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Square wave anodic stripping voltammetry determination of eco-toxic metals in samples of biological and environmental importance

Invited Paper

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Abstract: Square wave anodic stripping voltammetry was used in simultaneous determinations of eco-toxic metals (Pb, Cd, Cu and Zn) on bismuth film electrodes. The electrodes were prepared in situ on a glassy-carbon electrode (GCE) from 0.1 M acetate buffer (pH 4.5) containing 200 μg L^{-1} of bismuth (III), as well ex situ on electrochemically oxidized graphitized polyacrylonitrile carbon fibres from 200 mg L^{-1} $Bi(NO_3)_3$ in 1% HNO_3 (aqueous) solution. Preparation of a Bi-modified carbon fibre electrode (CFE) was by cation exchange of Bi^{+3} ions for H^+ of the acidic surface groups of the electro-oxidized carbon fibres, followed by electrochemical reduction to Bi^0. For the Bi-GCE the linear range was 20 - 280 μg L^{-1} for zinc, 10 - 100 μg L^{-1} for lead, 10 - 80 μg L^{-1} for copper, and 5 - 50 μg L^{-1} for cadmium. For the Bi-CFE it was 20 - 160 μg L^{-1} for zinc, 10 - 100 μg L^{-1} for lead, 10 - 100 μg L^{-1} for copper, and 2 - 120 μg L^{-1} for cadmium. For both kinds of bismuth modified carbon electrodes, low limits of detection and satisfactory precision were achieved. The method was successfully applied to certified reference materials of biological (bovine liver) and environmental (mussel tissue) importance.

Keywords: *Bismuth-film electrodes • Glassy carbon • PAN-based carbon fibres • Bovine liver • Mussel tissue*

1. Introduction

Metals such as Pb(II), Cd(II), Zn(II) and Cu(II) as well as some organics have been determined by anodic stripping voltammetry (ASV) on bismuth electrodes. Glassy carbon discs [1-15] carbon pastes [16], and screen-printed carbon inks [17-20] are often recommended as supports for Bi-FEs used in the square wave anodic stripping voltammetric (SWASV) mode. Bismuth can also be configured as the bismuth bulk electrode (Bi-BE) [21]. However, few reports consider carbon fibre electrodes (CFE) [22,23]. Their small size (7 µm diameter) permits measurements at low concentrations and in agitated solutions where charge transfer reactions are favored.
Metal electro-deposited on glassy carbon as a working electrode in SWASV is well known. Hence, emphasis here is on Bi-modified electrode preparation. Bi-FEs were prepared in situ on a glassy-carbon electrode (GCE) as well ex situ on electrochemically oxidized graphitized polyacrylonitrile carbon fibres. This oxidation formed carboxylic and phenolic groups, resulting in cation-exchange capacity. Bi^{+3} was cation-exchanged with H^+ ions then electrochemically reduced to Bi^0.

The GCE offers some advantages in addition to better resistance to water or organic solvents. By in-situ plating Bi on the GCE surface, analysis time is greatly reduced and the procedure simplified, as the initial Bi(III) preconcentration is omitted.

The use of such precise and sensitive electrodes in SWASV gives an opportunity to perform real-time measurements in ecology, bio-technology, clinical chemistry, and food chemistry. Heavy metals accumulated in organs such as liver or kidney can coordinate to proteins, lipids, and other components,

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permitting the study of enzymes' kinetic, structural or even immunological properties [31].

One of the major problems in ecology is the path of heavy metals in the aquatic environment. They may enter the food chain and accumulate in marine plants to endanger organisms like mussels, clams or shrimp, which concentrate them [24,25]. Analyses of these creatures serve as bio-indicators of metal pollution [26-30]. Such species can also be used in bio-detoxification and for checking high quality food [29].

2. Experimental Procedure

2.1. Apparatus

Stripping voltammetric measurements were carried out using the AUTOLAB modular electrochemical analysis system equipped with PGSTAT 30-Type III module and driven by a GPES 4.9 software package (Eco Chemie, Ultrecht, Netherlands) on a personal computer. Glassy-carbon disk electrodes (3 mm diameter) and electro-oxidized carbon fibres (bundles of highly oriented PAN-based fibres from BASF-Celanese Corp. U.S.A., type Celion GY-70, bundle length 3 cm, ca. 200 fibres with 7 μm diameter) served as supporting electrodes for the working Bi-FEs. The geometric surface of 1 mg bare or electrochemically oxidized carbon fibres was estimated from the data in Table 1, assuming a roughness factor of unity. The electrochemically active surface (1.2 cm^2) was determined from the peak currents of the reversible reaction

$$[Fe(CN)_6]^{3-} + e^- \rightarrow [Fe(CN)_6]^{4-}$$

performed on these electrodes and on a smooth Pt wire of known effective area, measured in the same solution as used for the experiments.

Table 1. Properties of PAN-based carbon fibers

Carbon fiber characteristics	Celion GY-70
Tensile strength (Gpa)	3.65
Tensile modulus (Gpa)	231
Density (g cm^{-3})	1.76
Filament diameter (μm)	7
Carbon assay (%)	92
Filaments per strand	3000
Linear density (g m^{-1})	0.198
Geometric surface (cm^2 mg^{-1})	3.3
Electrochemically active surface (cm^2 mg^{-1})	1.2(1)

(1) *Calculated by cyclic voltammetric measurements*

An Ag/AgCl electrode (saturated KCl) and a platinum wire served as reference and counter electrodes. Magnetic stirring was used as needed.

