg-C wire electrode (41 pM Pb at 1 min plating). Comparison to the RDE suggests that, after normalization of deposition time, the RDE would be more sensitive than the wire electrode, which is rather surprising in view of the much better mass transport and flux towards the wire electrode. An important difference is related to the conditioning of the electrode: the RDE requires polishing to a

high standard while the C microwire electrode used in this work required no physical pre-treatment at all.

Table 3.1. Optimized analytical conditions, limit of detection (LoD) and measurement reproducibility of voltammetric scans for Pb and Cd using Hg-C, Hg-Au and Hg-Ag electrodes in UV-digested, pH 4 buffered seawater. The carbon electrode was in-situ plated with Hg (the mercury removed after each scan), whereas the gold and silver electrodes were ex-situ plated (pre-plated electrodes were used). The LoD values were determined using a 60 s deposition time for 0.2 nM Pb and 1 nM Cd. The electrode stability was determined from the relative standard deviation (RSD) of the peak height of 100 scans.

Electrode	Diameter (μm)	Concentration of Hg and deposition potential (V)	LoD (pM) Pb/Cd	Ep (V) Pb/Cd	ΔEp (mV) between Cd and Pb	Stability RSD (%)
Carbon	7	In-situ, 20 μM Hg^{II} ; -0.6 V	41/68	-0.48/-0.74	260	2.7/10.2
Gold	10	Ex-situ, 200 μM Hg^{II} ; -0.4 V	87/680	-0.45/-0.56	110	1.6/8.1
Silver	12.5	Ex-situ, 2 mM Hg^{II} ; -0.4 V	18/74	-0.45/-0.64	190	1.5/3.4

3.3.6. Stability of the Hg-coated electrodes for metal measurements throughout the day

The stability of the measurements using the Hg-coated electrodes was assessed by calculation of the standard deviation of 100 consecutive measurements of 2 nM Pb and 10 nM Cd. The measurement stability was good at all three electrodes though best on the Hg-Ag electrode (1.5 % for Pb and 3.4% for Cd, for $n = 100$). The scans using the three electrodes (Figure 3.8) show good peak separation for the Hg-C and Hg-Ag electrodes, less good on the Hg-Au electrode. The Cd peak was close to the peak for Zn on the Hg-C electrode, suggesting that the Hg-Ag electrode is most suitable for determination of Pb and Cd in seawater.

3.4. Conclusions

Comparative measurements using ASV of Cd and Pb in seawater using bare C, Au and Ag microwire electrodes showed problems related to overlapping peaks and poor sensitivity. Resolution between the two metals was best on the Ag electrodes.

Coating the electrodes with Hg improved the analytical conditions for Pb and Cd, lowering the limit of detection and much improving the peak separation. All three electrodes can then be used to determine Pb and Cd in seawater when coated with Hg. Pre-plated coatings of Hg were found to be unstable on C, the Hg falling off the C wire within seconds of lifting it out of water. The Hg-coated Au wire electrodes can be used for Cd and Pb detection, but the coating weakened the Au electrode through dissolution of the gold by mercury. The pre-plated coating on the Ag electrode was most stable. For detection of Pb and Cd using the C and Au electrodes, it was best to use in-situ plating of the Hg coating, with subsequent removal in thiocyanate. The Hg coated Ag microwire has best prospects for use as pre-coated electrode for monitoring of Pb and Cd in natural waters.

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4

Chapter 4

**Determination of lead and cadmium in
seawater using a vibrating silver amalgam
microwire electrode**

4. Determination of lead and cadmium in seawater using a vibrating silver amalgam microwire electrode

Abstract

Silver amalgamated electrodes are a good substrate to determine lead (Pb) and cadmium (Cd) in seawater because they have properties similar to mercury but without the free mercury (Hg). Here a silver amalgamated microwire (SAM) electrode has been optimised for the determination of Pb and Cd in coastal waters and uncontaminated ocean waters. The SAM was vibrated during the deposition step to increase the sensitivity, and electroanalytical parameters were optimised. The Hg coating required plating from a relatively concentrated (millimolar) solution, much greater (500x) than used for instance to coat glassy carbon electrodes. However, the coating on the ex-situ amalgamated electrode was found to be stable and could be used for up to a week to determine trace levels of Pb in seawater of natural pH. The limit of detection square-wave ASV (50 Hz) using the pre-plated SAM electrode was 8 pM Pb using a 1-min plating time at pH 4.5. The limit of detection in pH2 seawater was 4 pM using a 5-min plating time, and it was 12 pM using a 10-min plating time at natural pH in the presence of air, using a square-wave frequency of 700 Hz. The vibrating SAM electrode was tested on the determination of Pb in reference seawater samples from the open Atlantic (at the 20 pM level), Pacific, and used for a study of Pb in samples collected over 24 h in Liverpool Bay (Irish Sea).

4.1. Introduction

The concentration of lead (Pb) in uncontaminated seawater is at the picomolar level (Biller and Bruland, 2012; Wu and Boyle, 1997a): 15 to 45 pM Pb in the Atlantic and < 10 to 80 pM Pb in the Pacific, considerably less than in estuarine or coastal waters where they can be in the low nM range (Omanovic et al., 2006; Windom and Smith, 1985). Cadmium has a greater range due to depletion in the surface waters to < 5 pM Cd whilst rising to ~1 nM Cd in deep Pacific waters (Biller and Bruland, 2012). Cd has a nutrient-like behaviour whilst Pb reflects variations in anthropogenic, contamination, which makes both metals of interest for investigation. Pb and Cd in seawater can be determined using isotope-dilution inductively-coupled mass-spectrometry (ICP-MS) (Wu and Boyle, 1997b), which provides

concentrations that are accepted as reference values. This technique requires expensive equipment and is not suitable for taking into the field. ICP-MS is laborious in that it requires a preconcentration step which can consist of $\text{Mg}(\text{OH})_2$ coprecipitation (Wu and Boyle, 1997b), a chelating resin (Biller and Bruland, 2012), or extraction (Kremling and Streu, 2001). On the other hand, the actual detection step is easier than by voltammetry and multiple elements can be determined simultaneously.

Electroanalytical methods are especially suitable for monitoring of metals, including lead, in saline waters (Mikkelsen et al., 2005), and to determine metal speciation. Anodic stripping voltammetry (ASV) has been used for many years to determine heavy metals in natural waters including seawater. Lead and cadmium are readily determined using mercury-based electrodes (mercury drop and mercury-coated solid electrodes). Best results have been obtained using mercury-coated glassy carbon rotating-disk electrodes (GC-RDE) using in-situ plating of a thin mercury film (Bruland et al., 1985; Florence, 1970; Mart et al., 1980) with sufficient sensitivity to analyse ocean waters. Pb and Cd can be detected in seawater using the mercury drop electrode (de Carvalho et al., 2007), bismuth film (de Carvalho et al., 2007; Kyrisoglou et al., 2012) and solid bismuth (Bi et al., 2010), electrodes by extending the deposition time, which makes them suitable to monitor Pb and Cd in coastal waters.

The GC-RDE electrode requires a long plating time (> 10 min) and requires polishing which makes the analyses laborious. For this reason a new electrode with superior sensitivity was developed to enable detection of Pb and Cd within a shorter analysis time and with less need for electrode handling, therefore enabling detection by placing the electrode in-situ using a buoy-mounted monitoring station (Chapman et al., 2012). Solid electrodes, with or without coating with Hg, are more convenient than the mercury drop electrode for measurements in the field, because the mercury drop can contaminate the environment upon its renewal. Bismuth, silver and gold electrodes can be used to detect lead to low levels (Bi et al., 2010; Bonfil and Kirowa-Eisner, 2002; Hwang et al., 2008; KirowaEisner et al., 1999) but the peaks for lead and cadmium tend to overlap on the gold and silver electrodes (Bi et al., 2013). Silver amalgam electrodes appear to combine the advantages of mercury (high hydrogen overpotential and good sensitivity for other amalgam forming metals) with that of solid electrodes (no free mercury and robustness) (Mikkelsen and Schroder, 2003). A limit of detection of ~ 5 nM Pb in dilute acid has been achieved using

the macro silver amalgam electrode (Mikkelsen and Schroder, 2001).

Microwire electrodes provide very good sensitivity as a result of microelectrode behaviour especially in combination with vibration during the deposition step as this lowers the diffusion layer thickness to $\sim 1 \mu\text{m}$, ensuring high mass transport and a low limit of detection at a short deposition time (Salaün et al., 2012). In preliminary work, microwire electrodes composed of gold, silver and carbon were tested and compared for the determination of lead and cadmium in seawater (Bi et al., 2013). That work confirmed that, like macro disk-electrodes, the bare microwire electrodes were not suitable due to peak overlap, but that mercury-coated electrodes gave good separation of the Cd and Pb peaks. The silver-amalgam microwire (SAM) electrode had the best peak resolution between Cd, Pb and zinc (Zn), and is here optimised for the determination of trace levels of Pb and Cd in uncontaminated seawater. In combination with high-frequency square-wave voltammetry this electrode could be used to determine Pb in-situ without oxygen interference, whilst with deaeration trace Pb is detected in uncontaminated ocean water.

4.2. Experimental

4.2.1. Chemicals and Reagents

Milli-Q water (Millipore, UK) of $18 \text{ M}\Omega\cdot\text{cm}^{-1}$ resistivity was used to prepare reagents and for dilutions. Pb and Cd metal stock solutions were prepared by dilution of atomic absorption standard solutions (BDH, UK) and acidified to pH 2 with HNO_3 . Mercury (II) nitrate 1-hydrate ($\text{Hg}(\text{NO}_3)_2\cdot\text{H}_2\text{O}$), sodium chloride (NaCl), sodium acetate 3-hydrate ($\text{CH}_3\text{COONa}\cdot 3\text{H}_2\text{O}$) were Analar grade from BDH (UK). The mercury plating solution contained 2 mM Hg and was acidified with 10 mM HNO_3 . Hydrochloric and acetic acid were purified by triple sub-boiling distillation on a quartz condenser. Sodium hydrogen carbonate (NaHCO_3) and boric acid were from BDH (Aristar). Sodium dodecyl sulphate (SDS), ethylenediaminetetraacetic acid (EDTA), and Triton-X-100 were from BDH (UK).

Acetate buffer solution (4 M, pH 4.5) was prepared from 2 M acetic acid and 2 M sodium acetate 3-hydrate. The pH of water samples was buffered at pH 4.5 by addition of 100 μL acetate buffer into 10 mL seawater. Ammonia was added to partially neutralise the pH of acidified samples in addition to the acetate buffer addition.

Seawater samples were collected during a survey of the Irish Sea with the RV Prince

Madog (July 2009) using a rosette-mounted, Niskin bottle (General Oceanics, 5 L), modified to obtain samples uncontaminated with metals (Teflon coated spring on the outside, no rubber inside). The samples were filtered (0.45 + 0.2 μm Sartobran 300 cartridge) into 500 mL acid washed Nalgene low-density polyethylene bottles, and acidified to pH 1.9 by addition of 10 μL HCl /10 mL seawater. Bottom (2 m above sediment) and surface (3 m below surface) were collected.

4.2.2. Apparatus

Electrodes were held in a 663VA electrode stand (Metrohm, Switzerland), connected via an IME 663 interface to a PGSTAT101 potentiostat (Metrohm) which was controlled by a PC using NOVA 1.6 software running on Windows 7 (32 bit). GPES software was used instead of NOVA (which is limited to ≤ 300 Hz) for experiments in which the square-wave frequency was varied up to 2000 Hz. The counter electrode (CE) was an iridium wire (2 cm length, 0.15 mm diameter) and the reference electrode (RE) was double-junction Ag/AgCl/KCl (3 M)//KCl (3 M). The working electrode (WE) was a 12.5 μm silver microwire (Goodfellow, UK) which was fitted in a polypropylene pipette tip as described before for a bismuth WE (Bi et al., 2013), using a method modified from that used before (Billon and van den Berg, 2004; Nyholm and Wikmark, 1992; Salaün and van den Berg, 2006). Briefly: the silver microwire was drawn into a 100 μl polypropylene pipette tip, heat-sealed, and fitted to a vibrator (Chapman and Van Den Berg, 2007) (JinLong Machinery). The vibrator was run at 1.5 V, which gave a frequency of approximately 200 Hz, and was controlled by the stirrer on/off control of the software.

The seawater samples were UV-digested (45 min) where indicated, using a 125 W high-pressure mercury vapour lamp (built at Liverpool University and available for purchase) in quartz tubes of 30 mL. The pH was measured using a Metrohm 605 pH-Meter. Solutions were purged using nitrogen gas when necessary.

4.2.3. Electrode preparation

The silver microwire was amalgamated with Hg prior to use by plating at -0.4 V (600 s) from a pre-purged, N_2 -blanketed, and unstirred solution of 2 mM Hg. The electrode was periodically re-activated by application of a negative potential (-3 V) for 2 s when the sensitivity was less than expected; hydrogen generation was then found to clean the

electrode surface. The electrode was stored in MQ, and overnight storage of a freshly made electrode was found to maintain the electrode stability. The Hg coating was renewed when this re-activation was unsuccessful, or when an interfering oxidation peak at -0.25 V began to build up. In-situ deposition was used to stabilize the response at low concentrations of Pb (< 0.1 nM) and Cd in acidified seawater by addition of a low concentration (10 μ M) of Hg, which was co-deposited with the Pb and Cd, to enable deposition times > 5 min.

4.2.4. *Optimized ASV procedure to determine Pb and Cd in seawater*

The pH was buffered to pH 4.5 with 40-mM acetate buffer in preliminary, optimisation, experiments, whilst analysis of acidified samples was carried out at pH 2. In most experiment, the acidified seawater was UV-digested (to make UV-SW) to determine total dissolved Pb and Cd. 10 mL UV-SW was normally used for the analysis. The water was purged with N₂ (5 min) to remove dissolved oxygen (DO), prior to the deposition step. The square-wave mode was used for the ASV scan at a 50-Hz frequency, 5-mV step and 25-mV wave-amplitude. A conditioning potential of -0.2 V was applied for 20 s before the deposition step. The deposition potential was -1.2 V at pH > 4.5, and -1.0 V for acidified samples. The deposition time was between 1 and 5 min, whilst the electrode was vibrated. A plating time of 5 - 10 min was used to determine very low Pb levels of \sim 10 pM occurring in some ocean samples. A second cleaning step at -0.1 V (10 s) was used to remove plated Cu when the deposition time was > 5 min.

The N₂-purge was continued for the first 3 min of the plating step (at plating times of \geq 5 min) to ensure that the water remained DO-free. At the end of the each plating step, a quiescence time of 3 s was used at -0.8 V (vibration off), which was followed by the scan from -0.8 to -0.2 V. Each scan was followed by a background scan, preceded by 20 s conditioning at -0.2 V to remove previously plated metal, and the plating time was reduced to 1 s. The background scan was subtracted from the analytical scan to obtain the background-corrected scan, which was used for quantification of the Cd and Pb peaks.

A standby potential of -0.35 V was applied between measurements to prevent oxidation of Hg, which was found to improve the electrode stability. The ASV sensitivity was calibrated by standard additions of Pb and Cd (typically 2) with a minimum of 3 measurements at each metal concentration. Concentrations and associated standard deviation were calculated using the LINEST function in Excel using all the measurements.

4.3. Results and discussion

ASV of Pb and Cd in seawater using the silver amalgam microwire (SAM) electrode gives good separation (~ 0.2 V) between the peak potentials of Pb (-0.46 V) and Cd (-0.68 V) (Bi et al., 2013). This is similar to that on the mercury drop electrode (Florence, 1970) and makes the SAM electrode potentially a good alternative to liquid mercury electrodes. Problems that were identified during these scans were related to instability of the response when measurements were repeated, and of instability of the electrode between sample changes and when stored overnight. Also, depending on the thickness of the coating with mercury, the silver microwire was found to occasionally become brittle and break suddenly, whereas the bare wire was stable. A series of experiments were carried out to optimise the voltammetric conditions and the electrode was subsequently tested on reference seawater and uncontaminated ocean samples.

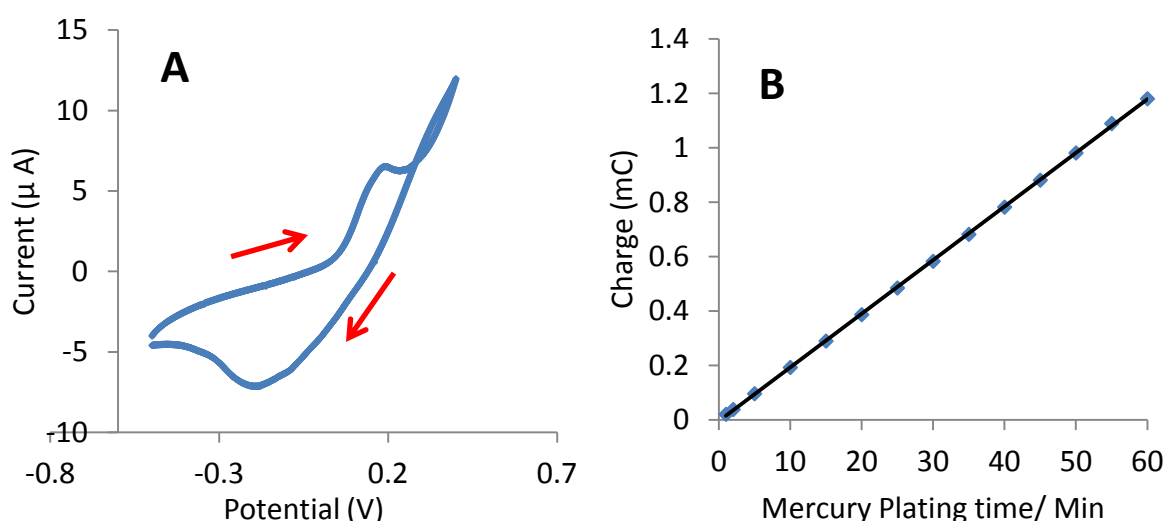


Figure 4.1. Mercury coating of the silver microwire electrode. The solution contained 1 mM Hg^{II} and 0.01 M HNO_3 . A) Cyclic voltammogram showing the oxidation wave for Hg at 0.2 V followed by the oxidation of the silver wire at more positive potential. B) Amperometric charge associated with the deposition of Hg at -0.4 V as a function of the Hg-plating time.

In preliminary experiments, the Hg coating of the silver microwire was optimised by varying the Hg concentration in the plating solution and the plating time. Cyclic voltammetry using the silver wire in a 2 mM Hg solution in 0.01 M HNO_3 showed two peaks: an oxidation peak at 0.2 V for the oxidation of Hg to Hg^{II} , which was followed by a further increase in the current at potential > 0.3 V due to the oxidation of silver (Ag). The returning scan showed a broad reduction wave with slight peaks at -0.1 and -0.2 V, respectively due to the reduction of silver and of mercury (Figure 4.1A). Similar peaks have been seen before (Stojek and

Kublik, 1975): the diffusion of Ag into Hg is kinetically slower than that of Hg into Ag, which causes a gradual ageing of the amalgam electrode. This is the cause of gradually shifting of these waves, and was found to cause interference with the Pb determination when an aged electrode was used repeatedly with a long deposition time.

The current of the negative-going scan returned to that of the forward scan near -0.4 V. This potential was selected as plating potential for Hg, as at this point the plating current should be maximal and without interference due to hydrogen generation.

4.3.1. *Effect of varying the thickness of the mercury layer*

The mercury coating on the silver wire was varied by using plating solutions between 1 and 5 mM Hg (in 0.01 M HNO₃) at varying plating times. The amount of Hg plated was measured by amperometry using the accumulative current during the plating step. The charge associated with Hg plating was found to be directly proportional to the electrolysis time of Hg up to 1 h electrolysis (for a plating solution containing 1 mM Hg and from unstirred solution) with a deposited charge of 327 nC s⁻¹ (r² = 0.999) (Figure 4.1B). This is in contrast with mercury deposition at Ir microelectrodes where the flux of Hg²⁺ was decreasing with increasing length of electrolysis, due to expansion of the microsphere and the resulting expansion of the diffusion layer (Belmont et al., 1996). The deposition charge was used to estimate whether the electrode diameter was changed significantly by the mercury deposition. The electrode length (2 mm) was estimated using a calliper. The volume of the deposited mercury (V_{Hg}) after 1 h electrolysis was calculated by:

$$V_{\text{Hg}} = Q M (n F \rho)^{-1} \quad (1)$$

where Q is the charge (C), F is the Faraday constant (96,500 C mol⁻¹), M is the atomic weight of mercury, ρ is the density (13.55 g cm⁻³) and n is the number of exchanged electrons (2). After 1 h electrolysis, the volume of Hg was estimated at 91,700 μm³ corresponding to an increase in diameter from 12.5 μm to 14.7 μm. This increase, which occurs over the course of the electrolysis time (1 h), does not affect significantly the diffusion layer size resulting in an apparent linear increase of the charge against electrolysis time. Although the diffusion layer size is predicted to continuously expand in quiescent solution, the experimental current reached a steady state due to thermal convection (Salaün and van den Berg, 2006). Assuming a constant mean diffusion layer size over the course of the electrolysis, its value was estimated using Equation (2):

$$\delta = L n F \Phi \pi D C t / Q \quad (2)$$

where δ is the diffusion coefficient of Hg^{2+} ($1.06 \cdot 10^{-5} \text{ cm}^2 \cdot \text{s}^{-1}$), C is the concentration (5 mM) and t is the electrolysis time. The diffusion layer size was estimated at 26 μm which is in reasonable agreement with previously data obtained at gold electrodes for 5 and 25 μm electrodes ($\delta = 10$ and 32 μm respectively) (Salaün and van den Berg, 2006). The Ag electrode still behaved as a Hg electrode even after re-oxidation of the deposited Hg, suggesting that some of the Hg remained in amalgamated form. However, this residual Hg was minor compared to the plated Hg, and the diameter of the Hg-Ag electrode is expected to be similar to that of the bare Ag electrode (12.5 μm).

