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Central European Journal of **Chemistry**

Optimization of analytical procedures for the simultaneous voltammetric determination of total Hg(II) in presence of Cu(II) in environmental matrices

Research Article

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Abstract: The present work reports the critical comparison about the employment of three different supporting electrolytes (0.1 mol L^{-1} $HClO_4$, 0.01 mol L^{-1} EDTA-Na_2 + 0.06 mol L^{-1} NaCl + 2.0 mol L^{-1} $HClO_4$ and 0.1 mol L^{-1} KSCN + 0.001 mol L^{-1} $HClO_4$) and their instrumental and chemical optimisation for the simultaneous voltammetric determination of total mercury(II) and copper(II) in sediments and sea water at gold electrode, especially discussing the reciprocal interference problems.

The differential pulse anodic stripping voltammetric (DPASV) measurements were carried out using a conventional three-electrode cell: a gold electrode (GE) as working electrode, a platinum wire and an Ag│AgCl│KCl$_{sat}$ as auxiliary and reference electrodes, respectively.

The analytical procedure was verified by the analysis of standard reference materials: Estuarine Sediment BCR-CRM 277, River Sediment BCR-CRM 320 and Mercury in Water NIST-SRM 1641d.

Once set up on the standard reference materials, the analytical procedure was transferred and applied to sediments and sea waters sampled in a lagoon ecosystem connected with Adriatic Sea (Ravenna area, Italy).

Keywords: Mercury • Copper • Voltammetry • Signal Interference • Environmental Samples

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1. Introduction

A large amount of mercury is released every year into the environment as a consequence of human activity. The danger posed to human health by this form of contamination comes mainly from food, i.e. from the ability of this element to enter the natural alimentary chains, to accumulate in progressively larger quantities at each trophic stage, and to reach highly toxic concentrations in the tissues of organisms that play a role in the human diet [1,2].

Concern over mercury contamination of the environment has promoted an intensive search for methods aimed at the determination of this metal.

Several methods have been described for mercury determination at low concentrations in clinical [3-5], biological [6-10] and environmental [11-14] samples. Such

methods are based on a wide range of techniques like inductively coupled plasma mass spectrometry (ICP-MS) [4-6,11,12,15,16], capillary column gas chromatography (CC-GC) [17,18], neutron activation analysis [19-21], nuclear magnetic resonance (NMR) [22], electrothermal atomisation atomic absorption spectrometry (ETA-AAS) [23,24], cold vapour atomic absorption spectrometry (CV-AAS) [25-31], cold vapour atomic fluorescence spectrometry (CV-AFS) [13,32-38] and potentiometric stripping analysis [39,40].

Voltammetric measurements are employed less even though voltammetry may be a good alternative to spectroscopy, since it allows for analytical determination without employment of too expensive equipment.

Such a technique provides for employment of gold [41-46], glassy carbon [47-51] and platinum electrodes [52], generally using acidic pH solutions as supporting electrolytes.

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Recently, also modified solid electrodes have been successfully employed for voltammetric determination of mercury(II) [53-59].

The present work reports analytical procedures based on the employment of different supporting electrolytes, and their critical comparison, for the voltammetric determination at gold electrode of total mercury(II) in presence of copper(II). Optimization of the chemical and instrumental parameters, in order to obtain the best voltammetric signal and consequently lower limits of detection, suitable for mercury(II) and copper(II) determinations in environmental matrices are also reported here.

The supporting electrolytes investigated were 0.1 mol L^{-1} $HClO_4$, 0.01 mol L^{-1} EDTA-Na_2 + 0.06 mol L^{-1} NaCl + 2.0 mol L^{-1} $HClO_4$ and 0.1 mol L^{-1} KSCN + 0.001 mol L^{-1} $HClO_4$.

The justification of the choices is based on the fact that two of these (acidic medium, preferably $HClO_4$, and KSCN in acidic medium) are already suggested in literature, certainly the former more than the latter.

The choice of a supporting electrolyte containing EDTA-Na_2 was based on the premise of obtaining more separated peaks and higher reversibility of the electrode processes following complexation. In other word to obtain a better resolution of the voltammetric peaks and a higher sensitivity of the method.