Potentiostatic square-wave pulses for the fibre oxidation were applied from a Bank-Elektronik double-pulse control generator; current was recorded with a Biomation Model 805 waveform recorder and a Hitachi VC 6025 digital storage oscilloscope. Details of the oxidation and the electro-oxidized carbon fibre structure have been presented elsewhere [32].

Cyclic voltammetric measurements were performed in a conventional three compartment cell with the compartments separated by sintered glass. The working electrode was carbon fibres immersed to a depth of 2.5 cm. A 2 cm^2 platinum wire was employed as the counter-electrode and a saturated calomel reference electrode (SCE) was placed close to the cathode through a Luggin capillary.

2.2. Reagents and solutions

All solutions were prepared with water purified by an Elix/Milli-Q system (Gradient, Millipore, Bedford, USA). The bismuth(III), lead(II), cadmium(II), zinc(II) and copper(II) standard solutions were obtained from pro analysi chemicals and diluted from standard stock solutions (1000 mg L^{-1} in 5% HNO_3, atomic absorption standard solutions). Hydrogen peroxide (30 volume %) was pro analysi, Merck. The supporting electrolyte was 0.1 M acetate buffer (pH= 4.5), prepared from CH_3COOH and NaOH. Methanol and acetone used for washing fibres were from Merck (Darmstadt, Germany). Electro-oxidation of the carbon fibres was carried out in aqueous Na_2SO_4 (pro analysi, Merck). Cation exchange was performed in $Bi(NO_3)_3{\cdot}5H_2O$ - HNO_3 (Merck). Electro-reduction of the exchanged Bi^{3+} ions on the oxidized fibres was done in 0.01 M $KHC_8H_4O_4$ - HCl, pH=4 buffer. This buffer was prepared from $KHC_8H_4O_4$ (≥99.5%, Sigma-Aldrich, Seize, Germany) and HCl (37%, Sigma-Aldrich). Acids used in the digestion (HNO_3, $HClO_4$ and H_2SO_4) were from Sigma-Aldrich. All chemicals used in this work were of analytical grade.

2.3. Samples

The technique was successfully applied to the determination of lead, cadmium, zinc and copper in biochemically and environmentally important certified reference materials: mussel tissue No.668, sample No.0096 (ERM®); mussel tissue No.278, sample No.1896 (ERM®) and bovine liver No.185, sample No.1242 (BCR®), using both kinds of Bi-FE.

Pre-treatment was necessary as SWASV is susceptible to errors from organic matter [33,34]. The matrices were dissolved in acid (HNO_3-$HClO_4$-H_2SO_4) (section 4.1) then diluted, as the analyte concentrations were high. The standard additions method was employed for the determination of Pb(II), Cd(II), Zn(II) and Cu(II).

2.4. Ex situ bismuth film deposition on the carbon fiber electrode (Bi-CF)

To achieve a constant electrode surface area the bundles were washed with 1:1 methanol-acetone for 3 h. They were next electro-oxidized in 1 N aqueous Na_2SO_4 by potentiostatic double pulses with E_{ox} = 2.3 V *vs.* SCE, E_{red} = -0.3 V, t_{ox} = 4.5 min, t_{red} = 0.75 min, t_{total} = 6 min [32]. Cation exchange was performed in aqueous 200 mg L^{-1} $Bi(NO_3)_3$ - 1%HNO_3. Exchanged Bi^{+3} electro-reduction was done in aqueous 0.01 M $KHC_8H_4O_4$ - HCl, pH=4, while the potential was swept from −2 to +2 V *vs.* SCE at 100 mV s^{-1}.

Fig. 1 is a representive cyclic voltammogram of the Bi modified electro-oxidized fibres. Current densities are expressed per mg of carbon fiber bundle. Cathodic peak (a) corresponds to the reduction of Bi^{+3} (at -0.45 V) while anodic peak (b) corresponds to the oxidation of Bi^0 to Bi^{+3} (at + 0.5 V). The system behaves almost reversibly. As can be seen, the oxidation peak is higher than the deposition peak because the kinetics of metal oxidation are faster than metal deposition.

2.5. SWASV procedure

Measurements were carried out by in situ co-deposition of a bismuth film with the target metals onto a glassy-carbon disk supporting electrode in the presence of dissolved oxygen, followed by square wave anodic stripping voltammetry.

The GCE was first well-polished on a felt pad with alumina slurry (0.3 μm). The three electrodes were immersed into a 20 mL electrochemical cell containing 0.1 M acetate buffer (pH 4.5) along with 200 μg L^{-1} of bismuth. The bismuth-modified CFE was prepared ex situ; the electrochemical cell then contained only buffer. After metal preconcentration hydrogen peroxide (0.02% v/v for the GCE and 0.10% v/v for the CFE) was added to the cell. Stirring was applied for 150 s before deposition and during film formation. For both Bi-FEs the SWASV parameters were: an initial potential of -1.4 V; a switching potential of +0.3 V; a deposition potential of -1.4 V for a deposition time of 180 s; a quiet time of 10 s; a SW amplitude of 0.020 V; a SW frequency of 50 Hz; a potential step of 0.005 V. Prior to each measurement, a “cleaning” step was applied at +0.3 V (for the GCE) or at -1.5 V (for the CFE) for 30 s to the stirred buffer solution. All experiments were carried out at room temperature (25±1ºC).

