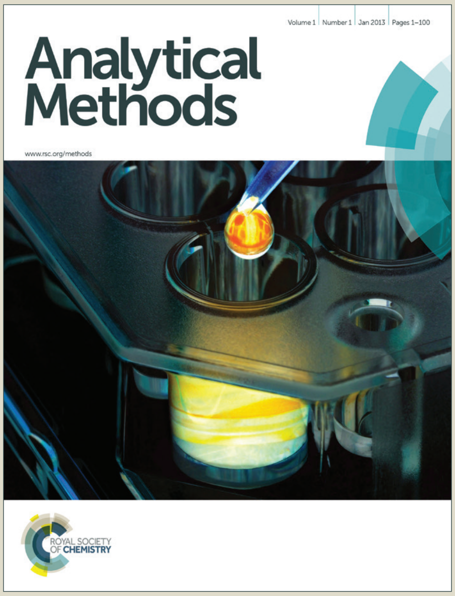
Analytical
Methods



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Applicability of the Bismuth Bulk Rotating Disk Electrode for heavy metal monitoring in undisturbed environmental and biological samples: Determination of Zn(II) in rainwater, tap water and urine.

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Abstract

The Bismuth Bulk Rotating Disk Electrode (BiB-RDE) is investigated here as a new and advantageous alternative to determine heavy metal ions in undisturbed environmental and biological samples (pH 7.5) by anodic stripping voltammetry, with demonstrated applicability to the determination of Zn(II) in rainwater, tap water and urine. The proposed method benefits from the insensitiveness of the bismuth bulk electrode to the Bi(III) hydrolysis that affects the common "in situ" Bi film approach preventing its use at intermediate to alkaline pH. Instrumental parameters such as the rotation speed, deposition time, frequency, amplitude and step potential were optimized for Zn(II) and Cd(II) stripping signal enhancement. The stripping response is lineal in 20-160 $\mu g.L^{-1}$ concentration range (for 120 s deposition) with detection limits of 8.09 and 11.5 $\mu g\ L^{-1}$ for Zn(II) and Cd(II), respectively. The accuracy of the results obtained was assessed successfully for Zn(II) determination by voltammetric measurements of surface water and wastewater certified reference materials. Applicability to Zn(II) determination in rainwater, tap water and urine was demonstrated by good correlation with ICP-MS. Our results open a new application field for stripping voltammetry at bismuth electrodes, for heavy metal determination and speciation in environmental and biological samples.

Keywords: Bismuth bulk rotating disk electrode; zinc; cadmium; neutral media; environmental samples; urine.

1. Introduction

A remarkable feature of voltammetric measurements is the minimum disturbance that collecting the analytical signal causes on the sample. It is well known that voltammetric currents are produced by electronic exchange at the electrode surface derived from oxidation or reduction processes suffered by the analytes, these phenomena occurring at the electrode - solution interface without affecting the bulk volume of the sample. These properties make voltammetric techniques especially suitable for "in situ" analytical measurements, a very valuable advantage in specific applications fields like environmental and biological monitoring. Moreover, careful adjustment of electrode potential applied to undisturbed or minimally disturbed samples permits the measurement of voltammetric responses from different species of the analytes, enabling the identification and quantification of e.g. oxidation states, complexation states or matrix bounded elemental ions in solution. Many examples of voltammetric measurement strategies, developed to attain the aforementioned goals have been published, as reviewed by Gonzalvez et al.[1] and Pesavento et al.[2].