2. Experimental procedure

2.1. Apparatus

A Multipolarograph AMEL (Milan, Italy) Mod. 433 was employed for all the voltammetric scans, using a conventional three electrode measuring cell: a gold electrode (GE) (surface area: 0.785 mm^2) as working electrode, activated following the procedure suggested by Bonfil *et al.* [60], an Ag│AgCl│KCl $_{satd..}$ electrode and a platinum wire as reference and auxiliary electrode, respectively.

The experimental conditions are reported in Table 1.

When gold electrode is employed, always it presents a problem linked to the presence of anions in solution. In fact, anions are known to adsorb strongly on gold surfaces [61,62], and this adsorption is dependent on the potential of the electrode and on the nature of the anion. According to Salaun and van den Berg [46], to minimize the excessive adsorption effect, in order to have higher voltammetric peaks and flatter voltammetric baseline, a negative potential of -0.8 V / Ag│AgCl│KCl $_{satd.}$ was applied between the deposition and the stripping

steps. It has been shown that, at this potential, Cl^-, Br^-, I^- and SO_4^{2-} do not adsorb on the gold electrode.

Before the measurements, to avoid accidental contamination, the Teflon voltammetric cell was rinsed with suprapure concentrated 1:1 HNO_3 and then many times with Milli-Q water.

The solutions were thermostated at 20±0.5°C and deaerated with Milli-Q water saturated pure nitrogen for 5 min prior to analysis, while a nitrogen blanket was maintained above the solutions during the experiments. The solutions were stirred with a Teflon-coated magnetic stirring bar in the purge step.

The atomic absorption spectrometry measurements were performed in the recirculation mode [63] employing stannous chloride as reducing agent and using a Perkin-Elmer Mod. Zeeman 5100 PC Atomic Absorption Spectrometer, equipped with a Zeeman background corrector. The absorption wavelength was fixed at 253.7 nm and the spectral band-width at 0.7 nm. Single-element Intensitron hollow-cathode lamps were used.

The instrumental parameters in the case of Cu(II) spectroscopic determination were: wavelength (nm): 324.8; slit (nm): 0.7; drying temperature (°C): 100; charring temperature (°C): 1100; atomization temperature (°C): 2300.

2.2. Reagents and reference solutions

All acids and chemicals were suprapure grade (Merck, Germany). Acidic stock metal solutions (1000 mg L^{-1}, Merck, Darmstadt, Germany) were respectively employed in the preparation of reference

Table 1. Instrumental parameters for the determination of Hg(II) and Cu(II) by Differential Pulse Anodic Stripping Voltammetry (DPASV).

	$HClO_4$	$HClO_4$-EDTA-Na_2	$HClO_4$-KSCN
E_i	+0.050	+0.050	-0.800
E_d	+0.050	+0.050	-0.800
E_{des}	-0.800	-0.800	-
E_f	+0.900	+0.900	+0.100
t_d	240	240	240
t_{des}	30	30	-
t_r	10	10	10
dE/dt	20	20	20
$\Delta\Delta E$	50	50	50
ττ	0.065	0.065	0.065
vv	0.250	0.250	0.250
r	600	600	600

Ei: initial potential (V/ Ag│AgCl│KCl $_{sat}$.); E_d: deposition potential (V/ Ag│AgCl│KCl $_{sat}$.); E_{des}: desorption potential (V/ Ag│AgCl│KCl $_{sat}$.); E_f: final potential (V/ Ag│AgCl│KCl $_{sat}$.); t_d: electrodeposition time (s); t_{des}: desorption time (s) t_r: delay time before the potential sweep (s); dE/dt: potential scan rate (mV s^{-1}); $\Delta\Delta E$: pulse amplitude (mV); ττ: pulse duration (s); vv: pulse repetition (s); r: stirring rate (r.p.m.).

solutions at varying concentrations for both elements, water demineralized through a Milli-Q system was used for all the dilutions.

The supporting electrolytes under study were a) 0.1 mol L^{-1} $HClO_4$, b) 0.01 mol L^{-1} EDTA-Na_2 + 0.06 mol L^{-1} NaCl + 2.0 mol L^{-1} $HClO_4$ and c) 0.1 mol L^{-1} KSCN + 0.001 mol L^{-1} $HClO_4$.