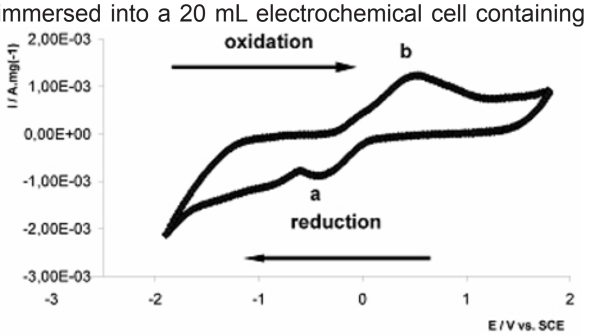

Figure 1. Cyclic voltammogram (υ = 100 mV s^{-1}) of Bi-modified-electro-oxidized carbon fibres in 0.01 M $KHC_8H_4O_4$ - HCl, pH=4. (a) $Bi^{+3} \rightarrow Bi^0$, (b) $Bi^0 \rightarrow Bi^{+3}$. Electrode modification: by dipping the electrochemically activated carbon fibres in 200 mg L^{-1} $Bi(NO_3)_3 \bullet 5H_2O$ - 1% HNO_3 for 24 h. Electrode weight: 2.0 mg / 2.5 cm (depth). Oxidation current (start): 19 μA, oxidation current (switching):14 μA.

3. Results and Discussion

3.1. Experimental parameters

Several parameters were varied to optimize sensitivity and precision. For the GCE, the bismuth concentration was optimized on the bases of peak height and separation: 200 μg L^{-1} bismuth worked well for both low and high (10 - 200 μg L^{-1}) metal concentrations. The effect of varying amounts of H_2O_2 on Cu(II) peak current was studied using 10, 50 and 100 μg L^{-1}Cu(II), Zn(II), Pb(II) and Cd(II). To remove Bi(III) interference in the determination of Cu(II) at the CFE, 0.10% v/v H_2O_2 was required; 0.020% v/v H_2O_2 was used for the GCE. The effect of preconcentration potential was studied over -1.8 - (-1) V. The optimum for both electrodes was -1.4 V. Finally, the peaks increased linearly with preconcentration time up to 180 s.

A cleaning potential at +0.3 V was used for the Bi-GCE. For the CFE, the bismuth before and after “cleaning” at +0.3 V was 4.0 and 3.70% w/w, determined by electrothermic atomic adsorption spectroscopy. However, a trial employing a cleaning potential at –1.5 V (more negative than the Bi oxidation potential) gave 3.95% bismuth, showing that only a small quantity of Bi was stripped from the electrode surface. Thus, a “cleaning” potential at -1.5 V was adopted for subsequent analysis using the Bi-CFE.

3.2 Optimization of square wave voltammetry parameters

The effects of frequency and potential step were studied over 12.5 – 200 Hz and 0.001 - 0.020 V, respectively, while the amplitude was examined from 0.010 - 0.080 V. The best compromise among sensitivity, peak sharpness

Table 2. Accuracy and precision of zinc, cadmium, lead and copper determinations in spiked 0.1 M acetate buffer (pH 4.5). [1]

Analyte	Added (μg L^{-1})	Carbon Fiber Electrode (CFE)					
		Found±S_d[2] (μg L^{-1})	S_r(%) [3]	R%	Found ±S_d[2] (μg L^{-1})	S_r(%) [3]	R%
		Within – day (n=6)			Between – day (n=6)		
Cadmium	5	5.9±0.1	1.7	118.0	5.2±0.1	1.9	104.0
	50	55.4±1.1	2	111.0	50.3±1.0	2	101.0
	200	186±4.3	2.3	93.0	181±3.8	2.1	90.5
Lead	5	6.0±0.1	1.7	120.0	5.1±0.1	2	102.0
	50	54.6±1.4	2.5	109.0	48.8±0.8	1.6	97.6
	200	193±3.9	2	96.5	183±3.2	1.7	91.5
Zinc	5	6.2±0.3	4.8	124.0	5.6±0.2	3.6	112.0
	50	60.2±2.3	3.8	120.0	54.2±2.0	3.7	108.0
	200	182±3.4	1.7	91.0	179±2.8	1.6	89.5
Copper	5	6.3±0.2	3.2	105.0	5.8±0.3	5.8	122.0
	50	53.2±1.6	3	106.0	51.2±1.0	1.9	102.0
	200	188±2.8	1.5	94.0	181±2.1	1.2	90.5
		Glassy Carbon Electrode (GCE)					
Analyte	**Added (μg L^{-1})**	**Found ±S_d[2] (μg L^{-1})**	**S_r(%) [3]**	**R%**	**Found±S_d[2] (μg L^{-1})**	**S_r(%) [3]**	**R%**
		Within – day (n=6)			**Between – day (n=6)**		
Cadmium	5	5.4±0.3	5.6	108.0	5.0±0.1	2.0	100.0
	50	52.0±0.7	1.3	104.0	51.3±0.8	1.6	103.0
	200	193±3.1	1.6	96.5	189±3.0	1.6	94.5
Lead	5	5.8±0.2	3.4	116.0	5.2±0.3	5.8	104.0
	50	53.1±1.0	1.9	106.0	50.4±0.4	0.8	101.0
	200	195±3.2	1.6	97.5	187±2.6	1.4	93.5
Zinc	5	6.8±0.3	4.4	136.0	5.8±0.2	3.4	116.0
	50	57.2±2.0	3.5	114.0	55.6±1.4	2.5	111.0
	200	191±3.0	1.6	95.5	188±2.6	1.4	94.0
Copper	5	6.0±0.3	5.0	120.0	5.5±0.2	3.6	110.0
	50	54.0±1.2	2.2	108.0	52.2±0.9	1.7	104.0
	200	193±2.5	1.3	96.5	188±1.9	1.0	94.0