Upon prolonged use, sensitivities for Cd and Pb were found to slowly decrease with concomitant cathodic shifting of the peak potentials. After replating Hg for 60 s, sensitivities and peak potentials were back to their original values suggesting that Hg-loss was somehow still occurring, possibly during change of solutions when no potential is applied to the Hg film (in a similar way to the C electrode but to a smaller extent (Bi et al., 2013)). Typically, the same electrode could be used for one month with periodic (once every 2 - 5 days) Hg replating.

The optimal mercury thickness for the determination of Pb and Cd in seawater was evaluated from scans for Pb and Cd in seawater. At Hg plating times > 300 s from 5 mM Hg the baseline improved due to suppression of the hydrogen wave which caused the baseline to flatten towards more negative potentials. The peak height for Cd and Pb improved slightly at a longer Hg-plating time of 600 s, which was adopted for the preparation of the SAM electrodes.

4.3.2. *Effect of varying the pH*

In preliminary experiments, chemical and electrochemical conditions were varied to achieve optimal sensitivity, minimise interferences, and stabilise the electrode. Sometimes acetate buffer (pH 4 – 5) is used to buffer the pH (Florence, 1970) in weakly acidic condition, but more acidic conditions (pH 2) are also used (de Carvalho et al., 2007) to determine Pb and Cd in seawater by ASV. To evaluate the effect of the pH, the pH was varied by additions of HCl in seawater buffered by addition of 10 mM bicarbonate, 10 mM ammonium acetate and 10 mM boric acid to UV-SW. NaOH was added to set the initial pH to 8.2, and subsequently increments of HCl were added to lower the pH to 2.2 whilst monitoring the pH

and the ASV response for 2 nM Pb and 10 nM Cd at each point. The response for Cd and Pb was found to increase by 50 and 90 % respectively when the pH was lowered from 8.2 to 2.2 (Figure 4.2A). Although the sensitivity was generally greater at lower pH, that for Pb was especially increased when the pH was lowered from 8 to 7. The sensitivity for Cd was more stable over the varied pH range, apart from slightly higher sensitivity at pH > 8 (only one data point). The α -coefficients for inorganic complexation of Pb and Cd in seawater (calculated using an ion-pairing model with constants adjusted to the ionic strength of seawater (Turner et al., 1981) and with updated carbonate chemistry (Dickson and Millero, 1987)) show negligible variation for Cd, whilst that for Pb decreases relatively strongly between pH 8.5 and 7.5 when it stabilizes at pH 7.5 (Figure 4.2B). In pH 8.2 UV-SW, the inorganic speciation of Pb is dominated by carbonate species and acidification causes this to shift to chloride species. Cd predominately occurs as chloride species in the entire pH range, but the decrease in sensitivity at pH > 8 may suggest that there is more complexation with carbonate ions than suggested by the stability constants used in this model. The speciation of the model agrees at pH 8 well with that reviewed recently for IUPAC (Powell et al., 2009). The relatively large change in sensitivity for Pb between pH 8.2 and 7.5 suggests that the carbonate species are electrochemically less reversible than the chloride species. The minor increase in the sensitivity for Pb at lower pH values can perhaps be ascribed to other effects such as hydrogen evolution changes in the charge on the electrode, or changes in anion adsorption. Although Hg has a high hydrogen overpotential, the presence of even small amounts of H^0 at the surface at -1.2 V could act as chemical reducing agent and possibly catalyse the oxidation process of Pb by reducing the freshly oxidised Pb^{2+} back to Pb^0 (Munteanu et al., 2009). Optimum sensitivity was obtained for both metals at low pH.

4.3.3. Influence of varying the deposition potential (E_{dep}) and deposition time t_{dep}

The E_{dep} was varied from -0.4 to -1.6 V with a 100 mV resolution in UVSW of pH 4.5, spiked with 1 nM Pb and 5 nM Cd. Greatest sensitivity was obtained for both metals at $E_{dep} < -1.2$ V (Figure 4.2C). The shape of the curve suggests a half-wave potential of -0.8 V for Pb and of -1.1 V for Cd. These half-wave potentials are much more negative than the peak potentials (at -0.5 and -0.7 V) suggesting that the strong increase is due to changes in anion adsorption, interaction with the silver of the SAM electrode, or hydrogen development at the most negative potentials.

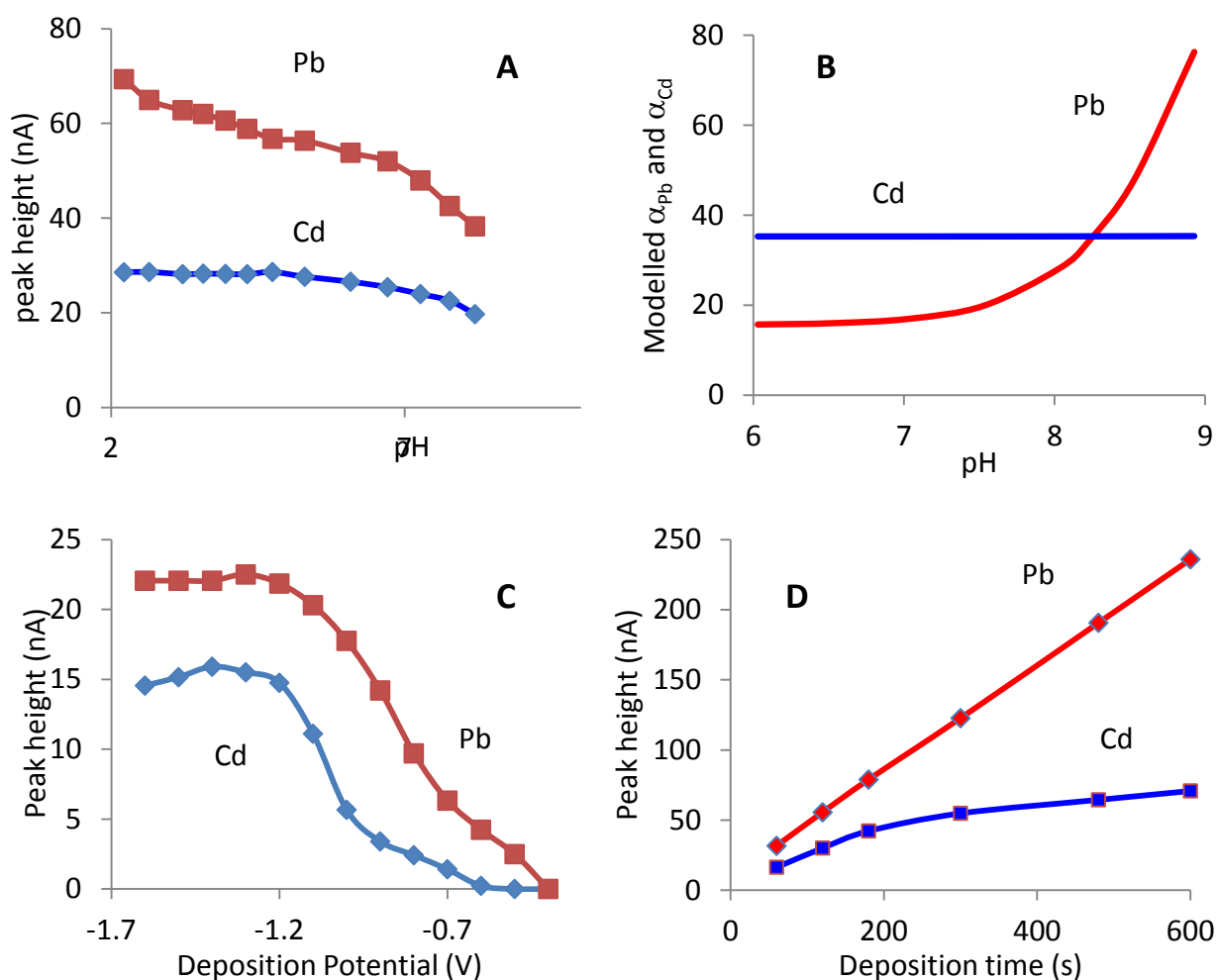


Figure 4.2. Optimisation of the voltammetric parameters for 1 nM Pb and 5 nM Cd in UV-digested seawater. The scanning mode was SWASV 50 Hz, 5 mV step height, 25 mV pulse amplitude, $t_{dep} = 1$ min, $E_{dep} = -1.2$ V. A) Effect of varying the pH on the SWASV peak heights ($E_{dep} = -1.2$ V for 60 s) in purged UVSW. The pH range was buffered with 10 mM NaHCO_3 , 10 mM $\text{CH}_3\text{COONH}_4$ and 10 mM H_3BO_3 . The pH was varied with additions of HCl starting from pH 8.4; B) calculated values of the α -coefficients of Pb and Cd as a function of the seawater pH; C) Effect of varying the deposition potential in UVSW of pH 4.5; D) Effect of varying the deposition time at pH 4.5.

Variation of the deposition time at a fixed deposition potential of -1.2 V showed a linear increase for 1 nM Pb up to at least 10 min, whereas the Cd (5 nM) increase was less and non-linear (Figure 4.2D). Both Pb and Cd are highly soluble in Hg (for instance the solubility of Pb in mercury is 1.5 % (Thompson, 1935)) and therefore a linear increase was expected for both metals. The non-linear increase for Cd suggests an interaction with the Ag of the electrode. This indicates that the amount of free Hg on the SAM electrode surface played an important role for metal determinations.

4.3.4. Interferences

Potential interferences from metals (Cu, Zn and Mn), surfactants (SDS, Triton X100) and EDTA were assessed in pH 4.5 UV SW spiked with 1 nM Pb and 5 nM Cd. Cu, Zn and Mn were added up to a concentration of 100 nM and did not affect the Pb and Cd peaks ($E_{\text{dep}} = -1.2$ V for 60 s). Mn is only deposited at very low deposition potentials on Hg ($E_{\text{dep}} < -1.3$ V) and is therefore not reduced in these conditions. Zn is not known to form intermetallic compounds with either Pb or Cd and was not expected for interfere. Cu could possibly interfere if accumulated continuously. The use of a cleaning step between scans at a potential of -0.1 V ensured that Cu was removed between scans with minimal oxidation of the Hg film.

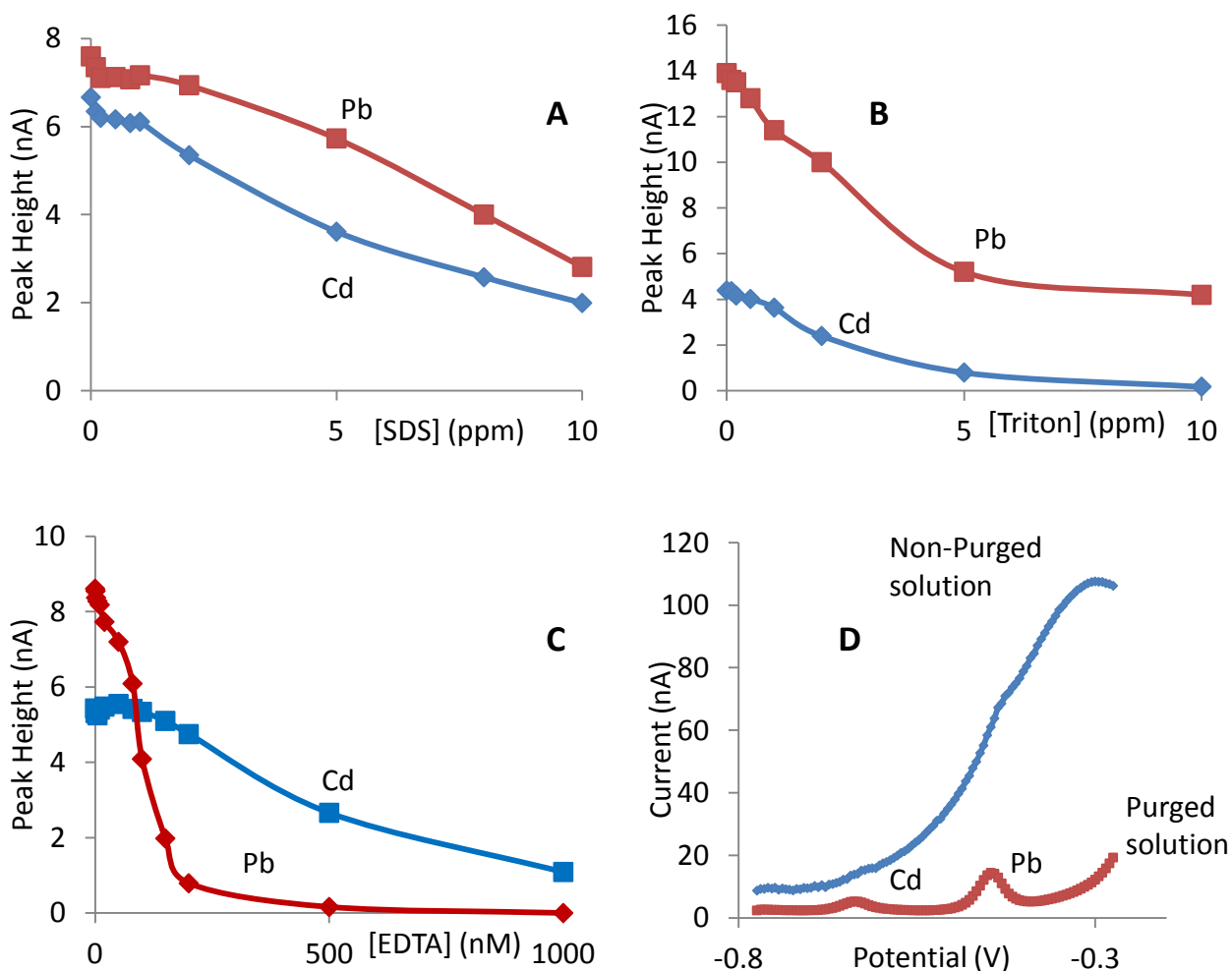


Figure 4.3. Effects of interference by the anionic surfactant SDS (A), Triton X100 (B), EDTA (C), and dissolved oxygen (D) on SWASV (50 Hz) peak heights of 1 nM Pb and 5 nM Cd in UV-seawater. A, B and C: $E_{\text{dep}} = -1.2$ V for 60 s in pH 8 seawater; D: acidified (pH 2) seawater, 0.2 nM Pb and 0.4 nM Cd, 3 min plating at -1 V, before and after purging to remove oxygen.

Variation of the concentration of chloride, and the ionic strength, by addition of 4 M

NaCl up to a final concentration of 0.7 M in 40 mM acetate buffer (pH 4.5) caused the peak for 1 nM Pb to increase at NaCl > 0.1 M: the Pb peak increased by 18 % from 0.1 M to 0.7 M NaCl (metal contamination from reagent were subtracted). This increase can be ascribed to a change in the Pb speciation from hydroxide to chloride species. The peak height for Cd did not vary significantly over the same range of chloride concentrations, which caused the chloride complexation of Cd to strengthen (the alpha-coefficient for Cd, α_{Cd} , went up from 4 to 35) but the Cd was not complexed by OH⁻ at this pH so the speciation change was minor.

SDS and Triton-X100, used as model species for anionic and un-charged surfactants respectively, were added up to 10 ppm. The peaks for Pb and Cd decreased with the surfactant additions (Figure 4.3), Triton causing a stronger effect than SDS at similar concentrations. Natural waters are known to contain concentrations between 0.1 and 0.5 ppm of non-ionic surfactants (Cosovic and Vojvodic, 1982) so the interference of surfactants should be minor for the conditions used here (60 s adsorption time for low nM levels of Cd and Pb) but could be significant for lower metal levels when longer plating times are used.

4.3.5. Influence of oxygen

Dissolved oxygen (DO) occurs at a concentration of ~0.2 mM (Millero et al., 2002) in air-equilibrated seawater of room temperature, and its reduction current on the Hg electrode tends to mask oxidation or reduction peaks for substances at much lower concentration (Figure 4.3D). For this reason it is necessary to purge the water with N₂ to remove the DO prior to voltammetric analysis. However, the electrochemical reduction of DO is electrochemically relatively irreversible compared to the redox couple of Pb⁰ / Pb^{II} at the mercury electrode, which means that high-speed techniques can be used to discriminate against DO (Wojciechowski et al., 1985). Square-wave ASV at high frequency was used to determine Pb in seawater without using deaeration. When the frequency was raised stepwise to 2000 Hz, the peak heights for Pb and Cd increased linearly with the frequency (Figure 4.4A). The linear increase with the square-wave frequency is characteristic for a thin mercury-film thickness (Kounaves et al., 1987). Although the Hg-Ag electrode is a wire, and thus does not benefit from truly spherical diffusion, its relatively low diameter (12.5 μm) ensures a cylindrical diffusion, which acts in a similar way. The linearity is aided by the thinness of the mercury film, which means that diffusion into the electrode is minimal.

The oxidation current for H₂O₂ (produced during reduction of DO) is increased much less (because of poor electrochemical reversibility) than the oxidation current for Pb, which causes the good discrimination. Secondly, at the end of the deposition step, oxygen is depleted at the electrode surface. By using a high stripping frequency, the scan is carried out before a significant amount of DO has reached the electrode (Wojciechowski et al., 1985).

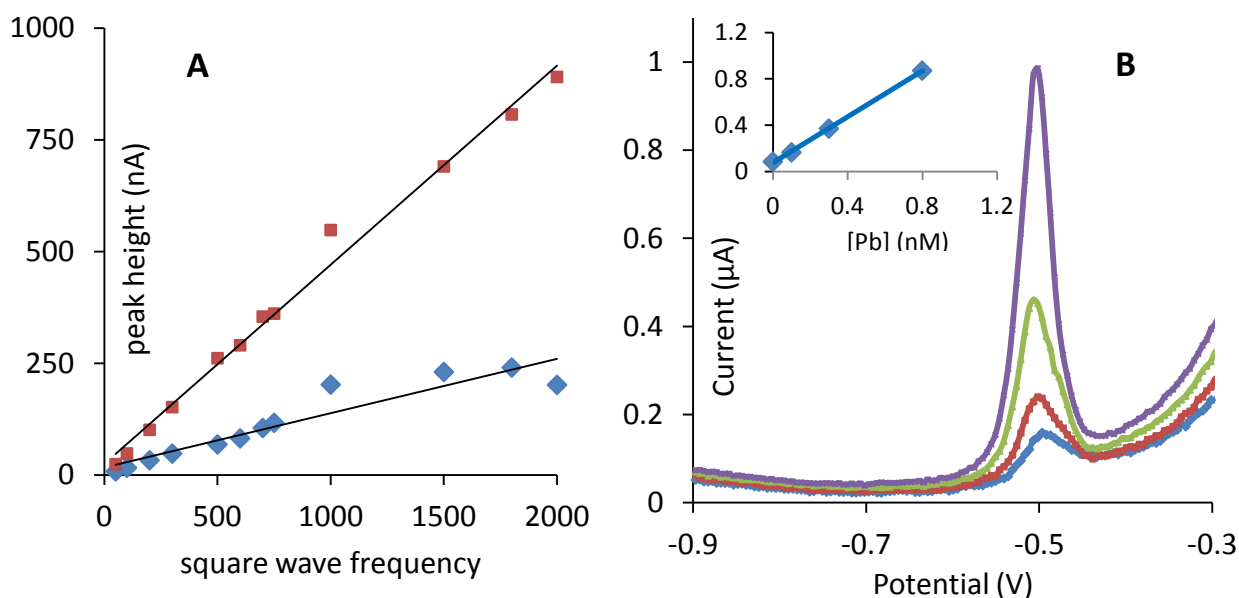


Figure 4.4. SWASV of Pb in pH4.5 seawater in the presence of air. A) Effect of varying the square-wave frequency; B) Voltammogram for 0.18 nM Pb and standard additions of 0.1, 0.3 and 0.8 nM Pb. Deposition time 10 min at -1.2 V, pH 8.2, scans at 700 Hz. Inset: linear response for Pb.

The scans became relatively noisy at square-wave frequencies > 700 Hz (scan rate of 3.5 V s⁻¹). 700 Hz was selected to determine Pb and Cd in seawater in the presence of DO in air equilibrated water. At 700 Hz the sensitivity for Pb was 11 x greater than that for Cd, whilst at 50 Hz, in deoxygenated seawater, the sensitivity for Pb was 16 x greater than that for Cd. The sensitivity for Pb at 700 Hz was sufficient to determine low Pb concentrations in seawater, but it was not possible to determine trace Cd due to the lower sensitivity. Scans for trace levels of Pb (0.18 nM Pb) at 700 Hz without deaeration in UV-SW (Figure 4.2B) show that trace Pb can be determined without deaeration and without the need for any reagents. The Pb concentration in this sample was 0.18 ± 0.03 nM, in agreement with that found after deaeration (0.20 ± 0.02), thus validating the high-frequency method.

The limit of detection for Pb, using the high-frequency method, in UV-SW from Liverpool Bay at natural pH (8.2) containing 0.26 nM Pb was found to be 12 pM Pb (n = 10)

using a deposition potential of -1.2 V for 10 min (relative standard deviation 2.3% for 0.26 nM Pb) (Table 4.1).

Table 4.1. Methods developed for sub-nM determination of Pb in seawater. GC-RDE = glassy carbon rotating disk electrode; VBiE = vibrating Bi electrode; HSCSV = high speed CSV; GIME = gel integrated microelectrode; SAM = vibrated silver amalgam electrode (this work, **).