To reading easier, throughout the text the three different electrolytes: a) $HClO_4$, b) $HClO_4$-EDTA-Na_2 and c) $HClO_4$-KSCN have been assigned the following abbreviations $HClO_4$, EDTA-Na_2 and KSCN, respectively.

Potassium dichromate was specially treated to render it virtually mercury-free: the salt was kept heated at 350°C for 4 days, then the temperature was raised to 410°C and the mass kept melted for 24 hours. The solidified salt was granulated and homogenized by corundum ball-milling.

The reducing agent $SnCl_2$. $2H_2O$ was dissolved in 10% (w/w) H_2SO_4 to give a 25% (w/w) solution which was bubbled with N_2 for 20 min to strip away any residual Hg and O_2.

Estuarine Sediment BCR-CRM 277, River Sediment BCR-CRM 320 and Mercury in Water NIST-SRM 1641d. were employed as standard reference materials for optimising and setting up the analytical procedure.

2.3. Sampling and sample preparation
2.3.1. Sediments
Sampling was performed by means of a plexiglass device: single carrots (section: 69.7 cm^2, height: 10 cm) were drawn out and put in polyethylene bottles, previously washed with a solution of 20% suprapure HNO_3 for 72 h and finally rinsed many times with Milli-Q deionised water. The sedimentary texture shows that clay and silt are the predominant size fraction (higher than 79% in all cases).

The samples, once dried at 45°C for 96 h, were passed through a 10 mesh inox sieve, to eliminate coarse material, powdered by means of a corundum ball mill, and passed through a 150 mesh inox sieve. Finally, they were dried at 50°C for 48 h prior to the sample preparation.

To solubilise the sediments, HNO_3-HCl acidic mixture has been employed.

Approximately 1.0 g of sediments and soils, accurately weighed, was placed in a Pyrex digestion tube calibrated at 25 mL and connected with a Vigreux column condenser together with 3 mL 69% (w/w) HNO_3 + 2 mL 37% (w/w) HCl. The tube was inserted into the cold home-made block digester, raising gradually the temperature up to 130-150°C, and keeping this temperature for the whole time of mineralisation (2 h). After cooling, the digest was filtered through Whatman N. 541 filter paper, evaporated to dryness and the soluble salts dissolved in 50 mL of the supporting electrolytes employed ($HClO_4$, $HClO_4$-EDTA-Na_2 and $HClO_4$-KSCN).

2.3.2. Sea Water
Sea water samples were taken with a portable suction pump made of stainless steel and pyrex glass, neither of which would contaminate the elements to be determined. Model experiments exactly simulating the sampling procedure were performed with artificial fresh and sea water, to verify that the samples would not be contaminated by the sampling device. The concentrations of both analytes in these artificial samples were found to be lower than the relevant limits of detection.

Water samples were immediately filtered on the spot through 0.22 μm membranes and transferred into polyethylene bottles, previously soaked in 1:1 nitric acid for 48 h and rinsed many times with deionised water (Milli-Q).

The samples were cooled to 4°C for transport, stored at this temperature and analysed within 72 hours.

$HClO_4$, $HClO_4$-EDTA-Na_2 and $HClO_4$-KSCN in water Standard Reference Material and sea water samples were employed as solutions for the voltammetric scans.

2.3.3. For the spectroscopic determination of Hg(II) in sediments and sea water, a different sample preparation procedure was followed [64]
2.3.3.1. Sediments
Approximately 1.0 g of sediment, accurately weighed, was placed in a digestion tube together with 1.2 g $K_2Cr_2O_7$ and 20 mL H_2O. A condenser was connected to the digestion tube and 20 mL H_2SO_4 were slowly added. The digestion tube was transferred to the hot block preheated at 180°C, and the digestion was allowed to proceed for 60 min to completion. After cooling at room temperature, the condenser was removed, rinsed with three 5 mL portions of H_2O and the washes were added to the digested matter. The open digestion tube, without the condenser, was replaced on the hot-block for a further 30 min boiling span. Finally, after cooling, the digested solution was diluted to 100 mL.