Due to their excellent sensitivity, electrochemical techniques with a preconcentration step are widely used for the measurement of trace metal. Thus, anodic stripping voltammetry (ASV) or adsorptive stripping voltammetry (AdSV) techniques with differential pulse (DPASV), or square wave (SWASV) sweeps are the common choices instead of direct reduction techniques, which are not sensitive enough for trace metal analysis. Platinum, gold and glassy carbon electrodes have been employed for voltammetric stripping determination and speciation analysis of various elements, but the mercury electrode (both in drop and film format) has been for decades the dominant tool in this field. In spite of advantageous electrochemical properties of mercury, its toxicity has prompted the development of alternative electrode materials. One of these alternatives is the bismuth electrode, introduced a decade ago, which opened a new and fruitful field in voltammetric stripping as demonstrated by a vast amount publications reviewed e.g. by Wang et al.[3]. These studies have demonstrated the feasibility of bismuth electrodes for analysis of trace heavy metals because it shows a similar performance than mercury electrodes and several advantages such as a low toxicity, a wide applicable potential window, insensibility to dissolved oxygen and good signal resolution. These properties have consolidated bismuth as a good alternative to mercury for the determination of heavy metals. The working cathodic limit of Bi electrodes ranges from -1.0 and -1.5 V, depending on pH. It allows the detection and determination of highly electronegative metals such as Zn, which are notoriously difficult to measure because its deposition and stripping are accompanied by hydrogen evolution at extreme negative potentials, especially in acid media.

Different types of bismuth-based electrodes have been used to achieve the voltammetric analysis of Zn(II), Cd(II) and Pb(II) commonly in slightly acid media: bismuth films on glassy carbon supports [4-6], screen-printed electrodes [7-9], Bi nano-powder modified carbon electrodes [10,11] and bismuth bulk electrodes [12, 13]. The determination of Zn(II), individually or in mixtures, has been possible in natural waters [14-18], waste water [19], tap water [20, 21] and biological samples [22-24]. Bi(III) hydrolysis prevents the use of the most common “in situ” Bi film approach (based on the codeposition of Bi with the analytes on the electrode surface) in neutral or alkaline media, so predeposited Bi films, bulk or microfabricated Bi surfaces are needed. Nevertheless, heavy metal determination on bismuth based electrodes in neutral media has not been attempted in environmental samples and rarely in biological matrices. Only two references have been found about Zn(II) anodic stripping determination in pancreatic islets at pH 7.4 [22], and serum at pH 6 [24]. The complexation of Cd(II) with thiol containing compounds on screen printed Bi film electrodes (predeposited film), at pH 7.5 to 8.5, has been recently described by Sosa et al [25]. The analysis of heavy metals in biological samples such as blood or urine provided us information about the environmental or occupational heavy metal exposure [26] and allows to evaluate the deficiency or excess of these metals in the organism. Several studies have demonstrated that Zn(II) metabolism is altered adversely in patients with type II diabetes mellitus, detecting a great loss of this metal through the urine [27-29] and a decrease in the plasma and serum Zn(II) concentration [30,31]. This leads to additional health problems for the subjects with this type of diabetes since Zn(II) is essential for growth [32], required for the normal function of numerous enzymes and hormones [33] and involved in biological processes such as DNA metabolism or neurotransmission [34].

In the present work we have explored the feasibility of the Bismuth Bulk Rotating Disk Electrode (BiB-RDE) for the voltammetric stripping of the heavy metals Zn(II), Cd(II) and Pb(II) in neutral (pH 7.5) medium by SWASV, with application to the determination of Zn(II) in rainwater, tap water and urine samples. We have previously reported analytical advantages of the BiB-RDE such as stability, robustness, stripping signal reproducibility, and favourable behaviour in neutral media [21], so we aim here to propose it as a new alternative for heavy metal determination in minimally disturbed environmental and biological neutral samples, with potential application to speciation studies and “in situ” measurements.

2. Experimental

2.1. Reagents and solutions

All chemicals used for the preparation of stock and standard solutions were of analytical grade. Working solutions were prepared by dilution of 1000 mg L^{-1} stock solution of Zn(II) (Scharlab ,Barcelona, Spain) with ultrapure water obtained from a Wasserlab Ultramatic equipment (Navarra de Tratamiento del Agua, Spain). “Reference Material for Measurement of elements in surface water SPS-SW Batch 121” and Reference Material for Measurement of elements in wastewater SPS-WW Batch 108”, both from Spectrapure Standards (Oslo, Norway), were used for accuracy evaluation. Certified reference materials were adjusted to pH 7.5 with phosphate buffer (final concentration 0.1 M), prepared with Suprapure grade di-sodium hydrogen phosphate and sodium dihydrogen phosphate, both supplied by Merk (Darmstadt, Germany).