[1] Experimental Conditions: Preconcentration potential -1.4 V for a preconcentration time of 180 s; supporting electrolyte: 0.1 M acetate buffer (pH 4.5) containing 200 μg L^{-1} bismuth (for GCE); see section 2.5 for other experimental details.
[2] Standard addition method was used for analyte estimation.
[3] Six replicate measurements.

and background was obtained at SW frequency 50 Hz; potential step 0.005 V; SW amplitude 0.020 V for both electrodes.

3.3. Analytical characteristics

3.3.1 Linearity

The linearity was investigated for both electrodes by increasing concentration levels from 2-300 μg L^{-1}. The optimized settings were: 0.1 M pH 4.5 acetate buffer (containing 200 μg L^{-1} Bi(III) for GCE), a deposition potential of -1.4 V for a preconcentration time of 180 s, square wave stripping frequency of 50 Hz, potential step of 0.005 V, amplitude of 0.020 V and electrode cleaning +0.3 V for 30 s (for the GCE) or -1.5 V (for the CFE). The utility of bismuth-film electrodes for simultaneously measuring lead, cadmium and zinc is illustrated in Fig. 2. Cu(II) was successfully determined in the presence of Bi(III) with both GCE and CFE electrodes. Fig. 2 displays the calibration plots and regression parameters.

The voltammograms showed well-defined SWASV signals for each metal, linear with increasing concentrations. In addition, the overall peak resolution remained constant over the course of the additions. Increasing metal levels had no effect on the highly reproducible bismuth peak. Cadmium and lead do not compete with bismuth for surface sites, but form binary alloys with bismuth. For both electrodes, H_2O_2 allowed interference-free determination of Cu(II) in the presence of Bi(III) due to the large shift in the re-dissolution copper peak (from -0.06 V to +0.212 V), yielding separation from the wide Bi(III) peak [15]. As the copper concentration increased, the zinc signal was suppressed by deposition of a zinc - copper intermetallic compound. This most affected the zinc peak. Thus the zinc calibration plot presented a very low slope and a low sensitivity on both electrodes (Fig. 2).

3.3.2 Limits of detection (LOD)

The detection limits were estimated by adding 3 times the standard deviation (S_d) of the blank to its average ($\overline{x_b}$ + 3 x S_d), utilizing the optimized settings above.

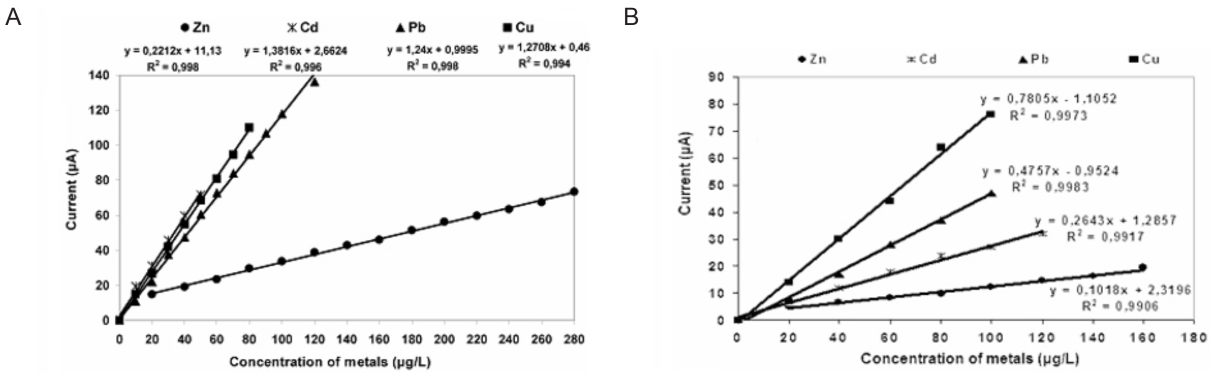

Figure 2. Calibration plots for increasing concentration of (A) copper, lead, cadmium in 10 μg L^{-1} steps and of zinc in 20 μg L^{-1} steps at the GCE and of (B) copper, lead, cadmium and zinc in 20 μg L^{-1} steps at the CFE.