2.3.3.2. Sea Water
In the case of sea water, 1 g L^{-1} of $K_2Cr_2O_7$ was added to the water samples before measurements by CV-AAS, using $SnCl_2$ as reducing agent.

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3. Results and discussion

3.1. Aqueous reference solutions

For the voltammetric determinations of Hg(II), a preliminary study was carried out employing the relevant aqueous reference solutions [the blank concentrations for both elements (Hg(II) and Cu(II) as eventual interfering metal, see section 3.1.2 "*Interference problems*") were lower than the respective limits of detection].

Particular attention was paid to the chemical conditions relevant to each supporting electrolyte, in order to obtain: a) best voltammetric instrumental signals; *i.e.*, well defined peak with flatter voltammetric baseline and consequently a better signal-to-noise ratio; b) high analytical sensitivities and, consequently; c) very low limits of detection.

3.1.1. Aqueous reference solutions

HCIO₄

The relationship between the Hg(II) peak current obtained in DPASV at different concentrations and the HCIO₄ concentration has been investigated (Fig. 1).

0.1 mol L⁻¹ HCIO₄ has been chosen as a good compromise, since, for HCIO₄ concentrations higher than 0.15 mol L⁻¹, the voltammetric signal slightly decreases.

HCIO₄-EDTA-Na₂

Fig. 2 shows the Hg(II) peak current obtained in DPASV *vs.* NaCl concentration behaviours, at different HCIO₄ concentrations. In all cases, the behaviours are very similar and have a maximum in the range 0.05 – 0.07 mol L⁻¹ NaCl concentration, so the best compromise seems to be 0.06 mol L⁻¹ NaCl.

It is important also to highlight that, increasing the HCIO₄ concentrations, the relationships Hg(II) *vs.* NaCl concentration show to have the same behaviour, but without improving significantly the i_p voltammetric signal. for HCIO₄ concentration higher than 2.0 mol L⁻¹, so much so that, the functions at 2.5 and 3.0 mol L⁻¹ HCIO₄ concentrations are not inserted in the Fig. 2, since they are practically superimposed on that at 2.0 mol L⁻¹ HCIO₄ concentration.

For these reasons, the supporting electrolyte employed results to be HCIO₄-EDTA-Na₂.

It is important to point out that the electrode process reversibility of both elements increases on increasing the EDTA-Na₂ concentration (see Table 3). Keeping constant the best HCIO₄ concentration (2.0 mol L⁻¹), the maximum possible EDTA-Na₂ concentration was 0.01 mol L⁻¹, experimentally obtained solubilizing the EDTA disodium salt under conditions of stirring for a long time.
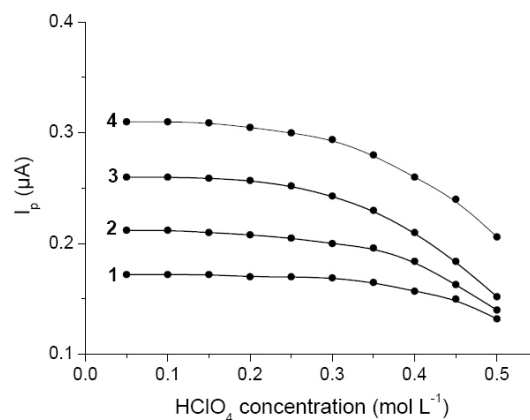

Figure 1. Relationship between the Hg(II) peak current obtained in DPASV and the HCIO₄ concentration. Hg(II) concentrations (µg L⁻¹): 1.07 (1); 2.30 (2); 3.96 (3); 5.03 (4). Experimental conditions: see section 2.1.
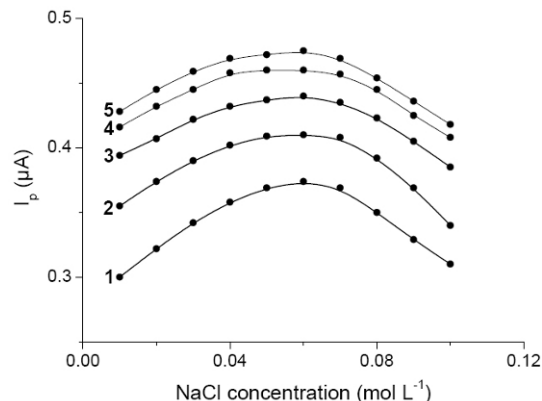