WE	Technique	Method	O ₂	UV	pH	t _{dep} (s)	E _{dep} (V)	LoD Cd (pM)	LoD Pb (pM)	Ref
Hg-GC RDE	DPASV	Ex-situ	No	No	2	900	-0.85	1*	5*	(Bruland et al., 1985)
Hg-GC	DPASV	In-situ	No	No	1.2	60	-0.9	383	290	(de Carvalho et al., 2007)
Hg-GC RDE	DPASV	In-situ	No	Yes	5.6	300	-1.5	5	8	(Fischer and van den Berg, 1999)
Hg-GC RDE	SCP	In-situ	No	No	3	900	-1.1	9*	14*	(Riso et al., 1997)
HMDE	DPCSV	n.a.	No	Yes	8	600	-1.1	12	30	(van den Berg, 1986)
HMDE	SWASV	n.a.	No	Yes	8	60	-1.2	100	200	(Colombo and van den Berg, 1997)
HMDE	HSCSV	n.a.	No	No	7.6	180	-0.3 to -2	27	39	(ColladoSanchez et al., 1996)
HMDE	HSCSV	n.a.	Yes	No	7.6	180	-0.3 to -2	36	48	(ColladoSanchez et al., 1996)
HMDE	CSV	n.a.	No	Yes	5	180	-1.2	n.d.	29	(Wu and Batley, 1995)
HMDE	DPASV	n.a.	Yes	No	1	3,600	-1.2	89	48	(Gillain et al., 1979)
Bi-Ir wire	SWASV	Ex-situ	yes	No	4.5	300	-1		5000	(Kyrissoglou et al., 2012)
VBiE	SWASV	n.a.	Yes	Yes	8.2	120	-1.2	5,000	500	(Bi et al., 2010)
GIME	SWASV	Ex-situ	No	No	2	900	-1.2	50	50	(Tercier-Waebler et al., 1998)
SAM	SWASV	Ex-situ	No	Yes	8	60	-1.2	79	8	**
SAM	SWASV	Ex-situ	Yes	Yes	8	600	-1.2	n.d.	12	**
SAM	SWASV	In-situ	No	Yes	2	300	-1.2	n.d	4	**

4.3.6. Limits of detection and electrode stability using ex-situ and in-situ prepared electrodes

The limit of detection using the pre-plated (ex-situ) vibrated SAM electrode was 8 pM Pb (n = 10) at a 1 min deposition time using a 50Hz square-wave frequency, in UV digested and de-oxygenated, seawater at pH 4.5 containing 0.09 nM Pb. This is much lower than obtained recently (~5 nM Pb at the same pH but using a longer, 5 min, plating time) using a

Bi-film coated wire electrode (Kyrissoglou et al., 2012); that wire was iridium and not vibrated, but the plating time was 5x longer, and the surface coated with Bi, indicating that for Pb detection the Hg-coating is superior.

It was attempted to lower this detection limit further to facilitate determination of open ocean samples where Pb concentrations can be in the low picomolar range (Wu and Boyle, 1997a). As this limit was obtained using just 60 s of plating, it was first attempted to reach lower values by extending the plating time to 5 and 10 min. Repeated measurement of Pb in acidified (pH 2) seawater using the longer plating times, showed that the response tended to decrease after several hours of use, and an oxidation peak appeared more positive than that of Pb, at ~ -0.2 V, which gradually shifted to ~ -0.3 V and then affected the shape of the Pb peak. This peak was not due to Cu (at -0.15 V), and it was found that the peak was removed if the electrode was re-plated with Hg. It is likely that this peak was due to a slow diffusion of Ag into the Hg coating (Stojek and Kublik, 1975). Because of this interference it was not convenient to use a longer plating time than 1 or 2 min for repeated sample analysis using the pre-plated electrode. In this condition the electrode can be used for several days without the development of this interference.

In-situ deposition of Hg on glassy carbon (GC) electrodes has been used for a long time to detect Pb and Cd (Florence, 1970). Here the Hg is plated during the deposition step of the metals, and is removed by oxidation at the end of each scan. An advantage of this method is that the mercury layer is very thin, causing a high concentration factor for plated metals, and a clean film is produced for each scan. This is best done in the presence of thiocyanate as then the Hg is completely removed (Fischer and van den Berg, 1999). On the SAM electrode the Hg is only partially removed due to diffusion of the Hg into the silver, and the thiocyanate was not found to improve electrode reproducibility or sensitivity. A gradual removal of Hg from the electrode in the presence of thiocyanate was found to lead to increased fragility of the electrode.

For concern of electrode safety, it was attempted to increase the electrode stability for longer plating periods by renewing the mercury deposit. Repeated measurements showed that baseline stability was improved by measuring in the presence of a low concentration of Hg ($10 \mu\text{M}$ Hg) which was added to the acidified seawater, thus continuously topping up the Hg coating during measurements. In this condition the plating time could be extended

without deterioration of the electrode, and the limit of detection was found to be 4 pM Pb using a plating time of 5 min.

The limit of detection obtained using the vibrating SAM electrode can be compared to that obtained using other electrodes (mercury drop and mercury coated, Bi coated, by ASV and cathodic stripping voltammetry (CSV)) in Table 4.1 (the compared methods were selected because they have a quoted, subnanomolar limit of detection of Pb). For the purpose of speciation it is best to work at natural pH and without Hg addition. In this case the limit of detection is 8 pM using just 1 min deposition using the vibrated SAM electrode. In the presence of air at a high square-wave frequency of 700 Hz, the limit of detection was found to be higher (at 12 pM) even using a longer (10 min) plating time. The lowest limit of detection (4 pM Pb) was obtained at pH 2 using the in-situ topping-up method (from 10 μ M Hg in seawater) with a 5-min plating time. This LoD was confirmed by measuring the Pb in 10mM HCl in which a small Pb peak was found for 5pM Pb (can is not shown). These limits of detection for Pb using the vibrated SAM electrode are lower than using other electrodes with the added advantage of the simplicity, robustness and low cost of the SAM electrode, which does not require frequent polishing like the glassy carbon electrodes. The detection limits of Cd were typically 10x higher than of Pb, due to the lower sensitivity for Cd.

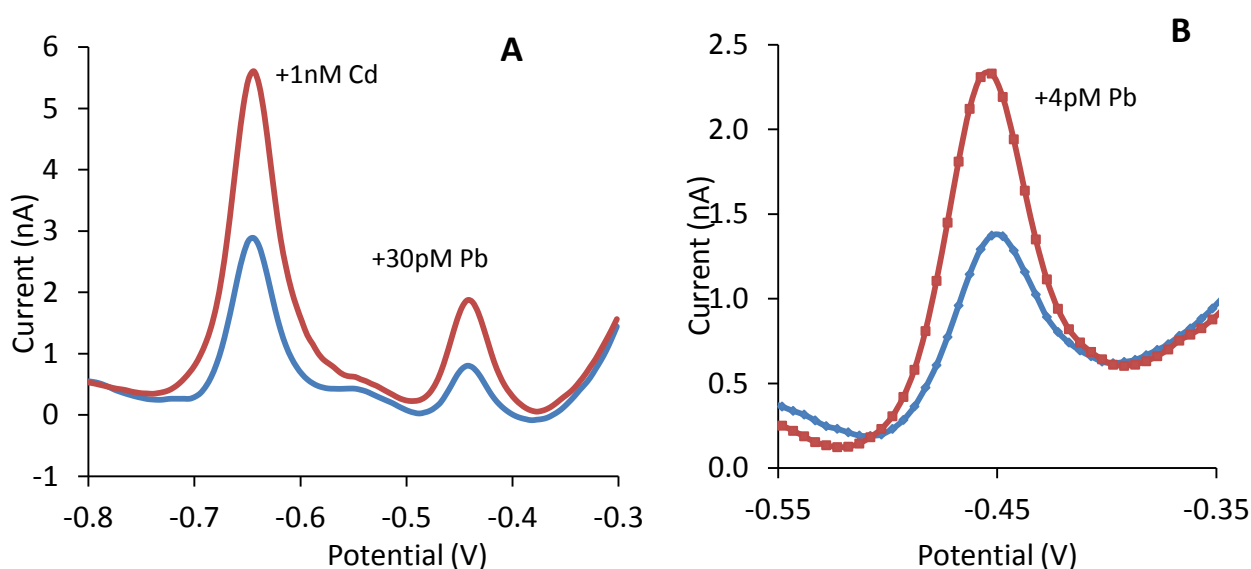


Figure 4.5. ASV scans for Pb and Cd in reference seawater. A) SAFe D2 reference seawater; pH 1.7; 5 min deposition at -1 V with in-situ Hg plating; the second scan after standard addition of 30 pM Pb and 1 nM Cd. B) The Pb peak in Atlantic GD water; 5 min plating using a pre-Hg-plated SAM electrode; the second scan after addition of 40 pM Pb.

4.3.7. *Application to the determination of Pb and Cd in reference seawater, and in estuarine and open ocean samples*

The vibrating SAM electrode was used to measure Pb in reference seawater, in coastal waters collected from Liverpool Bay (Irish Sea) and open ocean samples. The acidified reference samples was analysed at the original pH (pH 1.7), and 10 μM Hg was added to ensure a stable Hg coating. Good agreement was obtained for the reference waters (NASS-6 and SAFe) with overlapping standard deviations with the determined and certified, or consensus, concentrations (Table 4.2). The concentration of Pb obtained for the reference material from the Pacific ocean samples (SAFe D1 and D2) was the same as the consensus values, but for the Atlantic ocean GD water it was about 15% less than that found using ICP-MS (Biller and Bruland, 2012). Scans for the determination of Pb and Cd in the Atlantic water show the peak for Pb at a background concentration of 27 pM and an addition of 30 pM Pb, illustrating the good sensitivity for Pb using the SAM electrode (Figure 4.5). The sensitivity for Cd was much less, but was sufficient to correctly determine its concentration in all reference waters, giving very good agreement (within the standard deviation) with the consensus or certified values (Table 4.2).

Table 4.2. Analyses of lead in reference seawater samples. NASS-6 is available from NRCC, SAFe D1 and D2 was prepared during the SAFe cruise (Johnson et al., 2007), and GD91 and 92 is reference seawater obtained during a GEOTRACES cruise (Boyle et al., 2013). The comparative value for Cd in GD water is from Biller et al (Biller and Bruland, 2012). The Cd concentrations in GD water were calibrated using the calibration for the NASS-6 water.

Reference material	Found Pb (pM)	Reference value Pb (pM)	Found Cd (pM)	Reference value Cd (pM)
NASS-6	30.4 ± 0.6	29.8 ± 10	290 ± 10	277 ± 17
SAFe D1	27.2 ± 0.3	Pb: 26.0 ± 1.5	1000 ± 20	999 ± 27
SAFe D2	27.3 ± 0.4	Pb: 27.6 ± 1.9	990 ± 10	986 ± 42
GD 91 (Atlantic)	34 ± 3	43.9 ± 0.6	252 ± 20^a	276.2 ± 0.2
GD 92 (Atlantic)	33 ± 3		246 ± 14^a	

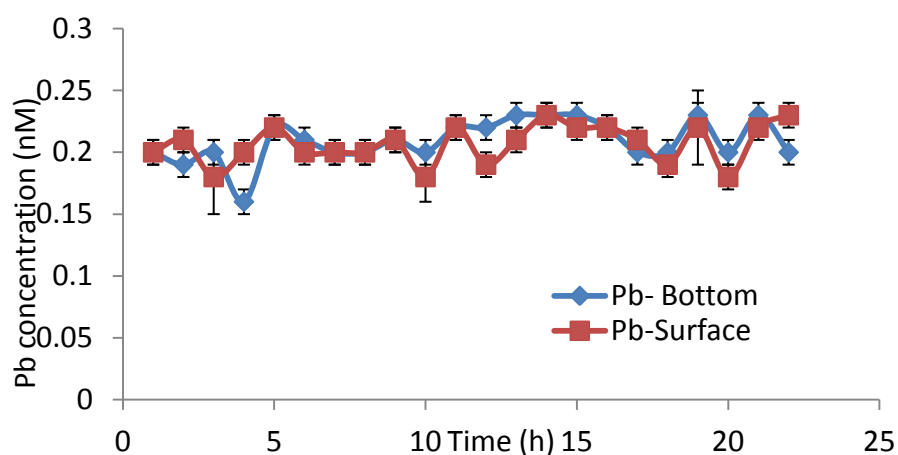


Figure 4.6. Dissolved Pb and Cd in Liverpool Bay measured in samples collected over 24 h at two depths (3 m and 10 m) at station 21. Samples were stored and analysed acidified to pH 2. The Cd concentrations were close to the limit of detection (0.2 to 0.3 nM Cd) which caused them to be noisy.

The vibrating SAM electrode was used to determine the concentration of Pb and Cd in coastal waters collected in Liverpool Bay (Irish Sea), on an hourly basis from bottom and surface waters (Figure 4.6). The samples had been acidified and UV-digested prior to analysis. Pb showed no significant variation with depth or time (salinity varied between 32.7 and 33.2) with an average concentration of 0.18 ± 0.02 . Cd showed variability between 0.14 and 0.56 nM Cd. A plating time of just 1 min had been used, because this was sufficient for Pb, but, in view of the large standard deviation of the Cd results, it is likely that this deposition time was too short and that the Cd data (not shown) were too close to the limit of detection, whereas the Pb concentration was readily determined. Cd could have been determined using a longer deposition time. The measurements in the reference seawater and Liverpool Bay show that the sensitivity for Pb using the SAM electrode is sufficient to determine Pb in uncontaminated ocean samples, whereas the sensitivity for Cd is sufficient for concentrations more typical for coastal waters.

4.4. Conclusion:

The vibrating SAM electrode was successfully used to determine the Pb and Cd concentrations in marine system. This electrode exhibited a high sensitivity and low detection limit: 4pM Pb with 5 minutes deposition time in acidified seawater. No obvious interference from other metals for Pb and Cd analysis and addition of organics were observed to decrease electrode sensitivity. However, this fouling effect could be removed by using a re-activation procedure. The good stability and sensitivity enable SAM be used for Pb speciation analysis in natural waters.

Acknowledgements

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5

Chapter 5

**The speciation of lead in seawater by
pseudopolarography using a vibrating silver
amalgam microwire electrode**

5. The speciation of lead in seawater by pseudopolarography using a vibrating silver amalgam microwire electrode

Abstract

A novel electrode based on a vibrating silver amalgam microwire was used for pseudopolarography of lead in seawater. The potential shift of electrochemically irreversible lead complexes was calibrated against the complex stability of several ligands in pH 8 seawater. The method was compared against the mercury drop electrode at various concentrations of lead and various deposition times, and the response was in general agreement with the theory. The new method enables the speciation of Pb at picomolar level in uncontaminated seawater. The method has been tested on samples from oceanic (Atlantic), coastal (Irish Sea) and estuarine (Mersey) origin at Pb concentrations between 30 pM and 2 nM. The pseudopolarograms were found to resolve the speciation of lead in terms of labile and irreversibly bound Pb species. The lead in the samples was largely in the labile component of the pseudopolarograms, indicating that it was reversibly complexed with values for $\log \alpha'_{\text{Pb}^{2+}\text{L}}$ of 2 for the oceanic sample, and of 4 for the coastal sample. A smaller fraction was resolved as stable, irreversibly bound species with values for the thermodynamic stability constant of $\log K_{\text{Pb}^{2+}\text{L}} = 15 - 16$. The speciation method was able to resolve metal species occurring at concentrations of only a fraction of total concentration of the metal. Unlike copper, where organic complexation is strong and electrochemically irreversible, and able to significantly decrease its bioavailability, the Pb mostly occurs in the labile complexed fraction and is therefore readily available for geochemical scavenging or biological uptake reactions.

5.1. Introduction

Lead occurs at levels of 10-80 pM in ocean waters where it behaves as a scavenged

element, its concentration decreasing as a function of depth (Biller and Bruland, 2012; Lee et al., 2011; Milne et al., 2010). Its concentration is usually determined by inductively-coupled plasma mass-spectrometry preceded by an off-line preconcentration step (Biller and Bruland, 2012; Lee et al., 2011; Milne et al., 2010). ICP-MS is not suitable to determine its chemical speciation (interaction with organic matter) for which voltammetric methods are typically used. The inorganic speciation of lead is dominated by carbonate species (Powell et al., 2009), with an α -coefficient of 28 for its inorganic species in pH8 seawater (calculated using an ion-pairing model with constants adjusted to the salinity of seawater (Turner et al., 1981), updated with recent stability constants for the carbonate system (Dickson and Millero, 1987)).

Several metals of biological importance (copper, iron, zinc, cobalt) are known to be strongly complexed with organic matter in seawater (Ellwood and Van den Berg, 2000; Ellwood et al., 2005; Gledhill and van den Berg, 1994; Moffett, 1995). The organics are important as they affect the biogeochemistry of these elements. Very little is known about the organic complexation of lead. Measurements of the lead complexing capacity using anodic stripping voltammetry (ASV) have indicated that the lead, in Pacific surface waters, is weakly complexed with organic complexing matter at < 0.5 nM and a conditional stability constant ($\log K'_{Pb'L}$) of 9.7 (Capodaglio et al., 1990), which translates to an organic α -coefficient only slightly greater (2x) than that of the inorganic species.

Organic metal complexation in seawater is usually determined by competitive ligand exchange and cathodic stripping voltammetry (CLE-CSV)(e.g. Cu (Campos and van den Berg, 1994)), but despite the suitability of a ligand for lead in freshwater (Yokoi et al., 1995), there is no suitable CSV method for lead in seawater and CLE-CSV cannot be used. Alternatives are complexometric titrations with detection by ASV (Chau, 1973), and pseudopolarography (Shuman and Cromer, 1979), both methods have existed for a long time. Labile complexes dissociate during the deposition step in ASV, which means that they are not part of the complexing capacity unless their diffusion is significantly less than that of the inorganic metal. This means that the previous Pb speciation work may have underestimated the degree of its

complexation.

Pseudopolarography provides a measure of the *thermodynamic* complex stability (independent of pH and ligand concentration), and is suitable when the ratio of the concentration of the ligand over that of the metal is near to unity, as is the case for Pb. Pseudopolarography has previously been used for speciation of zinc (Zn) in seawater (Lewis et al., 1995), Cd (Tsang et al., 2006) and Pb in freshwater model solutions (Lam et al., 1997), and Cu in seawater (Croot et al., 1999; Gibbon-Walsh et al., 2012; Nicolau et al., 2008).

Because of the low concentration of Pb in seawater, it is analytically demanding to detect the labile Pb and pseudopolarography has been rarely attempted (Pizeta et al., 2005). In that study, sedimentary porewaters were analysed and strong Pb binding ligands were found. The findings illustrated that a more sensitive electrode was required for Pb speciation at the low concentrations occurring in seawater. Here a novel, vibrating silver amalgam microwire (SAM) electrode (Bi et al., 2012) was used to carry out pseudopolarography of Pb in seawater. The sensitivity is sufficiently high to enable pseudopolarography of Pb at its natural concentration. The method is operationally calibrated against a chelate scale of known complexing ligands in seawater and tested on seawater original from coastal and oceanic conditions.

5.2. Experimental

5.2.1. Reagents

Milli-Q water (Elix, Millipore UK) used to prepare the reagents was deionised to a resistivity of $18 \text{ M}\Omega\cdot\text{cm}^{-1}$. Pb stock solutions were prepared by dilution of atomic absorption standard solution (BDH, UK) and acidified to pH 2 with HNO_3 . Sodium hydroxide (NaOH, Aristar), and mercury (II) nitrate 1-hydrate ($\text{Hg}(\text{NO}_3)_2\cdot\text{H}_2\text{O}$, AnalarR) were from BDH (UK). The mercury plating solution contained 2 mM Hg (II) and was acidified with 10 mM HNO_3 .

HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, 99%, Acros Organics) buffer contained 1 M HEPES and 0.4 M NaOH, giving a pH of 7.7 in seawater. Solutions of

humic and fulvic acid were prepared from Suwannee River fulvic and humic acid, from the International Humic Substances Society. The model ligands ethylenediaminetetra-acetic acid disodium salt (EDTA, AnalaR, BDH), nitrilotriacetic acid (NTA, > 99%, Sigma-Aldrich), diethylenetriaminepentaacetic acid (DTPA, 98+%, ACROS Organics), L-cysteine (> 99%, Sigma-Aldrich), glutathione (Biochemical, BDH), 8-hydroxyquinoline-5-sulfonic acid (Sigma-Aldrich) were added to UV-digested seawater buffered with HEPES to pH 7.7.

5.2.2. Sample collection and pre-treatment

Seawater used for optimization and lead speciation was collected from the Atlantic, near the shelf of the Celtic Sea (collected during a cruise with the RV Discovery, June 2012, Latitude N: 48.33, Longitude W: 9.43) by pumped collection from an underwater inlet. The water was filtered immediately through a Sartobran cartridge (0.45 μm and 0.2 μm), and stored at room temperature in an acid rinsed 50 L Nalgene container. 1 nM Pb was added for optimisation experiments using the SAM, and 10 nM Pb for comparative experiments using a hanging mercury drop electrode (HMDE). The water was used without Pb addition to determine the pseudopolarogram for Pb in this water.

Seawater from Liverpool Bay was collected during a cruise with the RV Prince Madog (July 2009) using a rosette-mounted, Niskin bottle (General Oceanics, 5 L), modified to obtain samples uncontaminated with metals (Teflon coated spring on the outside, no rubber inside). The samples were filtered and transferred to 500 mL acid washed low-density polyethylene bottles (Nalgene) and stored frozen on board the ship. The salinity of the water was 33.1.

Estuarine water was collected from the Mersey Estuary, bordering Liverpool Bay during a cruise with the research vessel Marisa in summer 2011. The samples were collected using a hand-pump and treated in the same way as the Liverpool Bay samples. The salinity of the water was 27.3.

5.2.3. Apparatus

Electrodes were held in a 663VA electrode stand (Metrohm, Switzerland), connected

via an IME 663 interface to a PGSTAT101 potentiostat (Metrohm) controlled by a PC using NOVA 1.6 software running on Windows 7 (32 bit). The counter electrode (CE) was an iridium wire (2 cm length, 0.5 mm diameter) and the reference electrode (RE) was double-junction Ag/AgCl/KCl (3 M)//KCl (3 M). The working electrode (WE) was a 12.5 μm silver microwire (Goodfellow, UK) which was fitted in a polypropylene pipette tip as described previously (Bi et al., 2012) (Salaün and van den Berg, 2006). Briefly: the silver microwire (Goodfellow, UK) was drawn into a 100 μL polypropylene pipette tip and heat-sealed, with 2 mm protruding. An connecting electrical cable (copper wiring) was inserted in the top, and the tip was friction-fitted to a polypropylene tip (500 μL) with a vibrator (Chapman and Van Den Berg, 2007)(Chapman and Van Den Berg, 2007)(Chapman and Van Den Berg, 2007)(Chapman and Van Den Berg, 2007) (asymmetric rotor, 1.5 V, at ~ 150 Hz, from JinLong Machinery, China).