2.2. Instrumentation

Square-wave voltammetric measurements were performed using a Methrom 663 VA electrochemical stand connected to µAutolab III (EcoChemie, Kanalueg, The Netherlands). A three electrode configuration consisted of a home-made BiB-RDE as a working electrode, an Ag/AgCl reference electrode and a glassy carbon counter electrode. An EsayCon Kit (EasyCon Hellas, Ioannina, Greece) and 10 mm lenght × 2.5 mm diameter 99% bismuth rod from RGB Research (London, U.K) were used for the fabrication of BiB-RDE as described earlier [13]. Method validation was carried out with a PerkinElmer ELAN 9000 ICP-MS (Waltham, Massachussets, EE.UU.).

2.3. Methods

2.3.1. Sampling procedure

A clean glass beaker was exposed to rain outside our laboratory in the University of Extremadura, Badajoz campus, for collecting rain water samples. It was placed on the top of a 150 cm height pole during 24 h. The rain water sample was filtered by 0.45 µm PTFE filter to a recipient. The samples were kept in the fridge at 4 ºC until analysis.

Tap water samples were collected just before analysis from a running water tap in our lab at the Department of Analytical Chemistry, University of Extremadura.

Morning urine samples were collected from a healthy volunteer, filtered through a 0.45 µm PTFE membrane and immediately analysed. All experiments were performed in compliance with institutional guidelines as approved by the Bioethical and Biosecurity Commission of University of Extremadura. Informed consent was obtained for any experimentation with urine samples from human subjects.

2.3.2. Voltammetric Stripping Measurement

Before Zn(II) measurements, the surface of BiB-RDE was polished with 1.0, 0.3 and 0.05 µm alumina and rinsed with distilled water, and inserted in a glass voltammetric cell containing 10 mL samples. Water certified reference materials, rainwater and tap water samples were adjusted to pH 7.5 with 1 M phosphate buffer (9:1 ratio). Urine samples were diluted (1:10) with 0.1 M pH 7.5 phosphate buffer to minimize matrix effects during the voltammetric measurements. No sample de-aeration was required. A deposition potential of -1.4 V was applied to the BiB-RDE under a variable rotation speed. After the deposition time and 10 s equilibration time, the square wave anodic stripping voltammograms were recorded from -1.4 to -0.3 V under different frequency, step potential and amplitude values. Stripping peak heights were registered as analytical signals.

3. Results and discussion

3.1. Effect of pH on the stripping voltammetric signals

As mentioned in the introduction, most published applications of ASV on bismuth electrodes employ acid media, most frequently pH 4.5 acetate buffer. However, the use of bulk bismuth surfaces like the BiB-RDE allows to explore different pH intervals due to electrode insensitiveness to Bi(III) hydrolysis [13, 35], thus opening the way for ASV measurements in minimally disturbed environmental waters. Fig. 1 depicts the voltammetric stripping behaviour of a standard solution containing 500 µg/L Zn(II), Cd(II) and Pb(II) on the BiB-RDE in pH 4.5 0.1 M acetate buffer (dashed line), and in pH 7.0 0.1 M phosphate buffer (continuous line). The comparison of the curves in Fig. 1 shows some interesting features, the most relevant being the better resolution of the Zn(II) signal respect to the hydrogen evolution cathodic limit that is shifted to more negative values when the pH increases. Moreover, a better definition of the signals and a greater sensitivity is observed for the voltammetric signals of Zn(II), Cd(II) and Pb(II) in the neutral phosphate medium, with nearly double size in the case of Zn(II) and Cd(II). Signal irreproducibility was observed for the Pb(II), probably due to species reactivity in the phosphate medium, but Zn(II) and Cd(II) signals showed a good general behaviour so these two analytes were selected for method optimization.