Figure 2. Influence of NaCl concentration on the Hg(II) peak current obtained in DPASV at different HCIO₄ concentrations. Hg(II) concentration: 19.6 µg L⁻¹. HCIO₄ concentrations (mol L⁻¹): 0.3 (1); 0.5 (2); 1.0 (3); 1.5 (4); 2.0 (5). Experimental conditions: see section 2.1.

0.01 mol L⁻¹ EDTA-Na₂ turned out to be the optimal concentration since for EDTA-Na₂ concentrations lower than 0.01 mol L⁻¹, keeping the NaCl and HCIO₄ concentrations constant, the Hg(II) peak current tends to decrease slightly.

HCIO₄-KSCN

The relationship between Hg(II) peak current obtained in DPASV and KSCN concentration, at different HCIO₄ concentrations, is reported in Fig. 3. It is clearly a constant maximum in the range 0.1 – 0.7 mol L⁻¹ KSCN concentration. 0.1 mol L⁻¹ KSCN has therefore been chosen as the optimal value.

As opposed to the other electrolytes, in the case of HCIO₄ its concentration seems to not significantly influence the Hg(II) peak current, at least in the 0.1-0.7 mol L⁻¹ KSCN concentration range, although the voltammetric peak seems to slightly

Table 2. Experimental peak potentials (E_p, V, Ag│AgCl│KCl$_{sat}$) for the aqueous reference solutions and for the standard reference material solutions. Number of independent determinations: 5.

		Hg(II)	Cu(II)
HClO$_4$	a	0.569±0.010	0.418±0.015
HClO$_4$-EDTA-Na$_2$	b	0.523±0.010	0.377±0.015
HClO$_4$-KSCN	c	-0.198±0.005	-0.349±0.010
	a	0.585±0.005	0.439±0.010
Estuarine Sediment BCR-CRM 277	b	0.549±0.010	0.396±0.005
	c	-0.177±0.005	-0.323±0.015
	a	0.597±0.015	0.441±0.010
River Sediment BCR-CRM 320	b	0.558±0.005	0.403±0.010
	c	-0.203±0.005	-0.358±0.015
Mercury in Water	a	0.577±0.010	0.427±0.015
NIST-SRM 1641d	b	0.512±0.005	0.369±0.005
	c	-0.215±0.010	-0.363±0.015

Table 3. Half peak width $w_{1/2}$ (mV) for the aqueous reference solutions and for the solution obtained by digestion of the standard reference materials.

		Hg(II)	Cu(II)
HClO$_4$	a	49	53
HClO$_4$-EDTA-Na$_2$	b	46	50
HClO$_4$-KSCN	c	73	81
	a	53	56
Estuarine Sediment BCR-CRM 277	b	47	52
	c	77	85
	a	55	58
River Sediment BCR-CRM 320	b	50	52
	c	75	83
Mercury in Water	a	52	55
NIST-SRM 1641d	b	48	51
	c	77	83
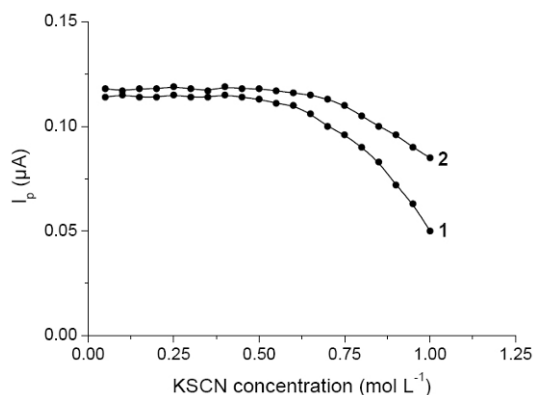
Figure 3. Influence of KSCN concentration on the Hg(II) peak current obtained in DPASV at different HClO$_4$ concentrations. Hg(II) concentration: 19.6 μg L^{-1}. HClO$_4$ concentrations (mol L^{-1}): 1.0 (1). 1.0x10^{-3} (2); Experimental conditions: see section 2.1.