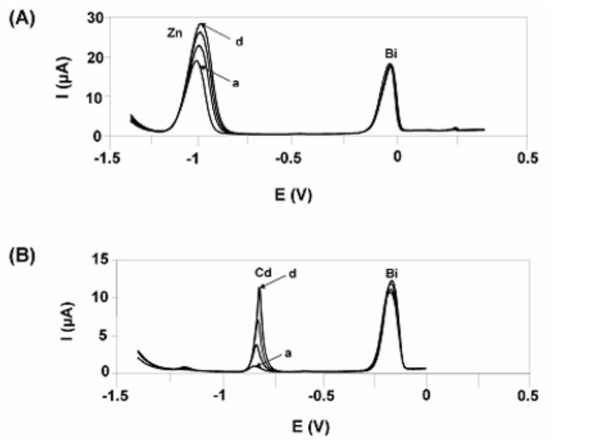

Figure 3. Measurements of (A) Zn(II) and (B) Cd(II) in mussel tissue ERM-CRM 668. Square-wave stripping voltammograms show a mussel tissue sample (a) and three standard additions (b - d) of 5 μg L^{-1} of each metal. Preconcentration potential -1.4 V for a preconcentration time of 180 s; "cleaning" for 30 s at +0.3 V (for the GCE) or at -1.5 V (for the CFE). Other experimental conditions as described in sections 2.5 and 4.1.

For Bi-GCE the detection limits were: zinc (2.80 μg L^{-1}), cadmium (0.10 μg L^{-1}), lead (0.40 μg L^{-1}) and copper (0.20 μg L^{-1}). For Bi-CFE they were: zinc (4.10 μg L^{-1}), cadmium (0.30 μg L^{-1}), lead (0.50 μg L^{-1}) and copper (0.60 μg L^{-1}). The determination of the zinc detection limit presented difficulties due to both its presence in the supporting electrolyte and its interaction with copper.

3.3.3 Reproducibility and precision

The recovery and precision were tested by determining the metals in a spiked 0.1 M acetate buffer (pH 4.5). Measurements were made in low, middle and high concentrations (5, 50 and 200 μg L^{-1}) in non-deaerated medium, employing a 180 s preconcentration time and 10 repeat scans. For the precision investigation, spiked buffers were analysed six times sequentially, and samples of 5, 50 and 200 μg L^{-1} were analyzed six times daily for a week.

Within-day and between-day precisions are presented in Table 2. Relative standard deviation (S_r%) values range between 1.2 – 5.8% for the CFE, and 1.0 – 5.8% for the GCE, while the average recovery values range between 89.5 - 124% for the CFE and 93.5 - 120% for the GCE.

We also found that mechanical polishing to remove the oxidized bismuth layer and initialize the Bi-GCE surface was only necessary after the electrode had been exposed to air for more than about 30 minutes. However, when the Bi-GCE was continuously immersed it was possible to perform reproducible consecutive stripping measurements by employing an electrochemical clean-up procedure only. This simply required maintaining the potential at + 0.3 V (*vs.* Ag/AgCl) for about 30 s after each stripping step.

Mechanical polishing of the Bi-CFE was not feasible. However, if it was continuously immersed in solution reproducible measurements were achieved by applying a simple "cleaning" step, holding the potential of the working electrode at -1.5 V for 30 s.

4. Analytical applicability

After obtaining encouraging calibration results for simultaneous SWASV measurement of trace Cd(II), Pb(II), Cu(II) and Zn(II) in buffer solution, the practical analytical applicability of the electrodes was investigated using low-volume samples with complex environmental and biological matrices.

4.1. Determination of zinc, cadmium, lead and copper in ERM mussel tissue certified reference materials

When complex matrices are analyzed by SWASV organic interferences must be removed prior to analysis [35-38].

Table 3. Determinations of Cd, Pb, Zn and Cu in mussel tissue certified reference materials ERM-278 and ERM-668.[(4)]

Standard	Analyte	Glassy Carbon Electrode (GCE) Determined value ±Sd (mg Kg^{-1})	S_r (%)	Certified value ±Sd (mg Kg^{-1})
Mussel tissue (ERM-278)	cadmium	0.300±0.005	1.7	0.348±0.007
Mussel tissue (ERM-668)	cadmium	0.245±0.002	0.8	0.275±0.004
Mussel tissue (ERM-278)	Lead	1.63±0.01	3.7	2.00±0.04
Mussel tissue (ERM-668)	Lead	-	-	-
Mussel tissue (ERM-278)	Zinc	79.5±1.3	1.6	83.1±1.7
Mussel tissue (ERM-668)	Zinc	65.3±0.8	1.2	70.7±1.1
Mussel tissue (ERM-278)	Copper	8.60±0.4	4.6	9.45±0.13
Mussel tissue (ERM-668)	Copper	-	-	-
Standard	**Analyte**	**Carbon Fiber Electrode (CFE) Determined value ±Sd (mg Kg^{-1})**	**S_r (%)**	**Certified value±Sd (mg Kg^{-1})**
Mussel tissue (ERM-278)	cadmium	0.315±0.009	2.9	0.348±0.007
Mussel tissue (ERM-668)	cadmium	0.210±0.003	1.4	0.275±0.004
Mussel tissue (ERM-278)	Lead	1.45±0.03	2.1	2.00±0.04
Mussel tissue (ERM-668)	Lead	-	-	-
Mussel tissue (ERM-278)	Zinc	75.6±1.2	1.6	83.1±1.7
Mussel tissue (ERM-668)	Zinc	62.5±0.6	0.9	70.7±1.1
Mussel tissue (ERM-278)	Copper	8.45±0.3	3.5	9.45±0.13
Mussel tissue (ERM-668)	Copper	-	-	-

The values are the mean of ten independent determinations ± 99 % confidence interval [39].
[(4)] Experimental Conditions: Preconcentration potential -1.4 V for a preconcentration time of 180 s; supporting electrolyte: 0.1 M acetate buffer (pH 4.5) containing 200 μg L^{-1} bismuth (for GCE); see sections 2.5 and 4.1 for other experimental details.