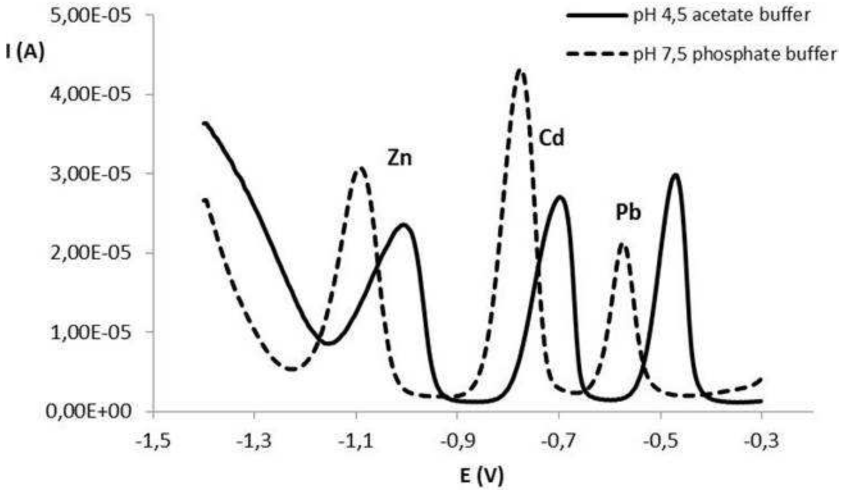

Fig. 1 Stripping voltammograms for 500 µg.L^{-1} of Zn(II) and Cd(II) registered in a 0.1 M phosphate buffer (pH=7.0) and in a 0.1M acetate buffer (pH=4.5) solutions. Experimental conditions: Deposition potential -1.4 V, stirring speed 1500 r.p.m., deposition time 120 s, step potential 5 mV, amplitude 25 mV and frequency 20 Hz.

3.2. Instrumental variables optimisation

The influence of relevant instrumental parameters affecting the voltammetric measurement was studied in order to obtain the optimized conditions for the determination of Zn(II) and Cd(II) on the BiB-RDE in neutral phosphate medium. First, the effect of the electrode rotating speed on the response was tested in the 0 to 3000 r.p.m range, using a standard solution containing 100 µg L^{-1} Zn(II) and Cd(II) prepared in pH 7.5 0.1 M phosphate buffer. Experimental conditions were 120 s accumulation time, 20 Hz SW frequency, 5 mV step potential and 25 mV amplitude. As shown in Fig. 2, and according with hydrodynamic theory, the signal intensity increased with the stirring speed increases up to a plateau. Turbulent regime was observed in the solution for rotating speed higher than 1500 r.p.m, producing irreproducible signals, so this rotating speed was selected as optimal.

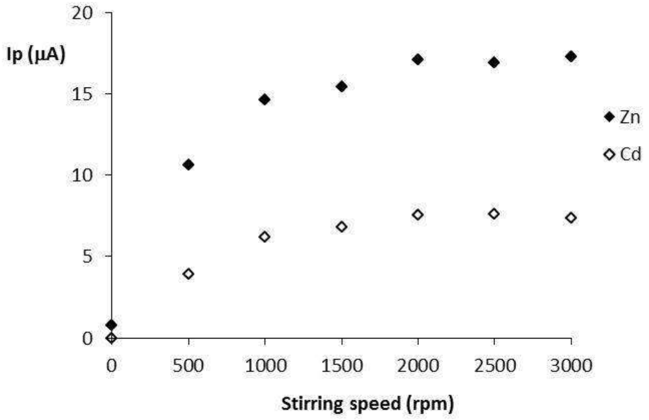

Fig. 2 Effect of stirring speed on the SWASV response of 100 μg.L^{-1} Zn(II) and Cd(II) at BiB-RDE. pH 7.5 0.1 M phosphate buffer, Deposition potential -1.4 V, deposition time 120 s, step potential 5 mV, amplitude 25 mV and frequency 20 Hz.