improve the voltammetric signal by employing 0.001 mol L^{-1} HClO$_4$ concentration. To support this, several measurements were carried out in the 10^{-5} – 1.0 mol L^{-1} HClO$_4$ concentration range and only part of the data is reported as example in Fig. 3. It is important to highlight that for HClO$_4$ concentrations lower than 0.001 mol L^{-1} the voltammetric signal keeps practically constant.

The voltammetric scans relevant to each supporting electrolyte, were carried out using the instrumental parameters listed in Table 1, while the experimental peak potentials for each supporting electrolyte are reported in Table 2.

3.1.2. Reversibility degree of the Hg(II) and Cu(II) electrode processes and possible interference problems

Many times when commonly used supporting electrolytes are employed the reduction peak potentials of each metal are very close making simultaneous voltammetric determination of neighbouring elements difficult.

However, it is important to highlight that the interference problem is strictly linked to the reversibility of the electrode process, since very reversible electrode processes imply well defined peaks, with half peak widths close to theoretical values.

In the case of Anodic Stripping Voltammetry [65-68], totally reversible electrode processes, for small pulse height [69], have been shown to have $w_{1/2}$ value independently of concentration, equal to 90.6/n mV at 25°C, where n is the number of electrons involved in the electrode process.
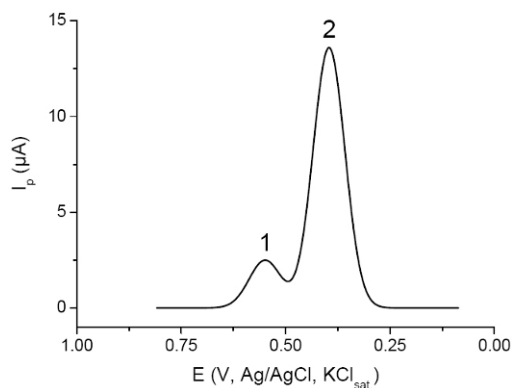
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Figure 4. Differential pulse anodic stripping voltammogram of Hg(II) (peak 1) and Cu(II) (peak 2) in Estuarine Sediment BCR-CRM 277 Standard Reference Material. Supporting electrolyte: $HClO_4$-EDTA-Na_2. Concentrations (see Table 4, $\mu g\ g^{-1}$): 1.77±0.06 [Hg(II)]; 101.7±1.6 [Cu(II)]. Experimental conditions: see section 2.1.
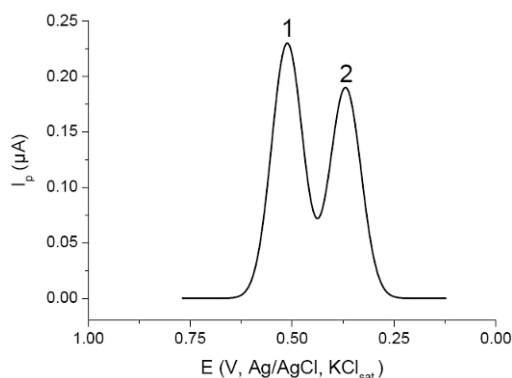

Figure 5. Differential pulse anodic stripping voltammogram of Hg(II) (peak 1) and Cu(II) (peak 2) in Mercury in Water NIST-SRM 1641d Standard Reference Material. Supporting electrolyte: $HClO_4$-EDTA-Na_2. Concentrations (see Table 4, $\mu g\ L^{-1}$): 1.59±0.02 [Hg(II)]; 1.23 (spiked) [Cu(II)]. Experimental conditions: see section 2.1.

The higher the value of $w_{1/2}$, lower is the reversibility of the electrode process, with very ill defined voltammetric signal. Considering that in the case of Hg(II) determination at gold electrode the more important interfering analyte seems to be Cu(II) [60], Table 3 reports the $w_{1/2}$ data for Hg(II) and Cu(II) relevant either to the aqueous reference solutions or to the standard reference materials.