Sample pre-treatment: Samples were oven dried for four hours at 103 ± 2ºC to constant weight. One gram of accurately weighed (±0.001 g) sample and fixed volumes of blank solutions were transferred to 100 mL Kjeldahl flasks and 7 mL of the digestion mixture (2 : 2 : 0.5 volume ratio of HNO_3 (d=1.41 g mL^{-1}), $HClO_4$ (d=1.32 g mL^{-1}), H_2SO_4 (d=1.81 g mL^{-1})) was added to each. The flasks were heated until white SO_3 vapours appeared. Heating was continued for 15 minutes to reduce the volume to 0.5 mL and the flasks were allowed to cool to room temperature (25 ± 1ºC). Finally, 5 mL of water was added, the contents were transferred to 25 mL cylinders and diluted to the mark.

SWASV measurements of mussel tissue ERM-CRM 668: The zinc and cadmium were determined separately, as the final zinc concentration was much higher

Table 4. Precision and recovery in zinc, cadmium, lead and copper determinations in mussel tissue certified reference materials [5]

Mussel tissue (ERM-278)									
Glassy Carbon Electrode (GCE)					Carbon Fibre Electrode (CFE)				
Analyte	Added (μg L^{-1})	Found±S_d (μg L^{-1})	S_r (%)[6]	R (%)	Analyte	Added (μg L^{-1})	Found±S_d (μg L^{-1})	S_r (%)[6]	R (%)
Cd	5	5.6±0.3	5.3	112.0	Cd	5	5.8±0.4	6.9	116.0
	50	53.2±1.1	2.1	106.0		50	54.0±1.3	2.4	106.0
	200	194.0±2.6	1.3	97.0		200	196.0±2.3	1.2	97.0
Pb	5	5.6±0.4	7.1	112.0	Pb	5	5.2±0.1	1.9	112.0
	50	46.2±0.3	0.6	92.0		50	52.1±0.8	1.5	92.0
	200	193.0±1.3	0.7	96.5		200	197.0±1.7	0.9	96.5
Zn	5	5.6±0.4	7.1	112.0	Zn	5	5.9±0.4	6.8	118.0
	50	53.1±2.4	4.5	106.0		50	51.4±2.1	4.0	106.0
	200	188.0±0.9	0.4	94.0		200	192.0±0.8	0.4	94.0
Cu	5	5.1±0.3	5.9	102.0	Cu	5	5.5±0.5	9.1	102.0
	50	53.1±2.2	4.1	106.0		50	54.3±2.0	3.7	106.0
	200	193±2.1	1.1	96.5		200	195±2.4	1.2	96.5
Mussel tissue (ERM-668)									
Glassy Carbon Electrode (GCE)					**Carbon Fibre Electrode (CFE)**				
Analyte	**Added (μg L^{-1})**	**Found±S_d (μg L^{-1})**	**S_r (%)[6]**	**R (%)**	**Analyte**	**Added (μg L^{-1})**	**Found±S_d (μg L^{-1})**	**S_r (%)[6]**	**R (%)**
Cd	5	5.2±0.5	9.6	104.0	Cd	5	5.8±0.4	6.9	116.0
	50	48.4±0.6	1.2	97.0		50	51.6±0.9	1.7	103.0
	200	195.0±1.5	0.8	97.5		200	198.0±1.4	0.7	99.0
Pb	5	-	-	-	Pb	5	-	-	-
	50	-	-	-		50	-	-	-
	200	-	-	-		200	-	-	-
Zn	5	5.7±0.2	3.5	114.0	Zn	5	5.4±0.3	5.6	108.0
	50	53.8±1.0	1.9	108.0		50	55.2±1.2	2.2	110.0
	200	192.0±2.1	1.1	96.0		200	194.0±2.0	1.0	97.0
Cu	5	-	-	-	Cu	5	-	-	-
	50	-	-	-		50	-	-	-
	200	-	-	-		200	-	-	-

[5] *Experimental Conditions: Preconcentration potential -1.4 V for a preconcentration time of 180 s; supporting electrolyte: 0.1 M acetate buffer (pH 4.5) containing 200 μg L^{-1} bismuth (for GCE); see sections 2.5 and 4.1 for other experimental details.*
[6] *Six replicate measurements.*

(2.828 µg L^{-1}) than the cadmium concentration (11 µg L^{-1}). For zinc determination using either electrode the sample and buffer were mixed in a 1:20 ratio prior to analysis to bring the zinc concentration within the linear range. In the cadmium determination samples were not diluted. A series of square-wave stripping voltammograms for the determinations in ERM-CRM 668 are presented in Fig. 3.