Seawater used for optimisation was UV-digested (45 min) using a 125W high-pressure mercury vapour lamp (Electroanalytical Accessories, UK) in quartz tubes of 30 mL. The pH was measured using a Metrohm 605 pH-meter calibrated using NBS pH standards. Solutions were purged during voltammetric analysis using water-saturated nitrogen gas.

5.2.4. *Electrode pre-treatment*

The silver micro-wire was conditioned in 0.1 M HNO_3 by application of a negative potential of -2 V for 1 min to produce hydrogen to clean the wire surface. This was followed by cyclic voltammetry (CV) scans between 0 and +0.35 V to oxidise and reduce the surface in an attempt to roughen it slightly. The mercury plating was then conducted at -0.4 V for 10 min in 2 mM Hg(II) solution (pH 2) from unstirred solution to produce the silver amalgam microwire (SAM) (Bi et al., 2012). The SAM was then transferred to Milli-Q water at open circuit for several hours (or overnight) to allow the amalgamation complete.

5.2.5. *Pseudopolarography*

The electrode was activated at -3 V (1 s) prior to each measurement. This short step was found important to maintain a good sensitivity and reproducibility over several hours, and was previously used with a Hg-coated gold microwire electrode (Gun et al., 2006).

Subsequently, the electrode was conditioned by application of a negative (reduction) potential (-1 V, 10 s) followed by a more positive, oxidation, potential (-0.2 V, 30 s) to remove any plated Pb prior to the deposition step. A desorption potential (-1.5V, 1 s) was applied before and after the deposition step to cause any adsorbed organic matter to desorb prior to the deposition step and again prior to the scan. The scans were in the square-wave mode, with a frequency of 50 Hz, a potential step of 5 mV, and 25 mV amplitude.

Each scan was followed by a background scan which was similar to the analytical scan but with the deposition step shortened to 1. The background scan was carried out after a 30s Pb-removal step at -0.2 V followed by a 1s desorption at -1.5 V, and then the deposition step (at the deposition potential of the analytical scan) and another 1s desorption step at -1.5 V. The background scan was subtracted from the analytical scan to obtain a background-corrected scan for each measurement, which was used for data treatment to obtain the peak height. The various step potentials are summarised in Table 5.1.

Table 5.1. Optimized procedure for pseudopolarography of lead in seawater. All steps are included with each scan to ensure a constant electrode surface.

SAM		HMDE	
Analytical Scan		Analytical Scan	
Reactivation	-3 V for 1 s		
Cleaning	-0.2 V for 5 s		
Conditioning	-1 V for 10 s		
Cleaning	-0.2 V for 30 s		
Desorption	-1.5 V for 1 s		
Deposition	E_{dep} for 60 s	Deposition	E_{dep} for 60 s
Desorption	-1.5 V for 1 s	Desorption	-1.5 V for 1 s
Equilibration	-0.8 V for 3 s	Equilibration	-0.8 V for 3 s
Scan	-0.8 V to -0.2 V	Scan	-0.8 V to -0.2 V
Background Scan		Background Scan	
Cleaning	-0.2 V for 30 s		
Desorption	-1.5 V for 1 s		
Deposition	E_{dep} for 1 s	Deposition	E_{dep} for 1 s
Desorption	-1.5 V for 1 s	Desorption	-1.5 V for 1 s
Equilibration	-0.8 V for 3 s	Equilibration	-0.8 V for 3 s
Scan	-0.8 V to -0.2 V	Scan	-0.8 V to -0.2 V

Comparative pseudopolarographic measurements were carried out using an HMDE. The optimised parameters of the square-wave scans were: 10 Hz, 10 mV step and 50 mV amplitude, and application of a desorption potential of -1.5 V (1 s) after the deposition step. The drop size was medium and no background scan was used.

Theory

The half-wave potential ($E'_{1/2}$) of the metal complex is expected to shift into a negative direction compared to that of the free metal ($E_{1/2}$) by an amount that is directly related to the thermodynamic complex stability (Croot et al., 1999):

$$E'_{1/2} = E_{1/2} - (0.059/n) \log K_{\text{PbL}} \quad (1)$$

where n is the number of electrons (2 for the oxidation of Pb to Pb^{II}) and K_{PbL} the thermodynamic stability constant for the complex of Pb^{2+} with ligands L. This equation indicates a linear relationship between the shift in $E_{1/2}$ and the log-value of the stability constant, and that the slope of this relationship is ~ 30 mV / log K unit. In this case the response is Nernstian. Previous work on copper using a mercury drop electrode (Croot et al., 1999) and a gold wire electrode (Gibbon-Walsh et al., 2012) have shown already that linear relationships were obtained, but that the slopes were not Nernstian. There was a large difference in the slope obtained for the two types of electrode. The slope and the shift were re-calibrated in this work for Pb.

The location of the half-wave potential was calculated from the pseudopolarogram using the following modified Lingane equation (Croot et al., 1999):

$$\text{Log} ((i_{\text{max}} - i) / i) = (E_{\text{dep}} - E'_{1/2}) (\alpha n / 0.059) \quad (2)$$

where i_{max} is the maximum current at the top of the wave, and α a transfer coefficient taking into account irreversibility of the complex. The α can be used to explain a non-Nernstian behaviour of the complexes at the electrode.

The $E'_{1/2}$ values were then obtained from plots of $\log ((i_{\max} - i) / i)$ as a function of the deposition potential (E_{dep}) by interpolation of the ratio to $E_{\text{dep}} = 0$.

5.3. Results and discussion

5.3.1. Potential sequence optimisation

The SAM electrode has in previous work been optimised to determine Pb and Cd by deposition at a fixed potential of -1.2 V at pH 8, or at -1 V at pH 2 (Bi et al., 2012). Conditioning steps, apart from a cleaning step at -0.2 V, were then not required as the deposition potential was already sufficiently negative to largely prevent adsorption of organic matter. However, due to varied nature of the deposition potentials used in this work, it was necessary to introduce a conditioning procedure that would return the electrode to reproducible condition prior to each following scan. Pseudopolarography of Cu using a gold electrode introduced a sequence of potential jumps (Gibbon-Walsh et al., 2012) to achieve this and a similar procedure was developed here. The procedure, here optimised for Pb, was tested by measuring the stability of the response for Pb (1 nM) in UV-digested seawater at various deposition potentials. Preliminary experiments, in which the deposition potential was varied, without using electrode conditioning, starting from more positive potential, showed that the measurements were affected by organic adsorption, which lowered the sensitivity and interfered with the pseudopolarography. Additionally, it was found to be necessary to occasionally reactivate the electrode as its sensitivity was found to gradually decrease, which was ascribed to slow changes in the amalgam due to electrode surface fouling as well as the presence of calomel. This was associated with the gradual appearance, and a gradual negative shift, of a wave starting about 0.2 V positive of Pb, with ageing of the electrode. The SAM electrode used here has a very thin mercury layer plated onto a silver wire at -0.4V. A significant change in the properties of this electrode over relatively short time could be explained by dissolution of silver into this very thin mercury layer.

An activation step consisting of briefly (1 s) applying a very negative potential (-3 V) to the electrode was found to restore the electrode response. This activation step was applied prior to each deposition step, to maintain constant sensitivity and a stable electrode surface

for the duration of the pseudopolarograms (typically 2.5 h).

Adsorption of organic matter (Louis et al., 2008) and major ions (Salaün and van den Berg, 2006) on the electrode surface varies with the applied potential (Shi and Lipkowski, 1996), due to the electrical charge on the electrode and on the organic and inorganic ions. Therefore the second aspect of the electrode conditioning involved application (1 s) of a desorption potential between the deposition potential and the scan, in order to minimise these adsorption effects. Variation of this desorption potential between -0.7 and -1.8 V, whilst measuring the response for 1 nM Pb in UV-SW (deposition at -1.8V) showed that the peak height for Pb increased with more negative desorption potential when these 1s pulses were applied. Greatest peak height was obtained with desorption potentials <-1.5 V (Figure 5.1A), and a desorption potential of -1.5 V was selected to minimise the production of hydrogen or other interfering substances that could potentially interfere. The peak height for Pb increased by ~30 % by application of this 1s step, which means that it eliminates an important systematic error in the pseudopolarograms, which might have led to incorrect interpretation of a negative wave to strong organic complexation. This desorption step is also required to improve the determination of Cu complexing parameters using the HMDE (Louis et al., 2008).

To minimise differences in the electrode behaviour due to deposition at different potentials, two further potential steps were introduced after the -3V activation step to cover positive and negative potentials: a cleaning step: -0.2 V (5 s, vibration on) and a plating step at -1 V (10 s), followed by a second cleaning step at -0.2 V (30 s). Then one desorption step (optimised to -1.5 V, 1 s). This was followed by the plating step in which the deposition potential was varied sequentially from -0.5 to -1.5 V. All these potential steps were with vibration on. The deposition step was followed by the desorption step (with vibration on) and this by a quiescence step (3 s, vibration off) at -0.8 V, prior to the scan which always started from -0.8 V and finished at -0.2 V. The sequence of potentials is summarised in Table 5.1.

A similar potential sequence was considered for the HMDE which was used in

comparative experiments: because a new electrode surface is produced by this electrode, this electrode required only the desorption step at -1.5 V between the deposition step and the scan. The solution was stirred during the deposition step and the quiescence time was 3 s.

The voltammetric scans were found to show significant curvature due to the low concentration of Pb in seawater. This curvature is due to variation in the electrode capacitance, which is minimal at the zero point of charge of the electrode near -0.5 V, near to the peak potential for Pb, and due to proximity of the mercury oxidation at more positive potentials. For this reason all scans were corrected by subtraction of a background scan, in which the deposition time was lowered to 1 s. The peak height for Pb was measured using a background-corrected scan.

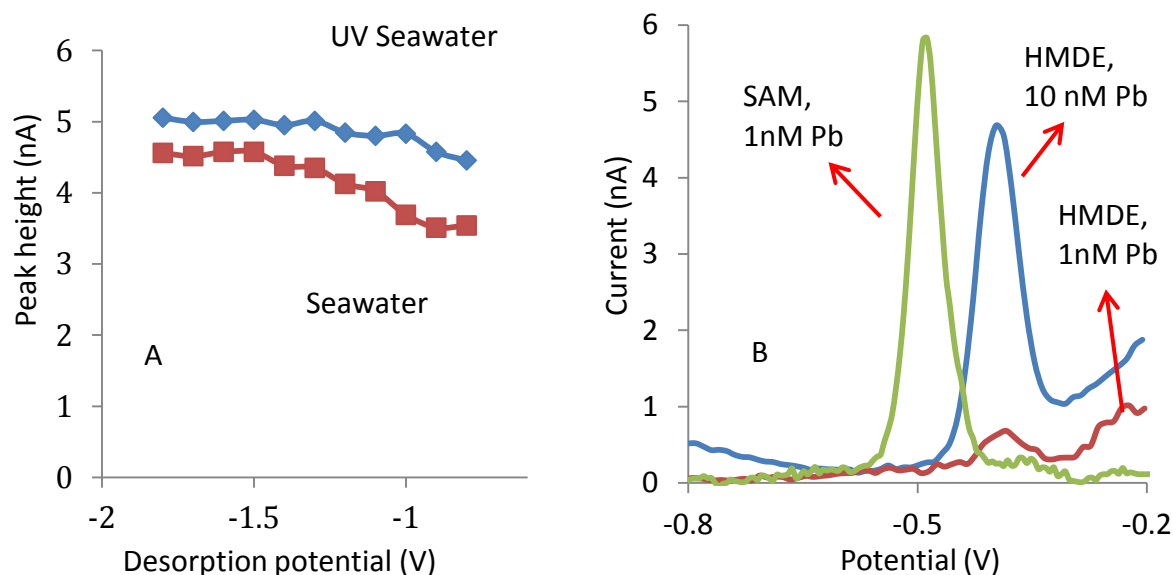


Figure 5.1, A). Optimisation of the desorption potential which is applied for 1 s immediately before and after the plating step of the pseudopolarogram, in UV-SW and in untreated seawater. Use of the desorption step at -1.5 V caused a 32% increase in sensitivity in untreated seawater, and of 13% in UVSW, compared to no desorption step. The scan was started always from -0.8 V. B) Scans for Pb (1 nM using the SAM and 10 nM using the HMDE) in UVSW, using a 60s plating time.

Scans for 1 nM Pb using the SAM, and 10 nM using the HMDE, after 1 min deposition (Figure 5.1B) show that the sensitivity of the SAM is about 13x greater in absolute terms (peak height). In addition the baseline of the SAM scan is flatter leading to a further reduction in the limit of detection compared to that with the HMDE. The Pb peak on the

SAM is more negative than on the HMDE because of the much smaller diffusion layer thickness in the electrode amalgam than in the HMDE: this means that the Pb is released more slowly from the HMDE.

5.3.2. Effects of pH, deposition time and Pb concentration on pseudopolarography of inorganic Pb

It was checked if the determined half-wave potentials for Pb are independent of the pH as they relate to the thermodynamic stability constant (Eqn. 1) which is independent of pH. Pseudopolarograms were made of Pb in UV-SW at different pH values to verify its effect (Figure 5.2A). Half-wave potentials on the SAM were found to vary slightly with the pH: pH 8.2: -0.465 V, pH 7.7: -0.450 V and pH 6.6: -0.446 V. The shift was greatest at pH 8.2. This region of the half-wave potentials, within about 100 mV of that for free Pb (this is the value obtained at lowest pH value) is where labile (or reversible) complexes are responsible for the half-wave shift. Irreversible can be expected to give a much greater shift in the region of 100s of mV (Gibbon-Walsh et al., 2012). The shift found here at higher pH is caused by stronger complexation of Pb by carbonate and hydroxide species at this pH. There was an increase in the overall wave height at the lowest pH which is due to the greater sensitivity at that pH.

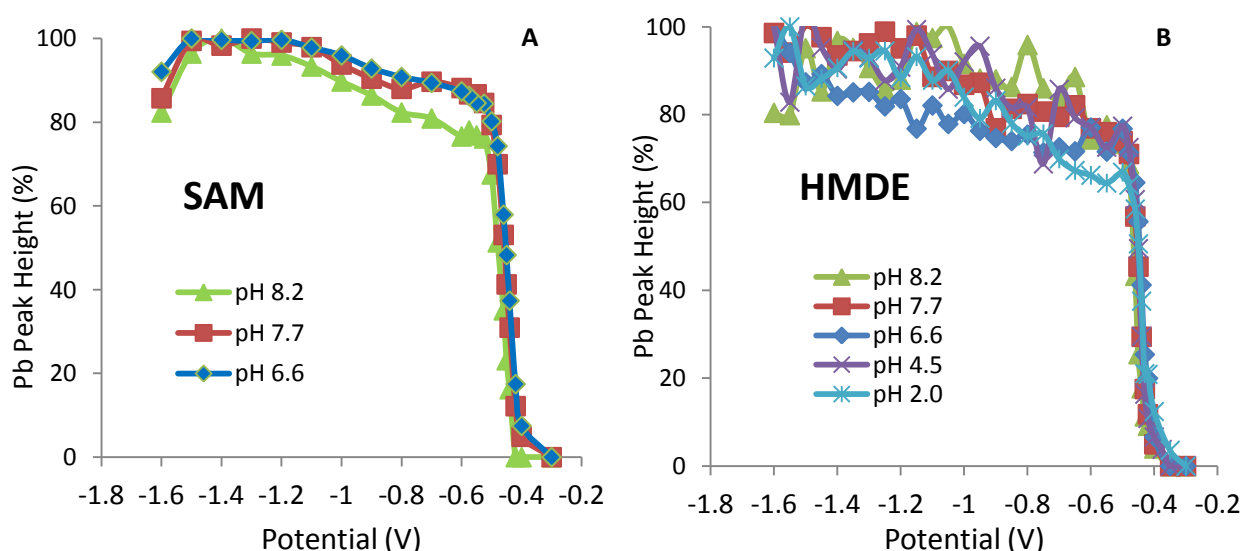


Figure 5.2: Effect of varying the pH on pseudopolarography of Pb in UVSW. A) using the SAM (1 nM Pb) and B) using the HMDE (10 nM Pb). The plating time was 60 s, with vibration for the SAM and with stirring for the HMDE. Half-wave potentials were using the SAM: pH 8.2: -0.465V, pH 7.7: -0.450V; pH 6.6: -0.446V. Using the HMDE: pH 8.2: -0.463 V; pH 7.7: -0.445 V; pH 6.6: -0.442 V.

The distribution of different metal species can be expected to change with the pH (free metal increasing with decreasing pH) and this information would be lost if the pH varies much from the natural pH of seawater. For this reason it is important to stabilize the pH near to the natural pH. HEPES pH buffer was used to set the seawater to pH 7.7. The HEPES was tested by pseudopolarography and was found not to cause any Pb complexation in seawater (no shift was found). The HEPES buffer was used in further experiments in seawater.

Comparative pseudopolarograms using the HMDE showed similar shifts to found using the SAM (Figure 5.2B): pH 8.2: -0.463 V; pH 7.7: -0.445 V, and pH 6.6: -0.442 V, showing again a relatively large shift (20 mV) for the highest pH and insignificant shift for the lower two pH values.

It is interesting that the half-wave potentials obtained using the HMDE are the same (within a few mV) as those obtained using the SAM, in view of the large difference in the peak potential (~100 mV) in the scans using the two electrodes (Figure 5.1B). This confirms that the half-wave potential is solely dependent on processes during the deposition step and is not affected by the scanning method.

Variation of the deposition time (30 s, 60 s and 120 s) for 1 nM Pb in UV-SW with pseudopolarography using the SAM electrode showed the pseudopolarographic wave increase with the deposition time (Figure 5.3C). The increase is expected because of the increased sensitivity with increasing deposition time. Normalisation of the peak height to the greatest peak height in each pseudopolarogram (Figure 5.3A) showed that the three waves obtained using the SAM are exactly superimposed which means that the same half-wave potential was obtained at each deposition time. The actual half-wave potentials were -0.448 V (30 s) -0.448 V (60 s) and -0.452 V (120 s). However, the same normalisation for the pseudopolarograms obtained using the HMDE (Figure 5.3D) gave pseudopolarograms that were shifted by a systematic amount: -0.429 V (30 s), -0.439 V (60 s) and -0.452 V (120 s). A shift with increasing deposition time is predicted for reversible metal complexes (Omanovic and Branica, 2003):

$$\delta E_{1/2} = (0.059/n) \log (t_{\text{dep1}}/t_{\text{dep2}}) \quad (3)$$

Eq. 3 predicts a shift of 17 mV in $E_{1/2}$ when the deposition time is increased from 30 s (t_{dep1}) to 120 s (t_{dep2}) in general agreement with a shift of 22 mV obtained here with the drop. For irreversible systems no shift is expected (Omanovic and Branica, 2004), while an intermediate response is obtained for quasi-reversible system. The same work also demonstrated that the reversibility of a system can be increased by increasing the diffusion layer thickness as it increases the time for the complex to dissociate within the diffusion layer. Consequently, a decrease of the diffusion layer thickness will act to increase the irreversibility.

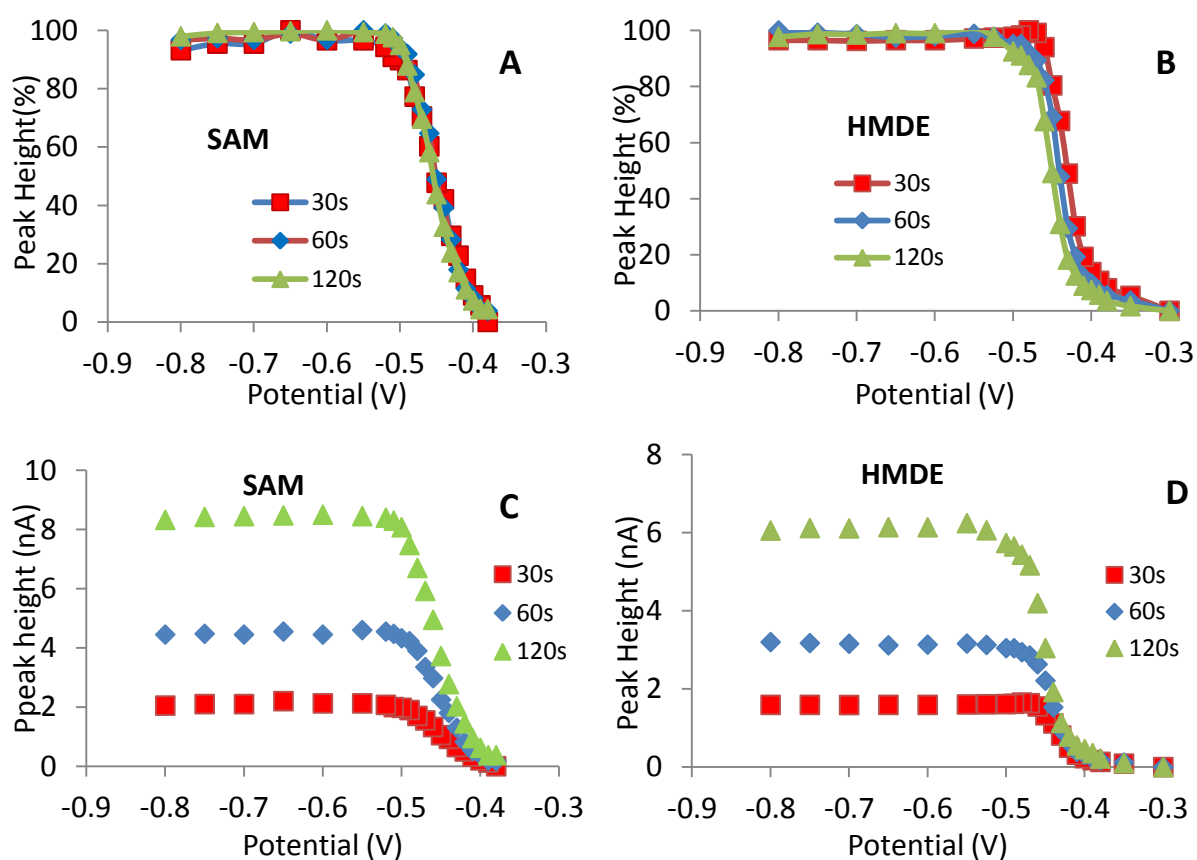


Figure 5.3. Effect of varying the deposition time on the pseudopolarograms of Pb (1 nM with the SAM and 10 nM with the HMDE) in UVSW using the SAM and the HMDE. A and B: Normalised peak currents as a function of deposition potential for the SAM (A) and the HMDE (B). C and D: actual peak heights for the pseudopolarograms.