On the basis of the $w_{1/2}$ values, certainly $HClO_4$-EDTA-Na_2, but also $HClO_4$, seem to be the best supporting electrolytes. In the case of these supporting electrolytes Hg(II) and Cu(II) present very good reversibility, since their $w_{1/2}$ values are very close to theoretical ones, and consequently also have lower interference problems. In this case qualitative investigations show that Hg(II)-Cu(II) concentration ratios higher than 250 cause strong interference of the voltammetric signals, thereby hindering the correct determination of both metals in the presence of each other.

In the $HClO_4$-KSCN supporting electrolyte, Hg(II) and Cu(II) $w_{1/2}$ data are decidedly higher and the interference can be very strong so the determination of both metals may be very difficult or perhaps impossible at low metal concentration ratios.

3.2. Quality control and quality assessment
3.2.1. Standard reference materials
The method set up in aqueous reference solutions was applied to standard reference materials: Estuarine Sediment BCR-CRM 277, River Sediment BCR-CRM 320 (from Institute for Reference Materials and Measurements, European Commission, Joint Research Centre, Belgium) and Mercury in Water NIST-SRM 1641d (from National Institute of Standards and Technology, Gaithersburg, MD, USA) in order to confirm and verify the applicability of the analytical procedure, and determine its accuracy and precision (Table 4).

However, it is important to point out that, in the case of Cu(II), the metal concentration listed in Table 4 has been spiked in the Mercury in Water NIST-SRM 1641d reference materials. This may seem an anomalous procedure, but in our opinion, it happens to be the only way, since the Standard Water Reference Materials containing certified concentrations of these metals together with Hg(II) are not available.

In the experimental conditions employed, precision as well as repeatability [70], expressed as relative standard deviation (s_r%) on five independent determinations, was satisfactory, in all cases it was lower than 5%, while accuracy expressed as relative error (e%) was generally in the order of 4-6%.

Figs. 4 and 5 show the square wave voltammograms of Hg(II) and Cu(II) in Estuarine Sediment BCR-CRM 277 and in Mercury in Water NIST-SRM 1641d Standard Reference Materials, respectively.

3.2.2. Limits of detection
The limits of detection (LOD) for both techniques (Table 5) for aqueous reference solution, solutions obtained by digestion of sediment and soil standard reference materials and superficial water standard reference materials were obtained by the equation LOD=$K\ s_{y/x}/b$ [70], where $s_{y/x}$ and b are the estimated standard deviation and the slope of the analytical calibration function of each element, respectively, with a 99.7% (K=3) confidence level [71].

In the case of voltammetric technique, since the analytical calibration functions were determined by standard addition method, it was also possible to directly obtain the LODs in the real matrices (Table 5).

Table 4. Accuracy and precision of the analytical procedure. The determined values are the mean of 5 independent determinations ± confidence interval at 95% probability level. Concentrations: $\mu g\ g^{-1}$ in the case of sediment Standard Reference Materials; $\mu g\ L^{-1}$ in the case of water Standard Reference Material. Experimental conditions: see Table 1.

	*	Element	Certified value	Determined value	e (%)	$s_{r\ (\%)}$
			Voltammetric measurements			
Estuarine Sediment **BCR-CRM 277**	a	Hg(II)	1.77±0.06	1.86±0.090	+5.1	4.3
		Cu(II)	101.7±1.60	96.0±5.900	-5.6	4.7
	b	Hg(II)	1.77±0.06	1.84±0.100	+4.0	3.9
		Cu(II)	101.7±1.60	95.9±6.300	-4.7	4.0
	c	Hg(II)	1.77±0.06	1.87±0.120	+5.6	5.1
		Cu(II)	101.7±1.60	107.1±5.700	+5.3	4.9
River Sediment **BCR-CRM 320**	a	Hg(II)	1.03±0.13	1.07±0.070	+3.9	4.1
		Cu(II)	44.1±1.00	41.7±2.000	-5.4	4.4
	b	Hg(II)	1.03±0.13	1.06±0.050	+2.9	4.3
		Cu(II)	44.1±1.00	45.8±1.900	+3.9	3.8
	c	Hg(II)	1.03±0.13	0.97±0.070	-5.8	5.0
		Cu(II)	44.1±1.00	46.5±2.700	+5.4	5.1
Mercury in Water **NIST-SRM 1641d**	a	Hg(II)	1.59±0.02	1.50±0.100	-5.7	4.7
		Cu(II)	1.23 **	1.29±0.080	+4.9	4.5
	b	Hg(II)	1.59±0.02	1.53±0.018	-3.8	4.2
		Cu(II)	1.23 **	1.18±0.090	-4.1	4.5
	c	Hg(II)	1.59±0.02	< LOD	-	-
		Cu(II)	1.23 **	< LOD	-	-