SWASV signals for each metal are well-defined and linear with concentration at the low µg L^{-1} level. The Bi-GCE and the Bi-CFE gave peak areas S_r% of 1.2 and 0.9% for Zn; 0.8 and 1.4% for Cd (Table 3). Their recoveries are presented in Table 4. In addition, the measured concentrations compared very well with the certified values, showing that the method was unbiased.

SWASV measurements in mussel tissue ERM-CRM 278: Sample pre-treatment was exactly the same as in ERM-CRM 668. Cadmium and lead were simultaneously determined without dilution. Zinc and copper were separately determined using 1:20 and 1:10 dilutions.

For copper(II) determinations in the presence of Bi(III), hydrogen peroxide (0.02% v/v for the GCE and 0.10% v/v for the CFE) was added to resolve bismuth and copper stripping signals and permit Cu(II) determination [15]. Fig. 4 displays voltammograms for

Table 5. Precision and recovery in zinc, cadmium, lead and copper determinations in bovine liver certified reference material [7]

Bovine liver (BCR-185)									
Glassy Carbon Electrode (GCE)					Carbon Fibre Electrode (CFE)				
Analyte	Added (μg L^{-1})	Found±S_d (μg L^{-1})	S_r (%)[8]	R (%)	Analyte	Added (μg L^{-1})	Found±S_d (μg L^{-1})	S_r (%)[8]	R (%)
Cd	5	5.6±0.4	7.1	112.0	Cd	5	5.4±0.3	5.5	108.0
	50	54.8±1.3	2.4	110.0		50	52.0±0.8	1.5	104.0
	200	196.0±2.2	1.1	98.0		200	194.0±2.0	1.0	97.0
Pb	5	5.2±0.2	3.8	104.0	Pb	5	5.6±0.4	7.1	112.0
	50	48.2±0.5	1.0	96.0		50	51.7±0.6	1.2	103.0
	200	194.0±1.0	0.5	97.0		200	197.0±0.9	0.4	98.5
Zn	5	5.7±0.4	7,0	114.0	Zn	5	5.3±0.3	5.7	106.0
	50	55.1±2.2	4.0	110.0		50	53.2±1.6	3.0	106.0
	200	190.0±1.0	0.5	95.0		200	194.0±0.8	0.4	97.0
Cu	5	5.5±0.3	5.4	110.0	Cu	5	5.3±0.4	7.5	106.0
	50	52.8±1.6	3.0	106.0		50	53.3±0.8	1.5	107.0
	200	189±2.4	1.3	94.5		200	194±1.8	0.9	97.0

[7] Experimental Conditions: Preconcentration potential -1.4 V for a preconcentration time of 180 s; supporting electrolyte: 0.1 M acetate buffer (pH 4.5) containing 200 μg L^{-1} bismuth (for GCE); see sections 2.5 and 4.2 for other experimental details.
[8] Six replicate measurements.

Table 6. Determinations of Cd, Pb, Zn and Cu in bovine liver certified reference material BCR-278.[9]

Standard	Glassy Carbon Electrode (GCE)			
	Analyte	Determined value ±Sd (mg Kg^{-1})	S_r (%)	Certified value ±Sd (mg Kg^{-1})
Bovine Liver (BCR-278)	cadmium	0.490±0.014	2.6	0.544±0.017
	lead	0.140±0.006	4.3	0.172±0.009
	zinc	131±1.6	1.2	138.6±2.1
	copper	235±3.5	1.5	277±5
Standard	Carbon Fibre Electrode (CFE)			
	Analyte	Determined value ±Sd (mg Kg^{-1})	S_r (%)	Certified value±Sd (mg Kg^{-1})
Bovine Liver (BCR-278)	cadmium	0.475±0.012	2.5	0.544±0.017
	Lead	0.152±0.008	5.3	0.172±0.009
	Zinc	129±1.4	1.1	138.6±2.1
	copper	250±4.0	1.6	277±5

The determined values are the mean of ten independent determinations ± 99 % confidence interval [39].
[9]Experimental Conditions: Preconcentration potential -1.4 V for a preconcentration time of 180 s; supporting electrolyte: 0.1 M acetate buffer (pH 4.5) containing 200 μg L^{-1} bismuth (for GCE); see sections 2.5 and 4.2 for other experimental details.

three successive concentration increments (5 μg L^{-1}) of Cd(II), Pb(II), Cu(II) and Zn(II) (curves b – d). SWASV signals are well-defined and linear with increasing concentration. In addition, the overall peak resolution remains constant over the course of the additions. The excellent reproducibilities are shown in Table 3, and the recoveries in Table 4. The measured concentrations were in very good agreement with those certified (Table 3).

The absence of organic or other contaminants is evident from the excellent recoveries (Table 4) and very similar to those obtained in pure buffer (Table 2).

4.2. Determination of zinc, cadmium, lead and copper in BCR bovine liver certified reference material.

The procedure's overall accuracy was further assessed by four repetitive analyses of bovine liver reference material (BCR-CRM 185). In this material toxic metals such as lead and cadmium are usually present in very low concentrations, otherwise metal poisoning occurs.