The negligible shift in the pseudopolarograms with the SAM is perhaps related to the much thinner diffusion layer thickness of the 12 μm diameter, vibrated, SAM ($\sim 1 \mu\text{m}$) (Salaün

et al., 2012)) as opposed to that at the HMDE ($\sim 8 \mu\text{m}$) (Omanovic and Branica, 2003)).

Variation of the concentration of Pb to 1, 2 and 3 nM with pseudopolarography using the vibrated SAM showed that the pseudopolarograms increased as well as shifted: $E_{1/2}$ was for 1 nM Pb: -0.448 V, 2 nM Pb: -0.457 V, and for 3 nM Pb: -0.462 V. The shift was small but systematic. On the other hand, the normalized pseudopolarograms using the HMDE were superimposed and were without systematic shift: $E_{1/2}$ for 5 nM Pb: -0.442 V, for 10 nM Pb: -0.443 V, and for 15 nM Pb: -0.443 V. Again the HMDE electrode behaves according to the theoretical prediction (Omanovic and Branica, 2004) whilst the SAM electrode does not. The small shifts of half-wave potentials on SAM electrode are probably due to the experimental errors. It can be lowered by using a higher resolution of scanning deposition potentials. The details are shown on Figure 5.4.

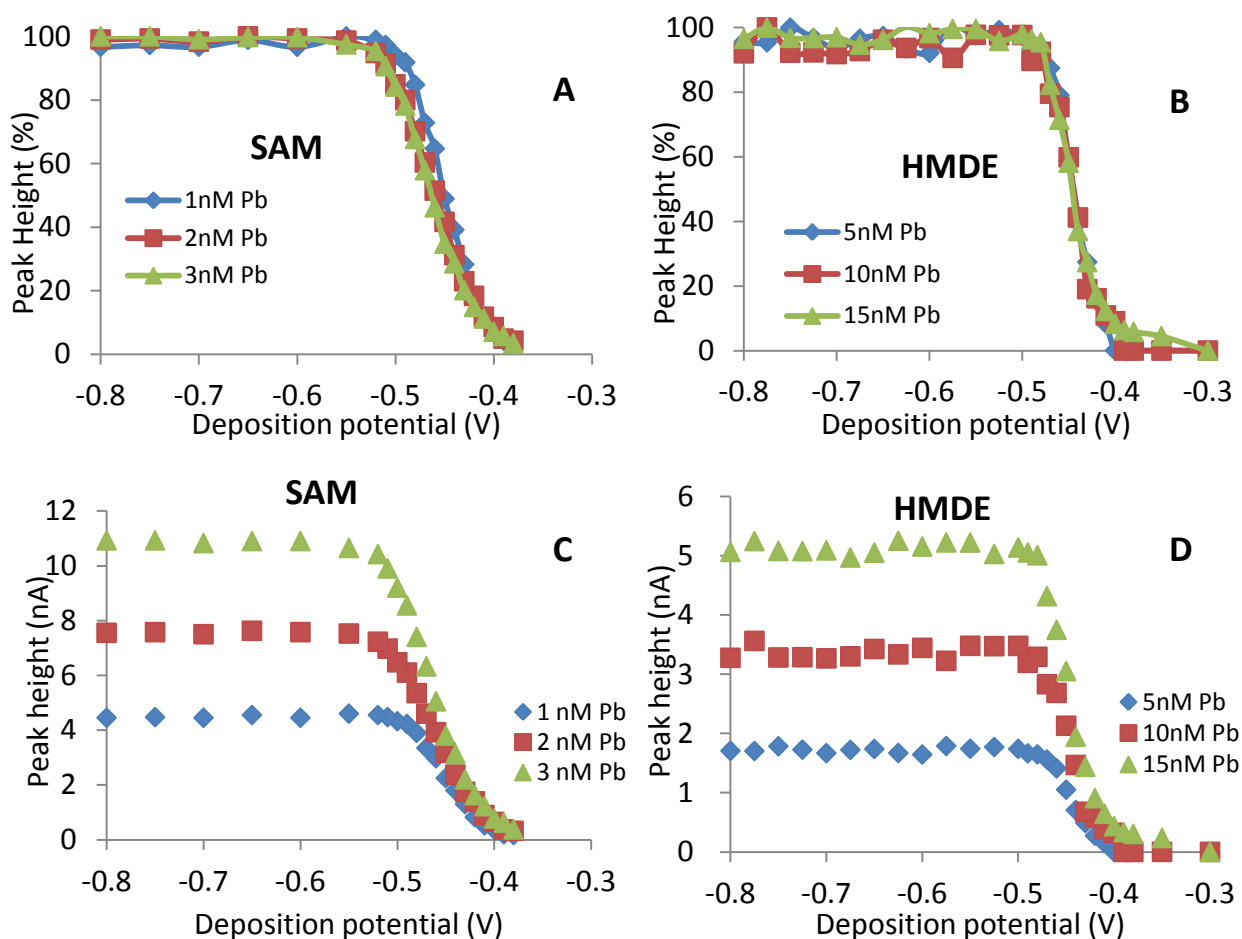


Figure 5.4: Effect of varying the concentration of Pb on pseudopolarography using the SAM and the HMDE. Pb was varied to 1, 2 and 3 nM using the SAM, and 5, 10 and 15 nM using the HMDE. $E_{1/2}$ values were: on SAM: 1 nM Pb: -0.448 V, 2 nM Pb: -0.457 V, 3 nM Pb: -0.462 V. on the HMDE: 5 nM Pb: -0.442 V, 10 nM Pb: -0.443 V, and 15 nM Pb: -0.443 V.

The previous experiments show that any effects of varying the deposition time or Pb concentration on the half-wave potentials are minor compared to the large shifts (100's of mV) that can be expected for organic metal species. Nevertheless the instrumental parameters were held constant in further experiments using model compounds and seawater samples.

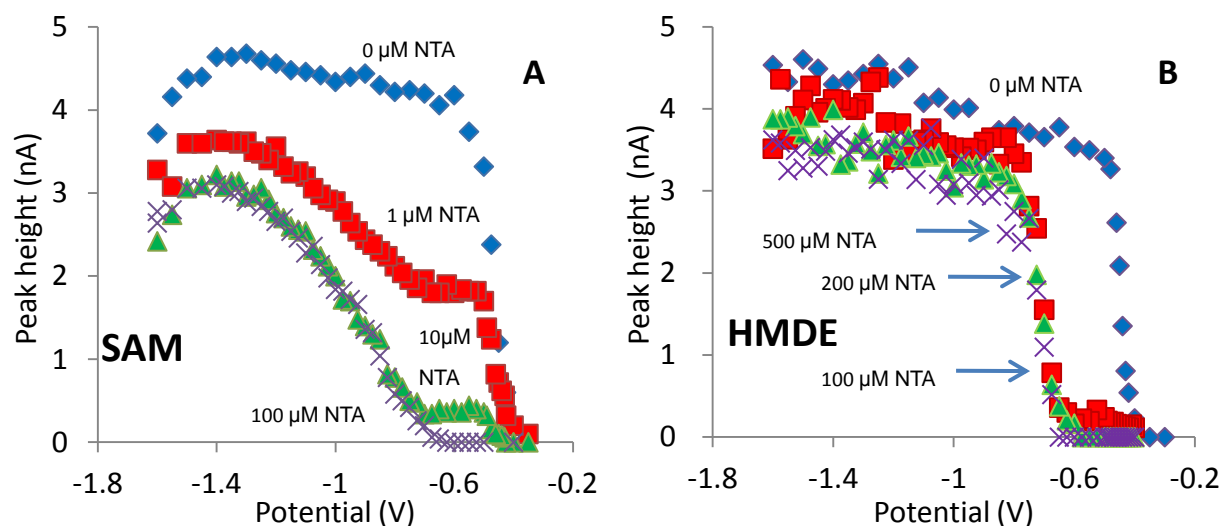


Figure 5.5. Pseudopolarography of Pb in the presence of NTA (at 1, 10 and 100 μM) in UVSW of pH 7.7, using the SAM (1 nM Pb) and the HMDE (10 nM Pb).

5.3.3. Calibration of the half-wave shift of irreversible Pb complexes using model compounds

Various model compounds were selected on the basis of complex stability with Pb in seawater and subjected to pseudopolarography using the SAM (with 1 nM Pb) and the HMDE (with 10 nM Pb). Although the shift in the half-wave potential is related to the thermodynamic complex stability, the complex has to exist in seawater conditions, which means that sufficient ligand had to be added overcome the effect of major cation competition and inorganic complexation by carbonate and hydroxide. The compounds were added to UV-SW of pH 7.7. In some experiments the concentration of the model ligand was varied to evaluate whether this affected the value of the half-wave potential. Using NTA, pseudopolarograms were found to shift and also much reduced in height in the potential region $> E_{1/2}$ (Figure 5.5 A). The maximum height of the wave at potentials past the half-wave potential (at values $< E_{1/2}$) was also reduced but by a smaller amount. The lowering of the

pseudopolarogram is caused by two effects: at potentials $> E_{1/2}$, the wave height represents the reactive metal that is not complexed, whereas at potentials $< E_{1/2}$ the wave height is controlled by the diffusion rate of the Pb-NTA. Essential all Pb was complexed at 100 μM NTA, whilst a smaller proportion was complexed at lower NTA (Figure 5.5 A).

The shift in the half-wave potential on the SAM electrode was much greater than that obtained using the HMDE (Figure 5.5 B): $E_{1/2}$ on SAM: 0 NTA: -0.452 V; 1 μM NTA: -0.907 V; 10 μM NTA: -0.911 V; 100 μM NTA: -0.913 V. On the HMDE the values for $E_{1/2}$ were: 0 μM NTA: -0.44 V, 100 μM NTA: -0.71 V, 200 μM NTA: -0.71 V, 500 μM NTA: -0.71 V. So, the half-wave shifts were independent of the concentration of NTA used, from -0.45 V without NTA to -0.91 V with NTA on the SAM, which is equivalent to a shift of -0.46 V, whereas the shift was -0.26 V on the HMDE. The NTA wave is also much extended along the potential axis of the pseudopolarogram of the SAM electrode, showing a strong irreversible behaviour, which is not apparent at the HMDE, because of the thinner diffusion layer at the SAM which enhances the irreversible character of the Pb-NTA complex over that obtained at the HMDE (Omanovic and Branica, 2004). An important confirmation of these experiments was that the half-wave potential for Pb-NTA at the SAM (-0.91 V) was independent of the NTA concentration, which is expected as this potential is directly related to the thermodynamic stability constant (Eqn. 1).

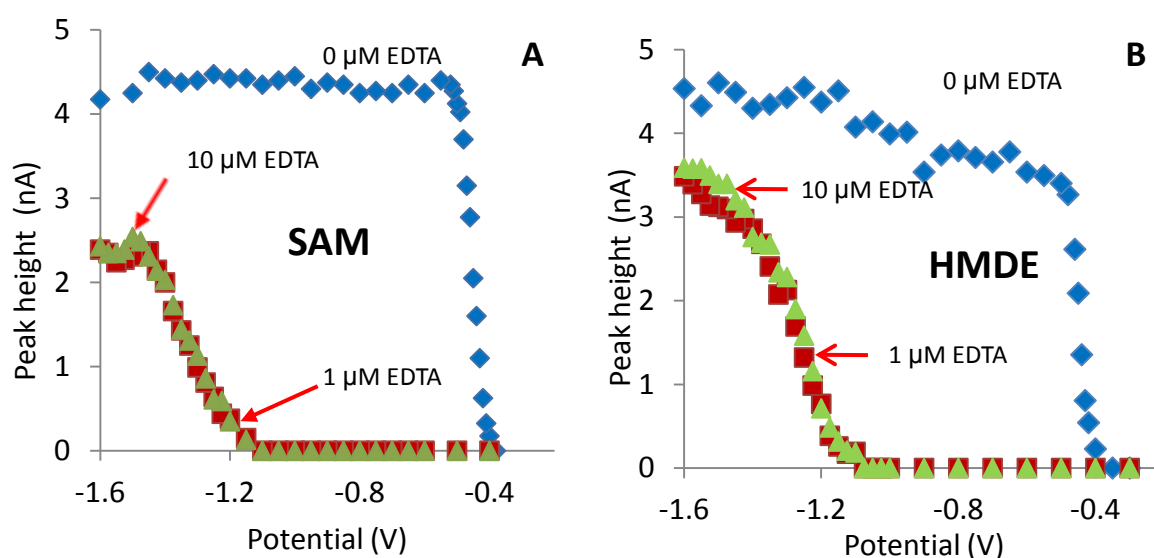


Figure 5.6. Pseudopolarography of Pb in the presence of EDTA (at 1 and 10 μM) in UVSW of pH 7.7, using the SAM (1 nM Pb) and the HMDE (10 nM Pb).

The pseudopolarograms of NTA were repeated at three deposition times (30 s, 60 s and 120 s). The values for $E_{1/2}$ were -0.886 V, -0.915 V and -0.918 V respectively. Therefore the value found at the shortest plating time was slightly more positive whilst the other two were the same, which was ascribed to the height of the wave being considerably lower at the 30 s deposition time.

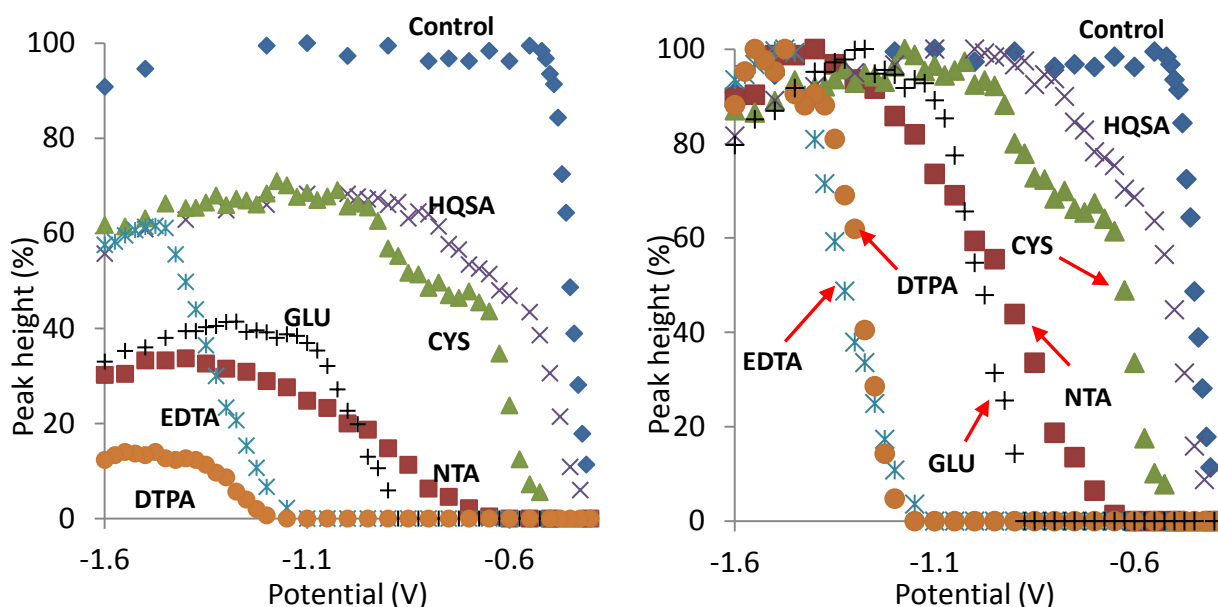


Figure 5.7. Pseudopolarograms of 1 nM Pb in pH 7.7 UVSW using the SAM (60 s plating time) in the presence of the model ligands added to UVSW, pH 7.7. A): peak height normalized to that of the control (UVSW) and B): peak heights normalized to the maximum of each species. Ligand concentrations: 100 μ M HQSA (8-Hydroxyquinoline-5-Sulfonic Acid), 200 μ M cysteine, 100 μ M NTA, 100 μ M glutathione, 100 μ M EDTA and 1 μ M DTPA.

Other ligands that were subjected to pseudopolarography using the SAM were EDTA, DTPA, glutathione (GLU), cysteine (CYS) and 8-hydroxyquinoline (HQSA), at various concentrations to ensure that strong Pb complexation was obtained for several of the experiments. Using EDTA the half-wave potential was shifted from -0.445 V (for free Pb) to -1.314 V for 1 μ M EDTA, and -1.315 V for 10 μ M EDTA. In line with the theoretical expectation, the same half-wave potential was found at the two EDTA concentrations. Also, the pseudopolarograms for 1 and 10 μ M EDTA were superimposed (Figure 5.6) as the Pb was

fully complexed at both concentrations of EDTA. The Pb peak height was fully masked at potentials $> E_{1/2}$ until it increased gradually at more negative potentials. Plateau level was reached from -1.5 V after which the peak height was constant at a height of about 40 % less than that for inorganic Pb, which is due to the lower diffusion coefficient of the Pb-EDTA complex. The value for $E_{1/2}$ Pb-EDTA obtained at the HMDE (Figure 5.6) was slightly more positive (at -1.28 V) than at the SAM. The difference was very small compared to the large difference that was obtained between the two electrodes using NTA.

The slope of the shift in the values for $E_{1/2}$ as a function of the thermodynamic complex stability was evaluated from a plot of $E_{1/2}$ as a function of $\log K_{PbL}$ (Figure 5.8). The slope was -0.056 ± 0.005 V. This slope (-56 mV/log K unit) is greater than expected for a 2-electron reaction (-30 mV/log K unit according to Eqn. 1).

Table 5.2. Half-wave potentials obtained for model compounds added to UV-digested seawater. All pseudopolarograms with 1 nM Pb using a 1min plating time.

Ligand	Concentration (μ M or ppm)	pK_{PbL}	$E_{1/2}$ (V)
DTPA	1 μ M	18.66	-1.282
EDTA	1 μ M	18.04	-1.314
	10 μ M	18.04	-1.315
Cysteine(CYS)	200 μ M	11.6	-0.891
Glutathione(GLU)	100 μ M	10.6	-0.982
8-HQ-5-SA(HQSA)	100 μ M	8.53	-0.75
NTA	1 μ M	11.34	-0.907
	10 μ M	11.34	-0.911
	100 μ M	11.34	-0.913

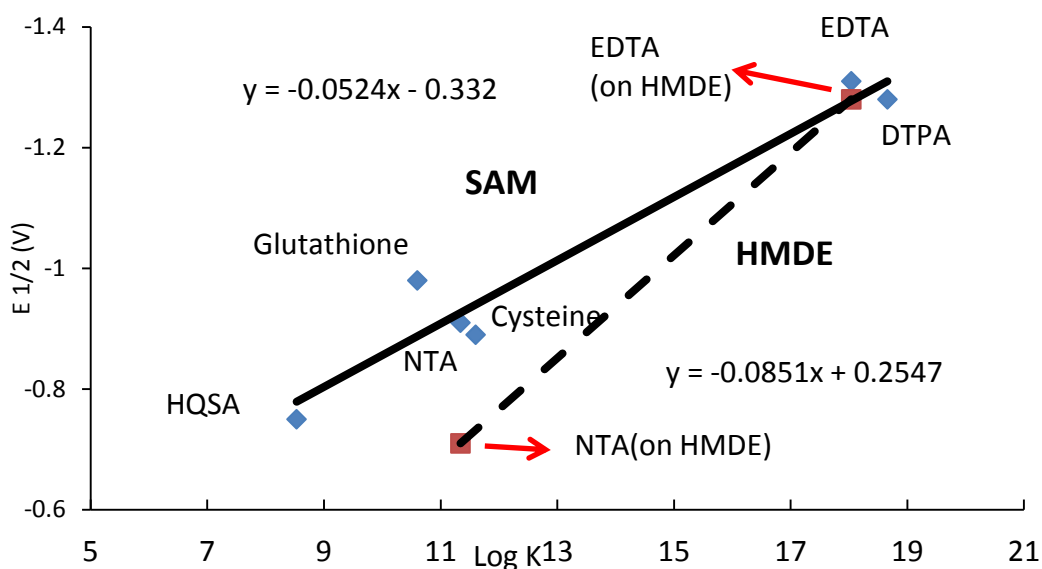


Figure 5.8. Half-wave potentials of model compounds in UV-SW obtained using the SAM (1 nM Pb) and the HMDE (10 nM Pb for EDTA and NTA), plotted as a function of the thermodynamic stability constant, K_{pBL} , of the species. The ligand concentrations and half-wave potentials are listed in Table 5.2.

The variation in $E_{1/2}$ was here obtained over a range of complex stabilities, with $\log K_{pBL}$ values between 9 and 19, and $E_{1/2}$ between -0.7 and -1.3 V. This is expected to be sufficient to cover the range of half-wave potentials found in the environment. A ligand at a concentration of ~ 1 nM (the ligands detected are irreversible species that can occur at concentrations below that of Pb) requires a value for $\log K_{pBL} \gg 9$ to bind the Pb significantly. That means that the highest potential (most positive) at which a suitable ligand can have its half-wave potential is at -0.8 V and probably more negative.

The new method was tested in pseudopolarography of humic substances added to seawater, and unaltered seawater samples from coastal and oceanic origins. Only the SAM was used in the remainder of this work as the sensitivity of the HMDE was insufficient for speciation of Pb in realistic natural conditions.