Next, the influence of deposition time on the analytical signals was inspected in the range from 0 to 480 s, on 0.1 M pH 7.5 phosphate standard solutions containing 100 μg L^{-1} Zn(II) and Cd(II). The obtained results are presented in Fig. 3A. Lineal correlations between peak currents and deposition time until 240 s were observed for both metals. 120 s was considered as adequate deposition time at the selected concentration level. The effect of the square wave voltammetric parameters amplitude, step potential and frequency was then tested. Optimal values for amplitude and step potential were 5 and 25 mV, respectively. The influence of frequency in the stripping signals was studied in the 8 to 110 Hz range. The peak intensity increases up to 80 Hz and then stabilizes. For values higher than 40 Hz, the reproducibility is worse so the results are not reliable. As a result, a frequency of 30 Hz was selected as optimal (Fig. 3B).

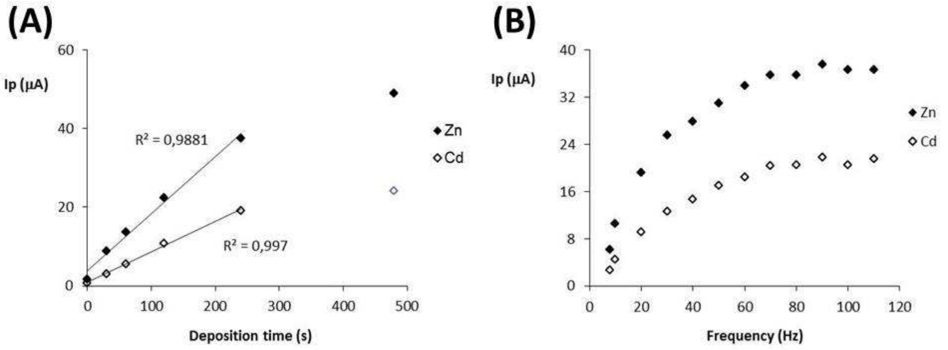

Fig. 3 Effect of (A) deposition time and (B) frequency on the SWASV response for 100 μg.L^{-1} Zn(II) and Cd(II) at BiB-RDE. pH 7.5 0,1 M phosphate buffer, stirring speed 1500 r.p.m., step potential 5 mV, amplitude 25 mV.

Signal stability is a key parameter to verify the applicability of the proposed BiB-RDE and voltammetric method, so 20 consecutive voltammetric runs on solutions containing 100 μg.L^{-1} of Zn(II) and Cd(II) were carried out to investigate the signal trend. The analytical signals remained stable, within less than 2% variability during the experiments, so the selected optimized conditions were considered appropriate for Zn(II) and Cd(II) calibration in the pH 7.5 phosphate medium.

3.3. Calibration data

A linear correlation between signal and concentration was found for both Zn(II) and Cd(II) in the 20 to 160 μg.L^{-1} range. The registered voltammograms are shown in Fig. 4. Table 1 summarizes the analytical parameters of the calibration lines (3 replicate measurements for each concentration level).