Prior to analysis pre-treatment was necessary to remove the organic matter. Lead and cadmium were measured without dilution while zinc and copper were separately determined after being diluted by 1:30 or 1:240 with buffer. The latter high dilution was necessary as the copper concentration was far above the linear concentration range. SW stripping voltammograms of (A) Pb(II) and Cd(II), (B) Zn(II) and (C) Cu(II) in bovine liver (BCR-CRM 185) are presented in Fig. 5. The procedure was repeated six times to examine its precision; results

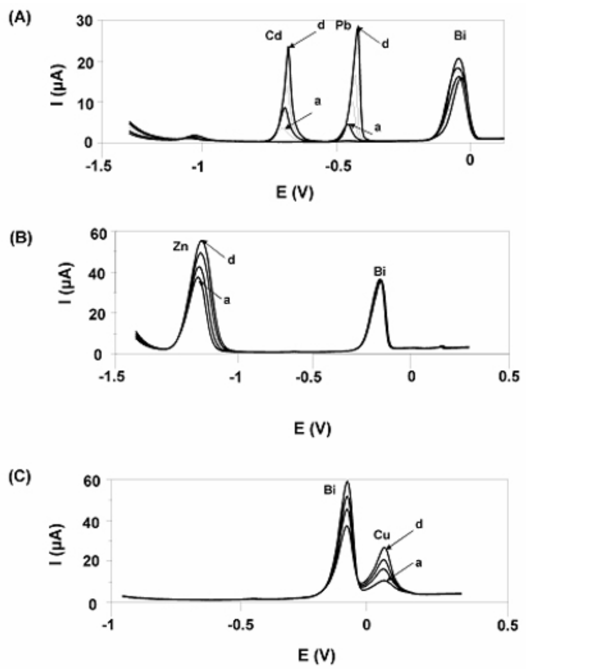

Figure 4. Measurements of (A) Pb(II) and Cd(II), (B) Zn(II) and (C) Cu(II) in mussel tissue ERM-CRM 278. Square-wave stripping voltammograms show a mussel tissue sample (a) and three standard additions (b - d) of 5 μg L^{-1} of each metal. Preconcentration potential -1.4 V for a preconcentration time of 180 s; "cleaning" for 30 s, at +0.3 V (for the GCE) or at -1.5 V (for the CFE). Other experimental conditions as described in sections 2.5 and 4.1.

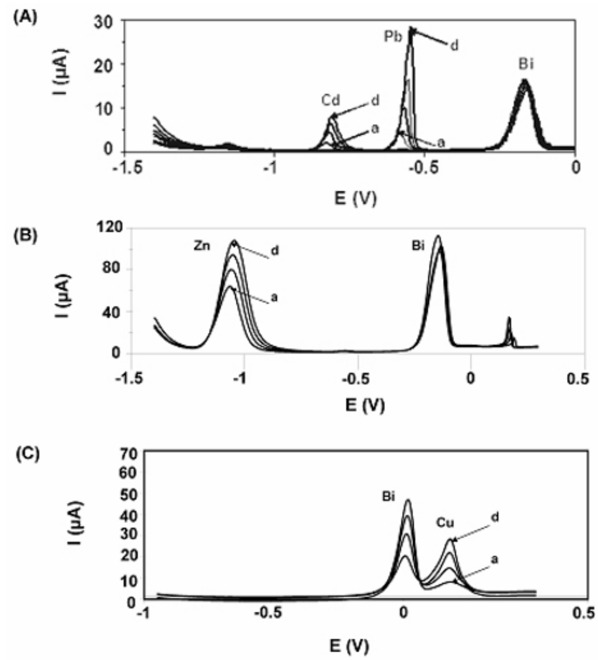

Figure 5. Measurements of (A) Pb(II) and Cd(II), (B) Zn(II) and (C) Cu(II) in bovine liver BCR-CRM 185. Square-wave stripping voltammograms show a mussel tissue sample (a) and three standard additions (b - d) of 5 μg L^{-1} of each metal. Preconcentration potential -1.4 V for a preconcentration time of 180 s; "cleaning" for 30 s at +0.3 V (for the GCE) or at -1.5 V (for the CFE). Other experimental conditions as described in sections 2.5 and 4.2.

are shown in Table 5.

The method proved capable of determining four elements in bovine liver. The precision and sensitivity of the method yields their detection down to μg L^{-1}. Analyte recoveries are very good even for the highly diluted copper, indicating that the proposed procedure is feasible for heavy metal determinations in complex biological samples. In addition, the concentrations found agree favourably with the certified values, with a low mean deviation (Table 6). This last observation indicates adequate decomposition of the bovine liver organic matter.

5. Conclusions

The analytical behavior of bismuth-film electrodes (Bi-FEs) in the determination of eco-toxic metals was studied. The Bi-FEs were prepared *in situ* on a glassy-carbon electrode (GCE) as well *ex situ* on electrochemically oxidized graphitized polyacrylonitrile carbon fibres. Interferences from organic substances were overcome by mineralization with HNO_3-$HClO_4$-H_2SO_4 and quantitation by standard additions. For both kinds of electrode, low limits of detection and satisfactory precision were achieved. The excellent results indicate their potential applicability in studies of Zn(II), Pb(II), Cd(II) and Cu(II) in environmental samples such as mussel tissue. Moreover, the electrodes' small size and long-term stability suggests their use in real-time metal monitoring in organs such as liver. The attractive behavior of these non-mercury electrodes indicates great promise for on-site environmental or clinical testing for heavy metals, avoiding the toxic effects of mercury.

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