5.3.4. Pseudopolarograms of Pb with humic substances added to seawater

Humic substances are known to bind metals in seawater (Laglera et al., 2007; Yang and van den Berg, 2009) and marine humics are thought to be an important ligand for iron in the

marine system (Laglera and van den Berg, 2009). For this reason binding of Pb by humic and fulvic acid were tested by pseudopolarography. The Pb complexes with humic and fulvic acid were found to dissociate in the labile region (Figure 5.9), within 100 mV of the half-wave potential for inorganic Pb. This means that the Pb-HA and Pb-FA species in seawater form reversible complexes that dissociate within the time frame of the experiment (milliseconds). This is interesting as one might have expected these to be irreversible because of the complex nature of humic matter. This finding confirms that copper, where similarly the complexes with FA and HA in seawater, had their half-wave potential in the labile region for that metal (Gibbon-Walsh et al., 2012). A previous pseudopolarographic study of Pb-FA in pH 5.5 buffer solutions of pure water found that this was an electrochemically reversible complex (Chakraborty et al., 2007) with the half-wave potential shifting in a negative direction with increasing FA.

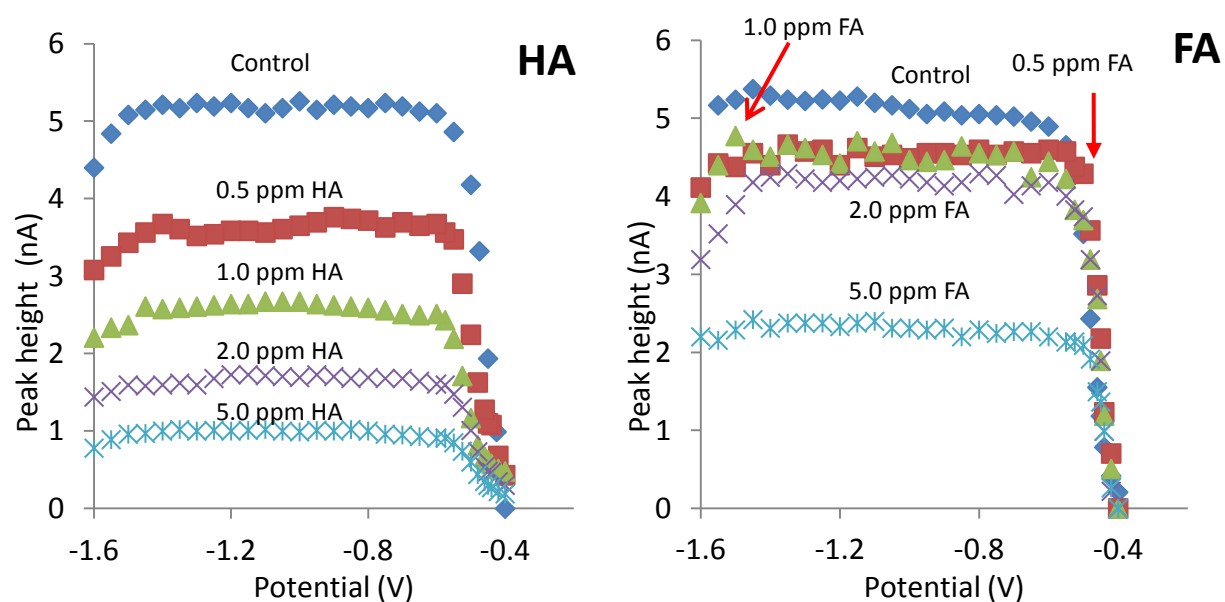


Figure 5.9. Humic substances. SR-HA and SR-FA were added to UV-SW along with 1 nM Pb. The solutions were equilibrated overnight prior to the pseudopolarography. The deposition time was 60 s. The found half-wave potentials are in Table 5.3 along with the complex stability.

In this work, the shifts in the half-wave potential for Pb-FA in seawater were in a positive direction, opposite that expected. A positive shift has been shown to occur in the event of surface active effects, and humic substances are known to have a surface active effect (Town, 1999), which may have played a role in the positive shift. It is likely that the

negative shifts with HA are smaller than they should be because of the surface effect, although the effect was minimised by the potential jumps to -1.5 V immediately before and after the deposition step. The HA data was used to calculate indicative values for the α coefficient of complexation of Pb' (inorganic Pb) with HA using a version of the Lingane equation valid for reversible complexes with a correction for the change in the mean diffusion current when inorganic Pb is complexed with an organic ligand L (Chakraborty et al., 2007; Gibbon-Walsh et al., 2012):

$$\text{Log } \alpha'_{\text{Pb}'\text{L}} = \Delta E_{1/2} / 30 + \log (i_{\text{LPb}'}/ i_{\text{LPbL}}) \quad (4)$$

where $\Delta E_{1/2}$ is the half-wave shift in mV, and $i_{\text{LPb}'}$ is the limiting current (the plateau level) for inorganic Pb, and i_{LPbL} for PbL, where L is HA, FA, or other electrochemically reversible complexes with a half-wave potential within about 120 mV of that for inorganic Pb (i.e. such species are considered labile). The data is summarised in Table 5.3. A value for the stability constant for Pb^{2+}HA was found of $\log K'_{\text{PbHA}} = 9.8 \pm 0.3$, which is at the high end of values found for Pb-HA before but which makes sense in view of the known heterogeneity of humic substances (Town and Filella, 2002), as a result of which stronger sites are detected at low metal-humic ratios as used here. This constant is conditional upon the composition of the solution (seawater of pH 8) as it was calculated from the half-wave shift. The calculation was corrected for the shift in the half-wave potential as a result of the lowering of the pseudopolarograms (Figure 5.9) due to the reduction in the mean diffusion coefficient as the inorganic Pb is complexed with HA or FA. Without the correction, the average value for $\log K'_{\text{PbHA}}$ would have been lower by 0.4 units at 9.4 ± 0.4 .

A value for K'_{PbFA} could not be calculated as there was no negative shift, which may be because the complex is weak, or because a positive shift (due to the lowering of the pseudopolarogram by the FA addition) masked a small negative shift. The lowering of the peak height by the FA additions suggests that there was significant complexation. This lowering by FA was much less than by HA (Figure 5.9) indicating that the Pb-FA is a weaker complex than Pb-HA, on a ppm basis.

Table 5.3. Pseudopolarography of humic substance (SRHA and SRFA) complexes with Pb. The equivalent ligand concentration (molar scale) associated with the concentrations of SRFA and SRHA was calculated from respectively 16.7 and 31.9 nmol mg⁻¹ binding sites HS(Laglera et al., 2007). The inorganic alpha for Pb (α_{Pb}) in seawater was taken as 28 (calculated for pH 8 seawater). K_{Pb2+L} is based on Pb²⁺.

HA (ppm)	[HA](nM)	$E_{1/2}$ (V)	$\delta E_{1/2}$ (V)	$\log \alpha_{Pb}$	α_{Pb}	$\log K'_{Pb2+L}$
0	0	-0.465				
0.5	16	-0.482	-0.017	0.69	4.9	9.9
1	31.9	-0.488	-0.023	1.07	11.7	10.0
2	63.8	-0.482	-0.017	1.09	12.3	9.7
5	160	-0.478	-0.013	1.13	13.6	9.4
Average						9.8 ±0.3
FA (ppm)	[FA] (nM)	$E_{1/2}$ (V)	$\delta E_{1/2}$ (V)			
0	0	-0.464				
0.5	8.4	-0.456	0.009	-	-	-
1	16.7	-0.456	0.009	-	-	-
2	33.4	-0.453	0.012	-	-	-
5	83.5	-0.441	0.024	-	-	-

The above calculation is based on the electrochemically reversible behaviour of Pb-HA. This was not obvious for Pb-FA because of the lack of an obvious shift. An alternative method would be to calculate the complex stability of Pb-HA and Pb-FA on basis of the drop in height of the plateau of the pseudopolarograms in Figure 5.8. This case assumes irreversibility and then the drop is caused by irreversible binding of Pb, which means that the complex stability is directly apparent from the ratio of the response for Pb in the presence and absence of the added ligand (HA or FA). Thus a value was obtained for $\log K'_{Pb/FA}$ of 9.2 ± 0.3 , and analogously a value of 9.2 ± 0.3 for $\log K'_{PbHA}$. This value for $\log K'_{PbHA}$ is less than that (9.8) obtained from the half-wave shift for PbHA, indicating that this procedure gives an underestimate, for instance because Pb-HA is actually a reversible species that dissociates near the $E_{1/2}$. It is likely that the value for $\log K'_{PbFA}$ is an underestimate similarly.

Irreversible species of Pb-HA or Pb-FA were not apparent within the range of potentials tested, so no use was made of the calibrated chelate scale.

5.3.5. Pseudopolarograms of Pb in seawater samples

The new pseudopolarographic method was applied to samples originating from estuarine, coastal and oceanic conditions. Pseudopolarograms were made of Pb in samples from the Mersey estuary, the Irish Sea (Liverpool Bay), and the Atlantic Ocean (Celtic Sea boundary). The measurements were at the original concentration of Pb, and were only undertaken using the SAM due to insufficient sensitivity of the HMDE. Each polarogram showed several waves, representing several species of Pb that are reduced at increasingly more negative reduction potential, reflecting binding by organic complexing ligands. The ligands are organic because they are not present in the UV-digested samples. Whether these species are irreversibly complexed, or reversibly, depends on the location of the half-wave potentials. To simplify the discussion the regions were subdivided into labile species (I) and irreversibly bound species (II), as indicated on Figure 5.10. There were more waves than these two, but these were small, amounting to < 10% of the Pb, and hard to identify. So, for now only these two broad groups were identified. Approximately, a ligand has to have a thermodynamic complex stability of $\log K_{\text{Pb}^{2+}\text{L}} \gg 10$ in order to bind any Pb at all in competition with inorganic complexation and at a nanomolar concentration. This is equivalent to a required shift of 300 mV (Eqn. 1) to at least -0.84 V or more negative. Species with more positive half-wave potential must therefore be labile species.

5.3.6. *Labile waves in the samples*

The pseudopolarograms have in common that there was a significant labile Pb fraction in all waters tested: the labile fraction amounted to 78 % (Atlantic Ocean), 92 % (Irish Sea) and 95 % (estuarine sample) (Figure 5.10, and the extracted half-wave potentials are in Table 5.4). The concentrations of Pb in the samples were measured by ASV using standard additions, greatly differing, at 32 pM, 0.2 nM and 2 nM respectively, so the actual amounts of the labile fraction differed greatly too. The location of the half-wave potential of the labile fraction determines whether it was organically bound or inorganic Pb, similar to the labile fraction of the humic species.

The labile wave was split in two for the samples from the Atlantic and Irish Sea. It is quite feasible that more than one reversible species is formed, but it is unexpected to get

more than one labile wave as the metal should be controlled by both ligands operating simultaneously. However, this scenario assumes that both ligands are present in excess. If at least one of the ligands is present at a concentration similar to that of the metal (or less), and if the two ligands have conditional stability constants that differ sufficiently (by a unit or more), then this could lead to a splitting of the wave, causing two step increases in the pseudopolarogram as first the weaker complex is dissociated and subsequently the stronger. This is the qualitative explanation that is ascribed to the presence of two labile waves to in these waters. It should also be kept in mind that the α -coefficient is not constant in this situation: its value should increase as the metal is drawn out of each species at increasingly negative deposition potential, which complicates a theoretical model of this scenario.

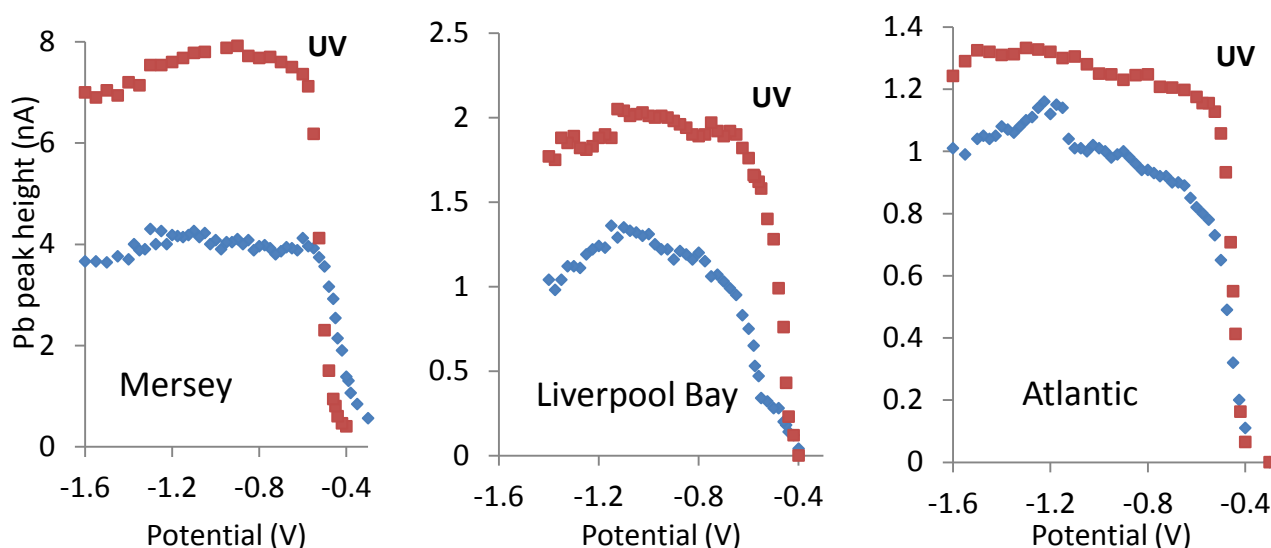


Figure 5.10. Pseudopolarograms of Pb in seawater samples. Mersey estuary (Sal 29): 2.6 nM Pb, 1 min plating; Liverpool Bay: 0.22 nM Pb; 2 min plating; Atlantic: 32 pM Pb; 3 min plating. The found half-wave potentials are shown in Table 5.4. The upper pseudopolarogram of each sample was obtained after UV-digestion.

The $E_{1/2}$ of the main labile wave (68%) in the Atlantic sample was at -0.472 V, shifted negative by 20 mV compared to the inorganic Pb wave (after UV-digestion) in the same water: this means that the labile Pb is bound with a binding strength of $\alpha_{Pb'} = 4.6$, i.e. 4.6x stronger than the inorganic complexation of Pb. On basis of Pb^{2+} the α -value is: $\alpha_{Pb^{2+}} = 130$ ($\log \alpha_{Pb^{2+}} = 2.1$). This α -value is valid for only part of the Pb^{2+} because of the presence of the second labile wave.

The second labile wave was located more negatively at -0.58 V which is still considerably more positive than required for an inert species and is therefore also a labile species. This complex is much more stable with $\log \alpha_{\text{Pb}^{2+}} = 5.7$, and amounting to 10% of the Pb.

The labile wave in the coastal water (Irish Sea) also consisted of two components, one with a positive shift due to adsorption effects (similar to the fulvic acid in Figure 5.9), amounting to 23%, and a second with a clear negative shift from -0.478 to -0.562 V. This is a large shift for a labile species, which means that this labile-bound Pb has $\alpha_{\text{Pb}'} = 630$ ($\log \alpha_{\text{Pb}'} = 2.8$, $\log \alpha_{\text{Pb}^{2+}} = 4.2$). This fraction amounts for 69% of the Pb. As the Pb concentration in this water (0.22 nM) is much greater than in the Atlantic sample (32 pM); this means that there is much more of this species in this water than in the Atlantic. These waters are known to have a high concentration of humic matter (0.2 – 0.5 ppm) that also binds with iron (Laglera et al., 2007) so ligands with a similar behaviour to humic acid are a plausible explanation as they bind the Pb similarly in a reversible species.

The labile wave in the estuarine water was shifted in a positive direction and could therefore not be used to assess whether the Pb is complexed with organic matter or is inorganic. The shift indicates the presence of a high concentration of organic matter with a surface active effect similar to that for the fulvic acid, so it is likely that the Pb is complexed as well, but the α_{Pb} of the labile fraction cannot be assessed without a method to correct for the positive offset. A possible improvement might be coating with Nafion (Strasunske et al., 2010) which could be attempted in further work.

5.3.7. *Strong, irreversibly bound species of Pb*

All three waters obviously showed evidence of a strong, irreversibly bound Pb species that was reduced at a potential in the region of -1 to 1.2 V (Table 5.4 and Figure 5.10). In the case of the Atlantic sample this fraction amounted to 22% of the Pb, in the case of the coastal water it was 8%, and for the estuarine sample 4%. The $\log K'_{\text{PbL}}$ values of this fraction were 16 (Atlantic), 15 (Irish Sea) and 15 (estuarine sample) (Table 5.4). This fraction is a small

proportion of the dissolved Pb but was resolved by the pseudopolarograms. The origin of these strong binding ligands is unknown. However, humic matter is heterogeneous and it is possible that it is all part of the same humic material, that binds metals with different binding strength at different sites (Filella and Town, 2001). However, that is a hypothesis that requires further work.

These values for $\log K_{Pb2+L}$ and $\log \alpha_{Pb2+L}$ can be compared to those found for natural organic complexing ligands in pH 8 seawater ($\log K'_{Pb^2+L} = 9.7$ and $\log K'_{Pb2+L} = 10.6$) by complexometric titration with detection by ASV (Capodaglio et al., 1990). The α_{Pb2+L} of those species at $\log \alpha_{Pb2+L} = \sim 2.6$ (estimated for the ~ 1 nM of ligands found in that work) is in the same range as that found here for the reversibly bound Pb in the Atlantic sample. It is not possible to compare the irreversibly bound species detected in this work as it would not have been found by the conventional complexometric titration. The conditional stability constant for the humic acid complex with Pb^{2+} ($\log K'_{Pb2+L} = 9.8$, Table 5.3) is smaller than that found by the complexometric technique.

Table 5.4. Half-wave potentials for lead complexes in seawater samples. The comparative $E_{1/2}$ value for UVSW was -0.46 V. $E_{1/2}$ values > -0.75 V are for labile species. Log α values were calculated assuming a shift of 30 mV/log α unit (Eqn. 1). Log K_{Pb2+L} values for irreversible species were calculated from the chelate scale relationship: $\log K_{Pb2+L} = (E_{1/2} + 0.289)/-0.0555$ (Figure 5.8). Log α_{Pb2+L} values were calculated from the shift in the labile wave based on Pb^{2+} .

Sample	Pb (nM)	Wave (I) (labile)				Wave (II) (irreversible)	
		$E_{1/2}$ inorganic	$E'_{1/2}$ PbL	Labile %	Log α_{Pb2+L}	$E_{1/2}$	log K_{Pb2+L}
Atlantic	0.032	-0.45	-0.47	68%	2.1	-1.2	16
			-0.58	10%	5.7		
Irish Sea	0.22	-0.48	-0.44	22%	-	-1.1	15
			-0.56	69%	4.2		
estuary	2.6	-0.52	-0.43	96%	-	-1.1	15

The pseudopolarogram of the oceanic sample is consistent with the Pb occurring largely

(~78%) in a reversibly, in part strongly, complexed species, with the remaining Pb in an irreversible species. The pseudopolarogram shows a general rise going from -0.8 to -1.1 V, which was unresolved but which suggests that more Pb was present in stable species. A similar rise is present in the coastal water sample, whilst the estuarine sample is dominated by the labile wave.

In addition to the Pb species that dissociate at potentials > -1.5 V, there is an inert fraction, amounting to 20 % (Atlantic), 40 % (coastal water) and 50 % (estuarine sample) that did not dissociate even at -1.5 V and could only be released by UV-digestion of the water (Figure 5.10). It is likely that this inert fraction includes colloidal material, and which will be interesting to investigate in further research.

The lead that occurs in the labile organic complex form (the major proportion of lead in all the waters tested) can be expected to participate readily in exchange reactions, whether related to biological uptake or to release Pb for adsorption on suspended particulate material. This description of the speciation of Pb in ocean waters is consistent with the known scavenged behaviour of lead in deep ocean waters (decreasing concentration with depth) that suggests that the geochemistry of lead is ultimately dominated by its inorganic reactivity.

The pseudopolarography of Pb in this work was found to provide information about the species (labile as well as irreversible) that it is occurring in at its natural, uncontaminated and un-augmented, condition. This information is different from that obtained by complexometric titrations from which the ligand concentration is obtained of species that by their nature are chemically reversible and occur in excess of the metal. Therefore the information obtained from pseudopolarograms is complementary to that from complexing capacity titrations. The pseudopolarograms are measured in a single sample aliquot, with the entire procedure under potentiostatic and computer control. The entire procedure is fully automated, apart from the sample transfer to the measuring cell. The only factor that complicates in-situ application of this technique is the need to remove dissolved oxygen as this lowers the limit of detection to the low picomolar levels. However, by using fast scanning

techniques(Wojciechowski et al., 1985) it may be possible to work in the presence of dissolved oxygen. The pseudopolarographic speciation technique is therefore relatively easy to implement for automated monitoring of the speciation of lead in natural waters using a vibrated SAM.

5.4. Conclusion:

Pseudopolarography was successfully applied for Pb speciation analysis in natural waters. Labile and strong complex by organics were observed from pseudopolarograms. Several model ligand were tested by this technique and a linear relationship between pKa and half wave potential $E_{1/2}$ were found. HA and FA, the important natural organic ligands, were added to the UV SW and their half waves with Pb were both in the labile regions. Combined with vibrating system, SAM electrode is suitable for in-situ speciation analysis.

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6

Chapter 6

**Determination of chromium in estuarine waters
by catalytic cathodic stripping voltammetry
using a vibrating silver amalgam microwire
electrode**

6. Determination of chromium in estuarine waters by catalytic cathodic stripping voltammetry using a vibrating silver amalgam microwire electrode

Abstract

Chromium (Cr^{VI}) in water can be determined by adsorptive catalytic cathodic stripping voltammetry in the presence of diethylenetriaminepentaacetic acid (DTPA) and nitrate on the hanging mercury drop electrode (HMDE). Predominately Cr^{VI} is detected and the water is UV-digested to convert all Cr to Cr^{VI} prior to analysis. An alternative to the HMDE by using a silver amalgam electrode based on a vibrating microwire was developed. The microwire electrodes were 12.5 μm diameter and electrochemically coated with mercury, and were stable for a week. Conditions were re-optimised, and I used a DTPA concentration of 5 mM, 30 mM acetate pH buffer (pH 5.5 in seawater and pH 5.8 in pure water), and 1.5 M nitrate solution. The microwire was reactivated prior to each scan by applying a negative potential (-3 V) for 2 s which removed all deposited Cr. The detection limit for chromium in pH buffer was found to be 0.2 nM Cr^{VI} and in seawater 0.3 nM Cr^{VI} at a deposition time of 30 s. The response increased linearly with the concentration of Cr^{VI} up to 60 nM Cr^{VI} in seawater. The limit of detection is less good than using the HMDE, but the linear range is good and the microwire electrode could form the basis of apparatus for flow-analysis. The method was successfully tested on water samples from the estuary of the river Mersey (Liverpool Bay) giving chromium concentrations between 1.48 nM and 2.29 nM.