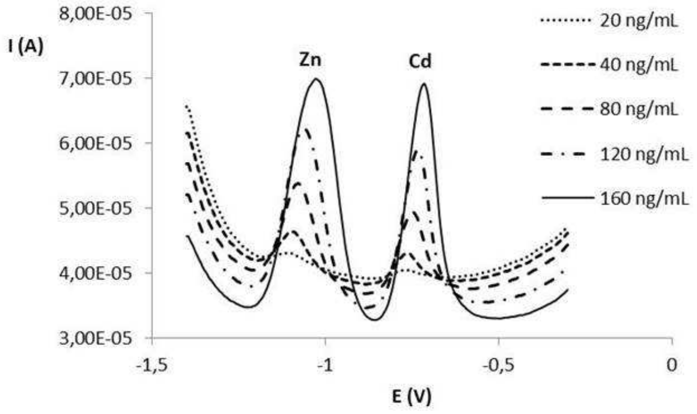

Fig. 4 SWASV calibration of Zn(II) and Cd(II) at the BiB-RDE. pH 7.5 0,1 M phosphate buffer, Deposition potential -1.4 V, stirring speed 1500 r.p.m., deposition time 120 s, step potential 5 mV, amplitude 25 mV and frequency 30 Hz.

Table 1.- Calibration data for the determination of Zn(II) and Cd(II) at the BiB-RDE in pH 7.5 phosphate buffer.

Metal ion	Slope (μA.L.μg^{-1})	Intercept (μA)	R^2	Linearity (%)	Analytical Sensitivity (μg.L^{-1})	Detection limit (μg.L^{-1})
Zn(II)	0.2399	-2.1439	0.9978	97.3	7.5	8.1
Cd(II)	0.2388	-3.2164	0.9873	95.7	10.1	11.5

Limits of detection (LOD), calculated according to the IUPAC definition, were estimated to be 8.1 μg.L^{-1} for Zn(II) and 11.5 μg.L^{-1} for Cd(II). No reference has been found in the literature about LODs obtained by ASV of Zn(II) and Cd(II) on bismuth bulk electrodes in neutral media, but both values are somewhat higher than the usual values reported for Bi film electrodes methods in acidic media [3, 12]. This is a common situation, assigned to the lower conductivity of the polycrystalline metal bismuth vs. glassy carbon generally used as a substrate for Bi film electrodes [35]. Table 2 shows the LODs obtained in some works where Cd, Zn or both of them were determined employing different types of bismuth-based electrodes. The analytical performance of the proposed methodology can also be compared with previously reported methods in a recently published review [36].

Table 2. Detection limits for Cd and Zn obtained by using bismuth electrodes under different conditions.

Bi Electrode	Conditions	LOD ($\mu g.L^{-1}$)	Reference
Ex situ BiFE	*Sonoelectroanalysis* pH=4.5 E_{dep}=-1.4V t_{dep}=60s	*0.011 (Cd)* *0.014 (Zn)*	[37]
In situ BiFE	*SWASV* pH=4.5 E_{dep}=-1.4V t_{dep}=600 s	*0.3(Cd)* *0.4 (Zn)*	[38]
	SWASV pH=4.5 E_{dep}=-1.2V t_{dep}=600 s	*0.2 (Cd)* *0.7 (Zn)*	[21]
Bi_{OX}SPCE	*SWASV* pH=4.5 E_{dep}=-1.5V t_{dep}=120 s	*5 (Cd)* *30 (Zn)*	[39]
	SWASV pH=4.5 E_{dep}=-1.5V t_{dep}=60s	*50 (Zn)*	[16]
Ex situ BiSPCE	*SIA-SWASV* pH=4.0 E_{dep}=-1.4V	*0.3 (Cd)* *17 (Zn)*	[40]
In situ BiSPCE	*SWASV* pH=4.5 E_{dep}=-1.4V t_{dep}=120 s	*3.6 (Cd)* *8.2 (Zn)*	[19]
	SWASV pH=4.5 E_{dep}=-1.5V t_{dep}=120 s	*2.9 (Cd)*	[41]

The obtained LOD for Zn(II) in this work improves those reported by studies in which different types of Bi films electrodes were employed and similar deposition times were established. However, LODs for Cd(II) at slightly acid pH are lower than the obtained value at neutral pH using the BiB-RDE. Higher deposition times provide lower LODs for both Zn(II) and Cd(II) but it involves extending the time needed for the analysis. Anyway, the method proposed here is still applicable for environmental and biological samples with the advantage of operation in undisturbed pH conditions and a short deposition time. The LODs for Zn(II) and Cd(II) are within the range of previously described values for bismuth bulk electrodes in acidic media [12, 13], sufficient for measuring the concentrations usually found in natural water or wastewater in the frame of water monitoring studies.

3.4. Analysis of certified reference materials

The accuracy of the proposed method was tested by the repeated analysis (3 samples each) of the certified reference material SPS-SW natural water, Batch 121 (Zn(II) concentration certified value: 100 ± 2 μg.L^{-1}, Cd(II) certified value, 2.61 ± 0.02 μg.L^{-1}) and SPS-WW waste water, Batch 108 (Zn(II) certified concentration value: 3000 ± 15 μg.L^{-1}, Cd(II) certified value, 100 ± 0.5 μg.L^{-1}). Fig. 5 shows the voltammograms obtained in the analysis of both reference materials by the standard addition method. Zn(II) signals were clearly identified but no Cd(II) signal was observed due to its low concentration level in the certified samples. Zn(II) concentration obtained by the proposed SWASV method was 96.08 ± 11.2 μg.L^{-1} in the SPS-SW samples and 2693 ± 290 μg.L^{-1} in the SPS-WW samples. These results showed good agreement with the certified values.

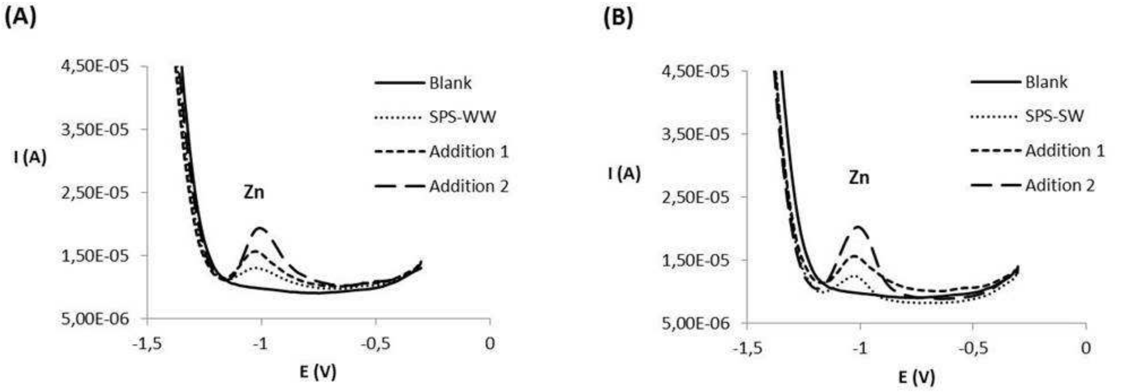

Fig. 5 Zn(II) stripping voltammetry response of (A) SPS-WW reference material and (B) SPS-SW reference material and response after two additions (30 μg.L^{-1} each). Experimental conditions as in Figure 4.

3.5. Analysis of real samples

Finally, the proposed method was tested for Zn(II) determination in untreated rainwater, tap water and urine (Table 3).

Table 3. Determination of Zn(II) in real samples. Standard deviation in brackets.

	Zn(II) concentration (μg.L^{-1})	
Sample (n = 3)	SWASV	ICP-MS
9/10 diluted rainwater	20.7 (4.0)	22.4 (1.3)
9/10 diluted tap water	48.1 (3.4)	51.2 (2.3)
1/10 diluted urine (sample A)	Not detected	4.42 (0.12)
1/10 diluted urine (sample B)	15.1 (3.5)	15.3 (6.2)

Zn(II) was undetectable in the rainwater samples so they were spiked with 20 μg.L^{-1} Zn(II) before analysis. Zn(II) concentration by SWASV was quantified by the standard addition method, the voltammetric response obtained for a rainwater sample is shown in Fig. 6.