6.1. Introduction

Chromium is an ecotoxic trace metal which is present in natural waters in two oxidation states: the reactive and toxic Cr^{VI} and the relatively inert Cr^{III} . Cr^{VI} is an inhaled carcinogen, toxic to humans and other mammals, while Cr^{III} is an essential mineral supplement at trace level (Li and Xue, 2001). Major sources of chromium contamination are metallurgy, electroplating industry, pigment production, tannery, mining and refractory materials (Bobrowski et al., 2009). Several sensitive methods exist in the literature for the

determination of chromium, some of these based on voltammetry, which has advantages related to sensitivity and ability to measure in seawater. Cathodic stripping voltammetry (CSV) makes use of adsorption of an electroactive complex with an added ligand. CSV has good sensitivity and can be used to determine metal speciation as well as its concentration in seawater. The CSV procedure for Cr in natural waters typically makes use of diethylenetriaminepentaacetic acid (DTPA) which forms a species with Cr^{III} that adsorbs on a hanging mercury drop electrode (HMDE) (Boussemart et al., 1992; Golimowski et al., 1985; Li and Xue, 2001). The Cr^{III} is freshly produced by reduction of Cr^{VI} at potentials < -0.1 V. The freshly produced Cr^{III} is 30x more reactive than existing Cr^{III} in the solution, so Cr^{VI} is the main contributor to the voltammetric response (Sander et al., 2003). Nitrate is added to increase the sensitivity through a catalytic reaction in which Cr^{III} is the catalyst, which causes reduction of nitrate when the voltammetric scan reaches its reduction potential. The CSV method mechanism has been developed further (Li and Xue, 2001; Sander et al., 2003) and modified using a different ligand (cupferron), and different electrode materials including a bismuth film electrode (Chatzitheodorou et al., 2004; Jorge et al., 2010) and a silver amalgam film (Bas, 2006; Bobrowski et al., 2011). Methods were recently reviewed (Bobrowski et al., 2009). An interesting modification has been the introduction of a more negative deposition potential in which the Cr^{VI} is plated as metallic Cr, then re-oxidised and re-adsorbed as the Cr^{III}-DTPA species (Korolczuk and Grabarczyk, 1999). This modification is included in this work.

The CSV procedure using the mercury is a “batch analysis” method, which is laborious because reagents are added to the voltammetric cell, either manually or using automated burets, for each analysis separately. This method would benefit from the introduction of flow analysis, but this is complicated with a mercury drop electrode as it is not easily adapted to flow analysis. Automation has been achieved using an HMDE (Korolczuk and Grabarczyk, 1999) making use of pumped medium exchange of a batch cell. Mercury drop electrodes have been incorporated in flow-cells (Daniel et al., 1997) but this has not been adopted on generally probably because of difficulties of stabilising a mercury drop in flow conditions and of environmental risk when using it in the field. Here a solid electrode was developed to determine Cr^{VI} by CSV. A solid electrode can be readily converted to flow analysis by fitting in a flow-cell, which is much easier than with the HMDE. Another advantage of the solid

electrode is that the use of mercury is much reduced although this electrode is mercury-coated.

Silver-amalgam electrodes (made similarly to dental amalgam) have been proposed before for voltammetry (Mikkelsen and Schroder, 2003) but that design is not readily made into a microelectrode. Solid electrodes have been used before for CSV. The mercury surface is particularly suitable for the adsorptive preconcentration step, but bismuth coated have also been used for several applications of cathodic stripping voltammetry (Svancara et al., 2010; Wang and Lu, 2000). A silver macro-electrode, physically coated with mercury prior to each scan, has been used before for the determination of Cr^{VI} (Bobrowski et al., 2011) and cobalt and nickel (Kapturski and Bobrowski, 2008) by CSV. Similarly a silver-bismuth alloy (Mikkelsen et al., 2003) and bismuth-coated carbon (Hutton et al., 2005) have been used for CSV of nickel and cobalt, and mercury-coated gold for CSV of iron (Gun et al., 2006). Alloys of silver and copper have been used for CSV of Ni (Skogvold et al., 2006).

Here a vibrating silver microwire electrode, electrochemically amalgamated with mercury, was used for CSV of Cr^{VI} . The surface is stable and suitable for CSV without requiring re-coating for a week, and its very thin diffusion layer thickness leads to rapid analysis with sensitivity sufficient for environmental monitoring.

6.2. Experimental

6.2.1. Apparatus

Electrochemical measurements were carried out using a μ AutolabIII voltammeter (Ecochemie, The Netherlands) connected to an IME663 interface and a VA663 electrode stand (Metrohm, Switzerland). The working electrode was a 12.5 μm diameter silver microwire (Goodfellow Company, UK), the reference electrode was a double-junction Ag/AgCl, 3 M KCl, and the counter electrode was an iridium wire of 0.15 mm diameter and 2 cm length. The instrument was controlled by the software GPES 4.9 using subtraction mode for analysis. Seawater and nitrate solution used for the experiments were UV-digested using a 125 W high-pressure mercury vapour lamp during 45 min to remove dissolved organic matter prior to use.

6.2.2. Chemicals

All reagents were of analytical reagent grade and all solutions were prepared using Milli-Q deionised water (Elix/Gradient Milli-Q water purification system, resistivity 18 M Ω cm⁻¹). Standard solutions of Cr^{VI} were prepared either weekly or at the beginning of a set of experiments by dilution of metal atomic absorption stock solutions (1000 mg Cr L⁻¹) with water. An aqueous solution of DTPA (Acros Organics) was prepared containing 0.25 M DTPA. The pH was adjusted using a pH buffer containing 3 M ammonium acetate (AnalaR grade, England). Addition of 0.03 M of the ammonium acetate solution to seawater gave a pH of 5.5. An aqueous solution of 5 M sodium nitrate (Fisher Scientific) was employed for the catalytic effect. Contaminating Cr in the nitrate was removed by co-precipitation with 0.1 mM iron(II) chloride followed by filtration (Boussemart et al., 1992). The mercury plating solution contained 2 mM monohydrated mercury nitrate (AnalaR grade, England) acidified with 10 mM HNO₃. Seawater samples (salinity 27 - 32) were collected using a polypropylene hand-pump in the Mersey Estuary in Liverpool Bay during a cruise with the Liverpool University research vessel Marisa in summer 2011. The samples were immediately gravity filtered through a 0.2 μ m cellulose acetate filter cartridge (Sartobran 300, Sartorius) and stored under refrigeration.

6.2.3. Microwire electrode preparation

The fabrication of the silver microwire electrode was similar to that of gold wire electrodes (Salaün and van den Berg, 2006). A copper wire was passed through a 100 μ L plastic pipette tip and dipped in a conductive silver solution. The end of the copper wire was then attached to a short length silver wire (12.5 or 25 μ m) by gently touching it and subsequently withdrawing it into the tip, with \sim 1 mm protruding. The tip was melted by holding 8 s in the mouth of a tubular oven set to 450 °C. Good sealing between plastic and the silver wire ensures long-term stability, which is obvious from a lack of water build-up in the electrode tip and from scans without noise. To obtain a vibrating electrode, a vibrating device was placed in the back of the tip (Bi et al., 2010) using an adapted method (Chapman and Van Den Berg, 2007).

6.2.4. Mercury plating

A scan in 10 mM HNO₃ was performed using cyclic voltammetry (CV) from 0 to +0.4 V to check for normal electrode behaviour, which showed silver oxidation beginning at +0.4 V. Subsequently mercury was added to the same solution to a final concentration of 2 mM Hg and purged with nitrogen during 5 min. Then, mercury was electrochemically plated on the silver microwire at -0.4 V (10 min) (Bi et al., 2013) resulting in a silver-amalgam electrode. The silver-amalgam electrode was used for about one week of experiments and was then replaced by a new one.

6.2.5. *Electrochemical reaction mechanism*

During the deposition step at -1 V dissolved Cr^{VI} is reduced to Cr^{III}. The Cr^{III} forms a complex with the DTPA which is adsorbed at the electrode surface. This mechanism is the same as on an HMDE (Boussemart et al., 1992). Subsequently, a potential scan was carried out to measure the amount of deposited Cr^{III} from its reduction current to Cr^{II} producing a peak at -1.2 V. The current was enhanced by a catalytic effect in the presence of nitrate ions, owing to the chemical reoxidation of Cr^{II} to Cr^{III} which is immediately re-reduced during the scan (Boussemart et al., 1992).

6.2.6. *General procedure to determine chromium*

The sample solution (10 mL) containing 5 mM DTPA, 0.03 M ammonium acetate buffer (pH 5.8 in Milli-Q water and 5.5 in seawater) and 3 mL 5 M nitrate (final concentration ~1.5 M nitrate) were pipetted into the voltammetric cell. The solution was deoxygenated by N₂-purging (5 min). The silver amalgam microwire electrode was activated at -3 V (2 s) (vibration on) and Cr^{III}-DTPA complexes were adsorbed at the deposition potential (E_{dep}) (30 s) (vibration on). E_{dep} was -1.0 V in seawater and -1.1 V in Milli-Q water due to a small difference in pH and peak potential. After a 2s equilibration time (vibrator off) the voltammogram was recorded by applying a differential pulse scan (DP) from -1.0 to -1.35 V. The electrochemical parameters were: interval time 0.1 s, step-potential 2 mV, modulation amplitude 50 mV and modulation time 0.002 s. The background scan used an adsorption time of just 1 s and was otherwise the same as the analytical scan. The background scan was subtracted from the analytical scan to obtain a background-corrected scan. This was automated using the Project function of the GPES software.

6.3. Results and discussion

6.3.1. Preliminary experiments

A peak was obtained for Cr^{VI} in water (seawater and pH buffers) using the silver amalgam electrode in the presence of DTPA and nitrate, under conditions used previously with mercury drop electrodes (Boussebart et al., 1992; Golimowski et al., 1985). Preliminary experiments were performed to optimise the conditions with the silver amalgam electrode for the determination of Cr^{VI} in 0.03 M ammonium acetate buffer (pH 5.8) and subsequently in seawater (pH 5.5). Differential-pulse was selected as stripping mode, as it was found to give better peak shape than the square-wave modulation. Silver microwires of 12.5 μm and 25 μm diameter were plated (10 min in unstirred condition) at various Hg concentrations in the plating solution (from 0 to 5 mM Hg). No signal was detected for Cr^{VI} without Hg on the microwire. The peak height for Cr^{VI} was found to increase with the mercury concentration, producing best response using 2 mM Hg for the 12.5 μm silver wire and 5 mM of Hg for the 25 μm silver wire. However, a high concentration of Hg for the plating solution (5 mM Hg) made the electrode unstable, causing it to break. A possible explanation for this is the gradual dissolution of the silver into the mercury causing it to weaken. For this reason a plating concentration of 2 mM Hg was selected. Comparative scans for 10 nM Cr^{VI} using a fixed concentration of 2 mM Hg for the plating solution, showed that the peak was greater using the 12.5 μm wire (Figure 6.1A), which may have been caused by a greater thickness of Hg on this wire. This wire was selected for further experiments in combination with a plating solution of 2 mM Hg.

The surface of the electrode was re-activated between scans by briefly applying a negative conditioning potential. Variation of this conditioning potential between -1.3 and -3 V (in pH 5.5 buffer in seawater) gave best result for -3 V, as the measurements became poorly reproducible at more negative values. It is likely that all Cr^{III} which is adsorbed on the electrode is reduced to Cr^{II} and then expelled due to hydrogen generation upon switching to -3 V; it is not deposited as Cr^{0} at -3 V as then it would not be removed at -3 V. Variation of the re-activation time between 1 and 10 s gave an optimal time of 2 s. Using this procedure scans were found to be reproducible and stable.

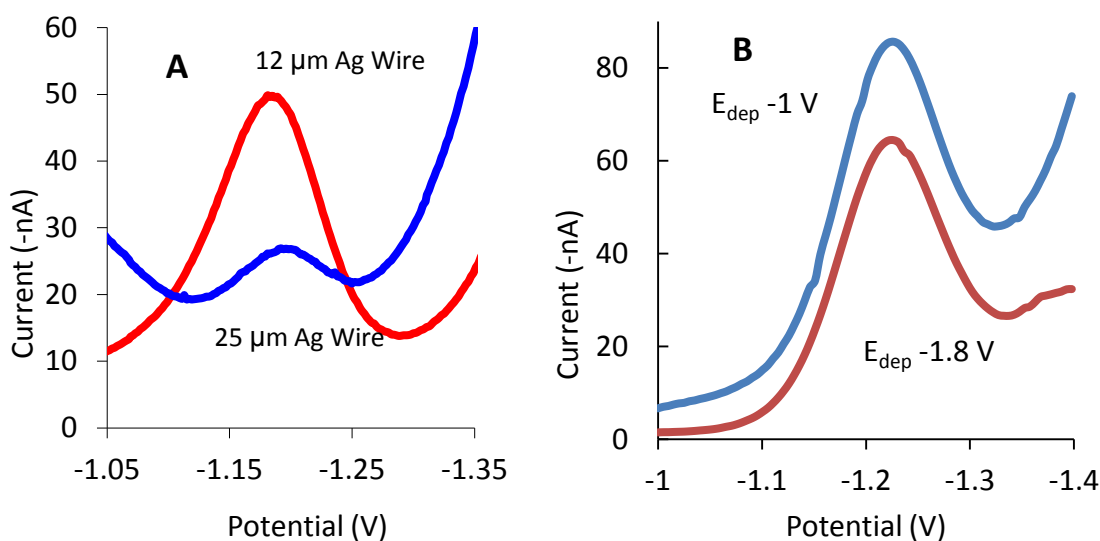


Figure 6.1. Voltammetric scans for Cr^{VI}. A) Effect of the diameter of the silver amalgam wire on the response for 10 nM of Cr^{VI} in pH 5.8 buffer. The silver wires had been coated by plating (10 min at -0.4 V) from a solution containing 2 mM Hg. Deposition time 10 s at -1 V; B) Effect of the deposition potential on the scan shape for 7 nM Cr^{VI} in seawater (pH 5.5). The deposition time was 30 s at the indicated potential using a 12.5 μm silver amalgam wire. Each scan was initiated from -1 V.

6.3.2. Optimisation of analytical parameters (DTPA, nitrate and modulation time)

The effect of varying the concentration of DTPA in pH buffer containing 10 nM Cr^{VI} showed an optimal concentration of 5 mM of DTPA (Figure 6.2 A), which is similar to slightly higher, than that (2.5 mM DTPA) on the mercury drop electrode (Boussemart et al., 1992). At higher ligand concentrations, the Cr^{VI} peak height was found to decrease, possibly due to competitive adsorption of the DTPA without Cr.

6.3.3. Effect of varying the nitrate concentration

The concentration of nitrate was varied at constant levels of Cr and DTPA. The peak height for Cr^{VI} was found to increase linearly with the nitrate concentration (Figure 6.2B). This is to be expected as the current is due to the reduction of nitrate, catalysed by the Cr^{III}/Cr^{II} redox couple: the current therefore is directly dependent on the diffusion of nitrate and therefore on its concentration. The downward curvature at high concentrations of nitrate in Figure 6.2B is due to sample dilution. A nitrate concentration of 1.5 M was used for further experiments (3 mL of 5 M nitrate per 10 mL of sample) to maximise the sensitivity with relatively minor sample dilution. Greater sensitivity could be achieved by increasing the

nitrate concentration further. This concentration of nitrate is 3x higher than used previously using the HMDE (Boussemart et al., 1992) in order to maximise the sensitivity of the silver-amalgam wire electrode.

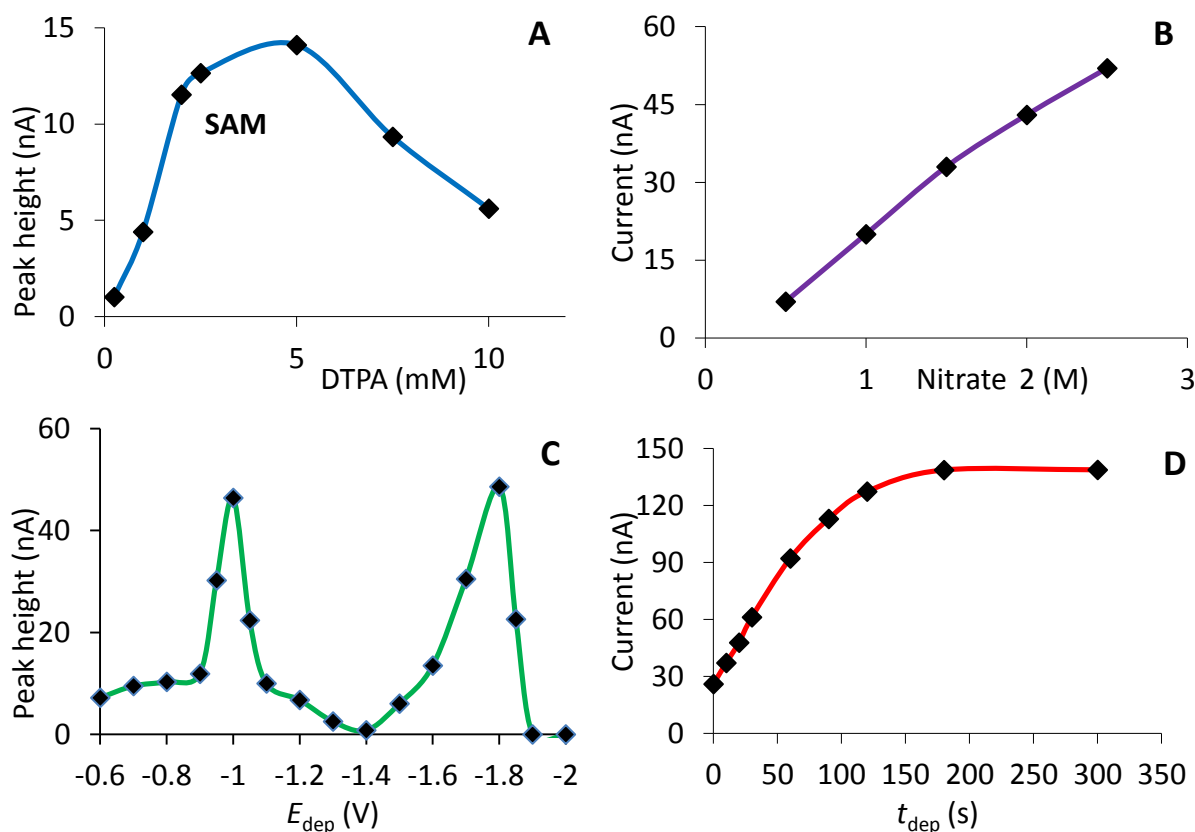


Figure 6.2. Experiments to optimise the voltammetric parameters of CSV using the vibrating amalgamated silver wire electrode of Cr^{VI} in seawater. The solutions contained 5 mM DTPA, 30 mM NH_4Ac , 1.5M NaNO_3 and 10 nM Cr^{VI} unless indicated differently. The deposition time was 30 s with vibration ON using an adsorption potential of -1 V; scans were in the differential-pulse mode after a 2s quiescence time at -1 V. A) Variation of the concentration of DTPA. B) Variation of the concentration of NaNO_3 . C) Variation of the deposition potential on the response for 6 nM Cr^{VI} in seawater. D) Variation of the deposition time on the response of 4 nM Cr^{VI} in seawater after plating at -1.8 V and 5 s equilibration at -1 V.

Preliminary experiments in which the concentration of nitrate was raised were found to give a smaller increase in the peak height, the increase strongly diminishing with greater nitrate concentrations, which was suspected to be due to the presence of interfering organic matter in the nitrate salt. This was removed by UV-digestion of the nitrate stock solution after which the linear response was obtained (Figure 6.2B). It was also found to be necessary to remove contaminating Cr^{VI} in the nitrate (by co-precipitation with Fe-hydroxide after reduction to Cr^{III}) as the background level contributed significantly to the water concentration of Cr^{VI} at low nM levels.

6.3.4. *Effect of modulation time*

The modulation time of the differential-pulse modulation was varied between 0.002 and 0.05 s. The peak height was found to decrease with the increasing modulation time. Highest peak current was obtained with the shortest modulation time of the instrumentation (0.002 s) which was selected for the Cr method. The decreasing response at longer modulation time is probably caused by a depletion of the diffusion layer as the reduction of Cr^{III} -DTPA is diffusion controlled (Sander et al., 2003).

6.3.5. *Optimisation of the deposition potential*

The deposition potential was varied in seawater containing 4 nM Cr^{VI} , from -0.5 to -2 V. Interestingly the response was best at two different potentials (Figure 6.2C): the first maximum was obtained at -1.0 V, which is similar as that found using the older HMDE method (Boussemart et al., 1992; Golimowski et al., 1985). However, at more negative potential a second maximum was obtained at -1.8 V, corresponding to deposition of metallic Cr^0 (Korolczuk and Grabarczyk, 1999). On an HMDE the response is the same after deposition at potentials between -1.5 and -1.7 V (Korolczuk and Grabarczyk, 1999) (more negative potentials were not tested, presumably due to instability of the HMDE). The data show that on the silver amalgam electrode the response is marginally greater at -1.8 V after which it decreases again after deposition at lower potentials. Other ions (major ions in seawater such as Na and Mg) could interfere with the Cr^{VI} deposition at these very negative potentials, which is perhaps the cause for the decrease, or hydrogen generation interferes with the complex adsorption at the negative potentials. The data shows that Cr^{VI} can be deposited on the amalgam silver wire with similar sensitivity at -1.0 V and at -1.8 V: at -1.0 V the deposition is as Cr^{III} -DTPA whilst it is as Cr^0 at -1.8 V. The deposition step at -1.8 V has to be followed by a reoxidation step at -1.0 V to generate the signal for Cr^{III} -DTPA. A repeat of this experiment in pH 5.8 acetate buffer showed that the maximum in response after deposition at -1.0 V shifted more negative to -1.1 V, along with similar shift of the peak potential, due to the slightly higher pH.

Though the peak height showed little difference between deposition at -1.0 and -1.8 V, the baseline under the peak tended to be flatter after deposition at -1.8 V (Figure 6.1B). Also,

measurement of Cr^{VI} in estuarine water samples showed that there was less interference from organic matter using deposition at -1.8 V. A possible reason for this is that organic matter tends to be rejected at negative deposition potentials (< -1 V). For this reason -1.8 V is preferred in the presence of organic matter.

6.3.6. Influence of deposition time

The deposition time was varied to evaluate at which point the wire electrode would be saturated. Using a deposition potential of -1.1 V the response for 4 nM Cr^{VI} in seawater was found to increase until about 60 s after which it first levelled off and then started to decrease. This was possibly caused by competitive adsorption of the free DTPA as this is also known to adsorb (Sander et al., 2003). Interestingly, using a deposition potential of -1.8 V, where the Cr^{VI} is reduced all the way to Cr^0 , the peak height continued to increase for a longer period, then stabilised and did not decrease (Figure 6.2D). The longer gradual increase is due to the continued accumulation of Cr^0 in the silver-amalgam of the electrode: possibly the free DTPA adsorbs less efficiently at this potential.

6.3.7. Linear range and limit of detection

The linear range and limit of detection for chromium was determined in both Milli-Q water and seawater, using ammonium acetate buffer and a deposition time of 30 s. Background subtraction was used to correct the scans for curvature in the baseline using the GPES software of the instrumentation. In Milli-Q water, the response was found to increase linearly with the concentration of Cr^{VI} to ~ 20 nM and non-linearly after that due to electrode saturation. The detection limit in this condition was ~ 0.2 nM Cr^{VI} (from 3 x the standard deviation). In seawater the peak height increased linearly to higher Cr^{VI} (60 nM) than in acetate buffer (Figure 6.3). The sensitivity in the seawater was about 25 % less than that in the acetate buffer. The detection limit for chromium in seawater in this condition was ~ 0.3 nM Cr^{VI} . The detection limits found here for MQ and seawater are higher than those found previously using the HMDE (0.01 nM Cr^{VI} in MQ and 0.1 nM Cr^{VI} in seawater) using the HMDE (Boussemart et al., 1992). The difference would be greater if the same concentration of nitrate had been used (1.5 M nitrate was used by the silver amalgam wire, whereas the previous HMDE work used 0.5 M nitrate). The reason for the higher limits of detection using

the solid (silver-amalgam) electrode is probably related to interference by dissolved organic matter that adsorbs on the electrode surface in spite of the negative potential (-3 V) used to regenerate the electrode between scans. A completely fresh surface is obtained prior to each measurement using the HMDE, which contrasts with the regenerated surface of the silver-amalgam microwire.

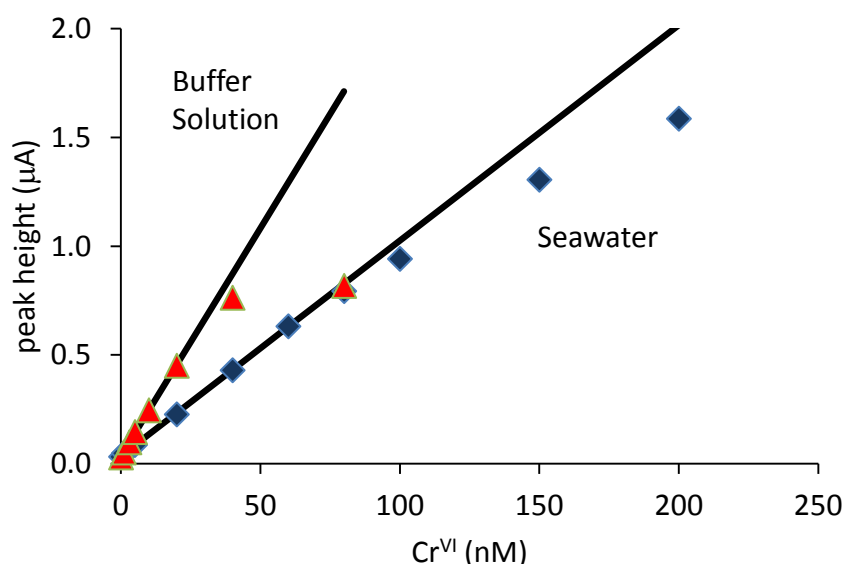


Figure 6.3. Response as a function of the concentration of Cr^{VI} in acetate pH buffer (A) and in seawater (B). Deposition 30 s at -1.1 V (A) and -1.8 V (B).

The sensitivity using the silver-amalgam wire is sufficient for the monitoring of Cr in fresh and coastal waters (typical concentrations of 2-4 nM Cr). The measurements using the silver-amalgam wire are quicker than using the HMDE because of the thin diffusion layer thickness (~1 μm) (Salaün et al., 2012). The response on the wire became non-linear after a deposition time of ~30 s at -1.1 V, which was extended to > 60 s at -1.8 V. It becomes non-linear after 1-2 min on the HMDE (Bobrowski et al., 2011; Boussemart et al., 1992), which was only slightly longer. So, an advantage of using a microwire is a shorter analysis time, but the overall sensitivity is less than achievable using the HMDE.

Table 6.1. Determination of Cr (nM) by CSV using the silver-amalgam electrode in water samples from the high salinity end of the estuary of the River Mersey (England). The samples were UV-digested prior to analysis. Samples stored at natural pH were analysed the day after sampling. Due to adsorption of Cr^{III} on the bottle walls these values represent the concentration of Cr^{VI}. Two samples were stored acidified to pH 2.2 to obtain the total dissolved Cr (Cr_T = Cr^{III} + Cr^{VI}); these were neutralised to neutral pH prior to UV-digestion.

Station	1	2	3	4	5	6
Natural pH (Cr ^{VI})	1.5 ± 0.1	1.7 ± 0.1	1.9 ± 0.1	2.3 ± 0.2	2.2 ± 0.1	2.2 ± 0.1
Acidified (Cr _T)	2.7 ± 0.1	2.5 ± 0.3				
Salinity	31.8	31.3	30.2	29.9	28.8	27.3

6.3.8. Application to seawater samples from Liverpool Bay

The method was tested on water samples collected from the estuary of the river Mersey. The samples had been UV-digested (45 min) at natural pH to remove organic matter and also to convert Cr^{III} to Cr^{VI} (Boussemart et al., 1992). In view of the particle-reactivity of Cr^{III} and the high particle load of the Mersey estuary, it is likely that most dissolved Cr in the estuary is Cr^{VI}. Any Cr^{III} in the water is likely to adsorb on the bottle walls upon storage, and would not be recovered by the UV-digestion. The filtered samples that were stored at natural pH therefore represent Cr^{VI}. The results (Table 6.1) show a concentration of Cr^{VI} between 1.48 nM and 2.29 nM. Some samples were stored acidified; the pH of these was neutralised immediately prior to UV-digestion and analysis, to obtain total dissolved Cr (Cr^{III} + Cr^{VI}). The total Cr in the acidified samples was greater than that in the samples stored at natural pH, indicating that samples should be stored acidified to prevent losses of Cr^{III}. The concentrations found are typical for clean coastal waters (Apte et al., 1998). It was attempted to determine Cr^{VI} in these samples without UV-digestion but this was not possible due to interference by dissolved organic matter. Separate experiments showed that measurement of Cr^{VI} without UV-digestion was possible using a HMDE (data not included) when a negative deposition potential (-1.8 V) was used due to better sensitivity with the HMDE.

6.4. Conclusions

The work shows that CSV of Cr^{VI} can be carried out using a silver amalgam microwire electrode. The solid electrode has advantages over the mercury drop electrode in that it should be easy to use as basis for a flow cell, and that very little mercury is released into the environment. Testing of the electrode on estuarine water samples showed that the wire electrode is sufficiently sensitive to monitor Cr in coastal waters. The work is an example of a replacement of a mercury drop based method to a solid electrode. It may be possible to apply the mercury drop substitution with the silver-amalgam wire to other metals that are detectable by CSV. A drawback is that the method has a higher limit of detection than the original method based on the mercury drop electrode.

Acknowledgements

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7

Chapter 7

General Conclusions & Future Perspectives

7. Conclusions and Future Perspectives

7.1 Conclusions:

- 1) Bismuth rods can be used as solid electrode for the determination of Pb and Cd in seawater. Bi electrode is insensitive towards the presence of dissolved oxygen agreeable for in-situ analysis. While it was found to suffer from the interference of copper and chloride. Moreover, its low sensitivity (LoD: 0.14 nM Pb using 10 min deposition) was not suitable for low metal concentration determination.
- 2) Comparison of microwire electrodes consisting of carbon, gold and silver, showed that none of these gives good separation between Pb and Cd in seawater. However, after coating with mercury, any of them can be used to determine Pb and Cd in seawater. However, the mercury coating is not stable on the carbon fibre, falling off when the electrode is transferred between solutions, which means that this electrode is not suitable for in-situ determinations without in-situ plating of the mercury coating. Gold wire was found to have a problem of being fragile due to high solubility of gold in mercury. Silver was found to be the most suitable material for Pb and Cd analysis in seawater even at pM level because of high stability and sensitivity (LoD: 0.012 nM, using a 10min deposition at natural pH for Pb). The limit of detection using the SAM electrode is very low in acidified water (0.018nM Pb and 0.074 nM Cd, using a 2 min plating time).
- 3) Because of its stability and high sensitivity for Pb, the SAM electrode was applied for Pb speciation analysis using pseudopolarography in uncontaminated seawater. The results showed that the majority of the Pb occurs in the labile part of the

pseudopolarogram: this means that the Pb is in reversible complexes and relatively readily available for adsorption on particles, and uptake by biota, as these species readily dissociate. A small fraction (< 22 % in the ocean sample, < 8 % in a coastal sample, and < 5 % in an estuarine sample) was found to occur strongly bound in inert species.

- 4) SAM electrode was also observed to be suitable for chromium determination using CSV in natural waters. A deposition at very negative potential was applied to reduce the organic interference. A comparable performance was found on SAM to the classic mercury drop electrode for Cr analysis.

7.2. Future Prospectives:

Since the electroanalytical performance mainly depends on the working electrode material, a suitable electrode with high sensitivity and stability, easy to fabricate and maintain, is in need for metal speciation analysis in natural environment. Combined with the vibrating system, bismuth film microwire electrodes such as Bismuth/Carbon Fibre, Bismuth/Gold, Bismuth/Silver, and mercury coated bismuth electrode need to be tested for Pb and Cd determination in the marine system.

Hydrogen evolution on electrode surface can confine the application of electrode. Thus, practical and theoretical approaches would be necessary to study the H^+ -metal binding on different metals. Future work would be carried out to find out the factors that interference the H_2 gas formation.

Pseudopolarography is successful to explain the lead species in seawaters. However, it is weak at distinguishing the weak complexes among the labile fraction. CSV which has been widely used on the mercury drop electrode to study metal speciation would be introduced together with the pseudopolarography.

Because the SAM electrode is very suitable for in-situ analysis, it would be applied in an in-situ equipment used in the field. Other chemicals would be tested in natural waters, such as iodine, nitrate and iron.

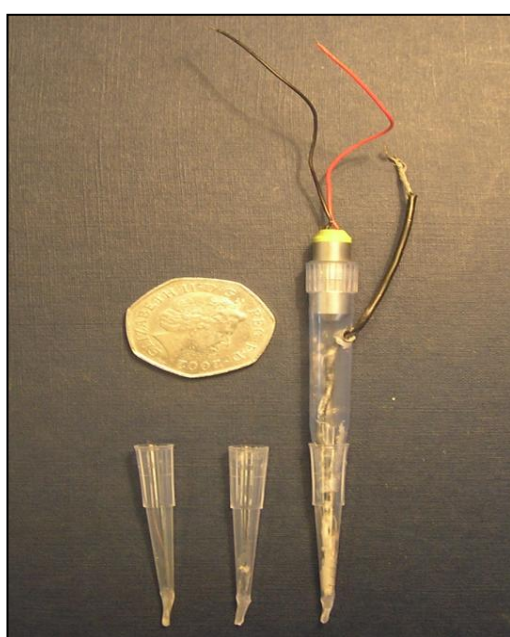
ANNEX

Annex I. Electrode Fabrication Details

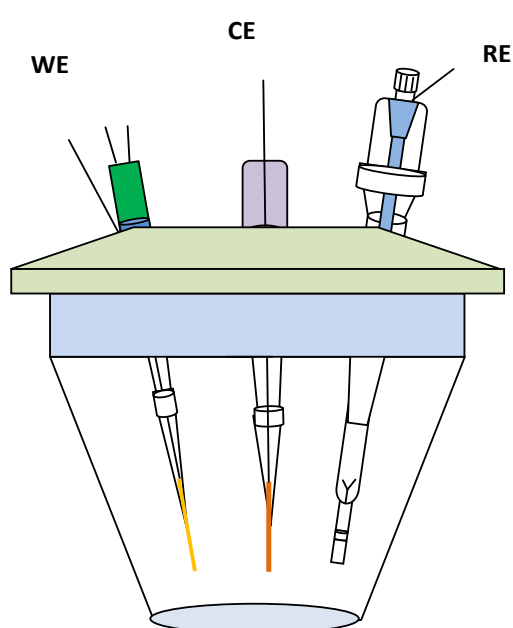
The vibrating disk and microwire electrodes consist of electrodes sealed in a pipette tip, which can be attached to a vibrating device. The electrode tip can be easily changed. The details of fabrication are shown below:

- (1) 5 cm length of 100 μm copper wire is passed through a 100 μL pipette tip (polyethylene, Fisher scientific, UK). The end of the copper wire is then dipped in a conductive silver resin (Leitsilber L100, Maplin, UK), which had been freshly agitated and acted as conductive adhesive used to create a connection between the electrode wires and Cu wire. The copper wire is then carefully pulled through the pipette tip until the electrode wires and Cu wire connection is at the tip of the pipette.
- (2) Seal the electrodes and Cu wire together in the tip by holding it for several seconds in the homemade oven (200~450 $^{\circ}\text{C}$), recheck the sealing till only electrode material is exposed ($\sim 2\text{mm}$)
- (3) The electrode tip can then be fitted onto a 1mL polypropylene pipette tip set. A cable through the 1mL pipette tip connects the Cu wire in the 100 μL tip and the WE cable
- (4) The vibrator is incorporated at the top of the 1mL pipette tip and it is driven by a 1.5V power supply.

The electrode size and Cell system are shown below: (A): Electrode (B): Cell System

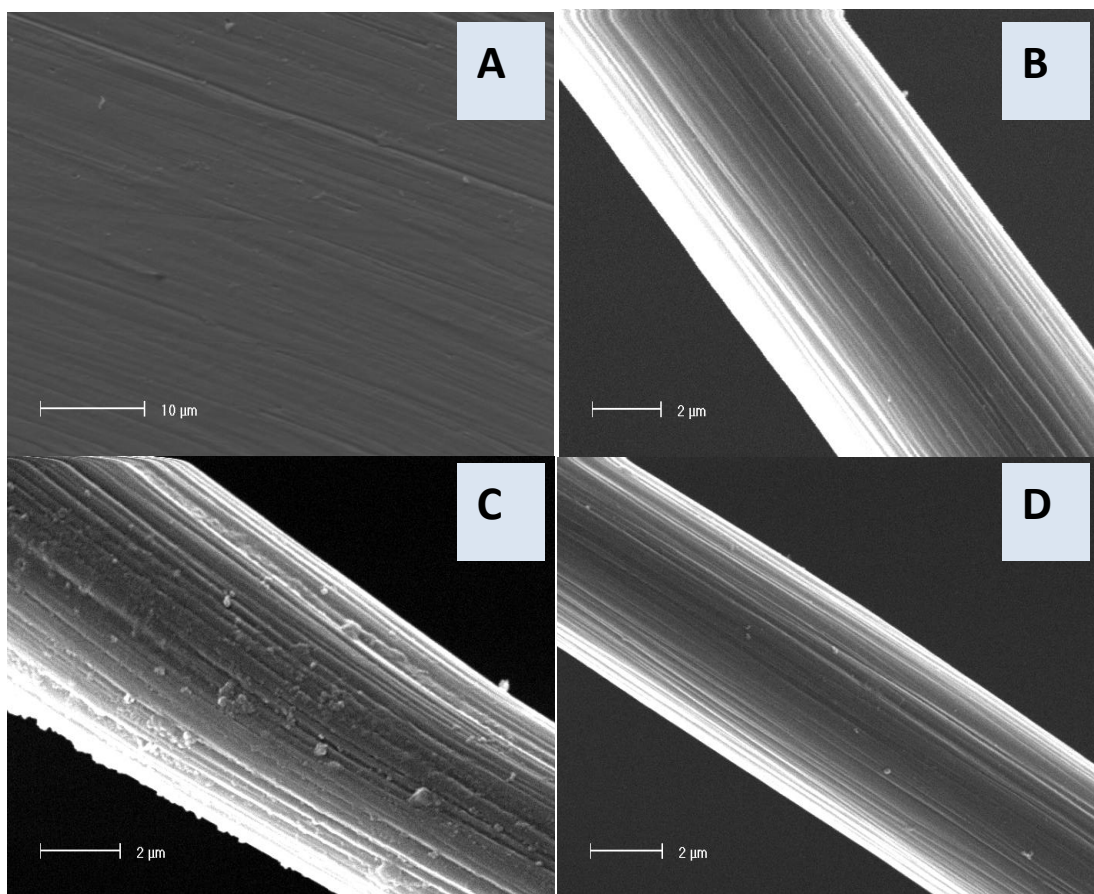


(A)



(B)

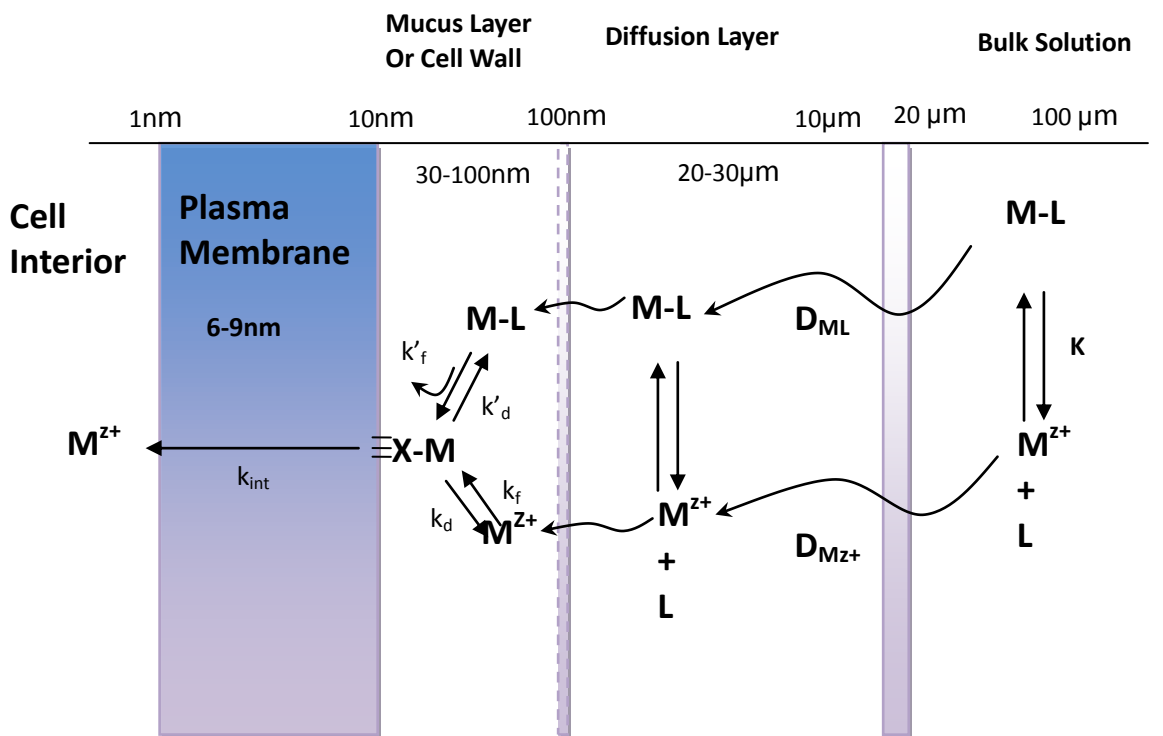
Annex2. Electrode Surface Condition by SEM



Bismuth electrode needs polishing before use. The polishing effect was checked by SEM (Photo A). The electrode surface is even and a little scratch is helpful to improve the sensitivity found in the experiment.

Carbon Fibre electrode was found to lose mercury during analysis. So, the mercury attaching stability was checked by SEM (Photo B to D). (B) A clean new carbon fibre before use. (C) Carbon fibre coated with mercury; (D) Mercury is removed using KSCN. These photos exhibit that a little mercury is still on the electrode even exposed to air and KSCN acts very effectively to remove the mercury from the electrode.

Annex3. Model of Metal Uptake by Organisms



M^{z+} : free metal ion

M-L: metal complex

M-X-membrane: surface metal complex

k_f, k'_f : rate constant for formation of the surface complex

k_d, k'_d : rate constant for dissociation of the surface complex

k_{int} : rate constant for internalization or transport of the metal across the biological membrane

Step of metal uptake:

- (1). Metal transports by advection or diffusion onto biological surface
- (2). Metal pass through a 'protective layer'
- (3). Adsorption or surface complexation binds at sites out of the plasma membrane
- (4). Final uptake or internalization

(From: Peter G.C. Campbell, 'Interaction between Trace Metals and Aquatic Organisms: A Critique of the Free-ion Activity Model' in Book: 'Metal speciation and bioavailability in aquatic systems' by A.Tessier and D.R.Turner, (1995), Wiley, Chichester, P47.)