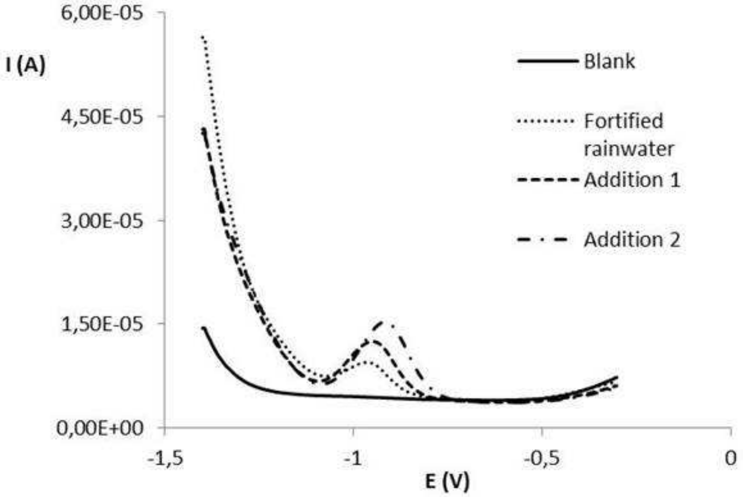

Fig. 6 Zn(II) stripping voltammetry response of a fortified rainwater and response after two additions (20 µg.L^{-1} each). Experimental conditions as in Figure 4.

The determination of Zn(II) in tap water sample was also tested by SWASV on the BiB-RDE. The samples were filtered and the pH was adjusted to 7.5 by phosphate buffer as previously mentioned. For both rainwater and tap water samples, the obtained results were in good agreement with the results provided by ICP-MS.

The proposed voltammetric method was also tested on two types of urine samples. The first (sample A) was collected from the volunteer without altering her habits, and the second (sample B) was collected after a week of daily ingesting of a multivitamin dietary supplement (1.5 mg Zn/tablet). Then, stripping analysis was carried out under the optimized conditions described in section 2.3.2. The Zn(II) concentration in the cell for sample A (4.42 µg.L^{-1} as measured by ICP-MS) is under the detection limit of the SWASV method, therefore Zn(II) could not be detected by the proposed method in sample A. Increasing of the deposition time in combination with dilution degree could be considered for extending the range of the method to normal urine samples. However, an excellent recovery was obtained when applying the voltammetric method to sample B (98%). This proves the suitability of the BiB-RDE for the analysis of urine samples with elevated Zn(II) levels, e.g. those collected from patients with type II diabetes mellitus where Zn(II) levels can exceed 1 mg.L-^{1}. Voltammetric measurement of Zn(II) in urine sample on a Bi electrode has only been described in a single previous work, using a Nafion-coated Bi film electrode, with a somewhat low recovery of 67% [20]. We envisage expanded applicability of the BiB-RDE by using it as part of rugged voltammetric sensors capable of performing direct measurements in undisturbed biological and environmental matrices, by combining proper media adjustment, sample dilution and tuning of voltammetric parameters, especially the deposition time.

4. Conclusions

The applicability of the bismuth bulk rotating disk electrode (BiB-RDE) has been demonstrated for voltammetric stripping measurement of Zn(II) and Cd(II) by SWASV in neutral media. The experimental variables of the proposed method were optimized for calibration of the selected metals in the low µg.L^{-1}, using a 0.1 M phosphate, pH 7.5 medium. The performance of the proposed method was demonstrated for Zn(II) with certified reference materials and environmental water samples and urine, this work being the first reported application of the bismuth bulk electrode to the determination of Zn(II) in environmental and biological samples without significant pH modification. These results are promising for analytical developments based on the Bi electrode oriented to in situ measurements and elemental speciation studies. Further investigation should be directed to enhance the sensitivity of the method and exploring other trace elements voltammetric responses in undisturbed environmental and biological samples.

Acknowledgements

This work is supported by the Spanish Ministry of Science and Innovation (project CTQ2011-25388), Junta de Extremadura, Spain (GR10091), Junta de Extremadura air quality network, and the European Union (FEDER).

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