	Element	Certified value	Determined value	e (%)	$s_{r\ (\%)}$
		Spectroscopic measurements			
Estuarine Sediment **BCR-CRM 277**	Hg(II)	1.77±0.06	1.87±0.11	+5.6	4.7
	Cu(II)	101.7±1.6	106.3 ±5.60	+4.5	4.3
River Sediment **BCR-CRM 320**	Hg(II)	1.03±0.13	0.96±0.10	-6.8	5.1
	Cu(II)	44.1±1.00	46.0±2.30	+4.3	4.5
Mercury in Water **NIST-SRM 1641d**	Hg(II)	1.59±0.02	1.67±0.12	+5.0	4.9
	Cu(II)	1.23 **	1.16±0.09	-5.7	5.3

* a: $HClO_4$; b: $HClO_4$-EDTA-Na_2; c: $HClO_4$-KSCN
** In the case of Cu(II), the concentration listed in the certified value column has been spiked to the Standard Reference Material at the beginning of the digestion step.

3.2.3. Comparison between voltammetric and spectroscopic measurements

To better validate the voltammetric analytical procedure proposed, the metal concentrations have also been determined by atomic absorption spectroscopy.

The voltammetric and spectroscopic results reported in Tables 4 and 6 relevant either to standard reference materials or to sediment, soils and superficial water sampled from sites A-F (see section 4), respectively, show very good agreement (differences generally lower than 7% in all cases).

4. Practical applications

Once the method for the voltammetric determination of Hg(II), and/or in presence of Cu(II) was set up in aqueous reference solutions and validated by analysis of standard reference materials, it was adapted for sediments and sea water (salinity 2.5-2.9%) samples that were drawn out at in five sites inside a lagoon ecosystem located in the proximity of Ravenna (Italy).

The Lagoon of Ravenna is a peculiar and protected natural ecosystem of tourist importance; it is also near an area of intense industrial and agricultural activity. During the 1950s, a very important industrial area was built in the southern border of the wetland. Unfortunately before 1973 due to the lack of environmental legislation, industrial wastes were released directly into the Lagoon without any treatment. It has been estimated that during the 1958-1973 period tens of tons of mercury coming from chemical plants, which produced acetaldehyde and vinyl chloride from acetylene using mercury salts as catalysts contaminated the Lagoon of Ravenna [72].

The experimental results reported in Table 6 show that the analytical procedures proposed are certainly applicable and transferable without problems to environmental matrices like sediments and superficial water.

The sampling sites A-F were chosen based on increasing distance from the point of maximum pollution impact of the seventies (distance: A<B<C<D<E<F).

Moreover it is also important to highlight that these data are in general agreement with the Hg(II)

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Table 5. Limits of detection (LOD)*, calculated in μg L^{-1} and expressed in μg g^{-1} only in the case of Estuarine Sediment BCR-CRM 277 and River Sediment BCR-CRM 320, determined in the Aqueous Reference Solutions and in the Standard Reference Materials. Number of independent determinations: 5. The limits of detection were obtained by the analytical calibration functions [70] of each element (K=3, 99.7 % confidence level [70,71]) (see Section 3.2.2).

		Hg(II)	Cu(II)
HClO$_4$	a	0.76	0.91
HClO$_4$-EDTA-Na$_2$	b	0.63	0.70
HClO$_4$-KSCN	c	3.09	3.15
Estuarine Sediment BCR-CRM 277	a	0.17	0.22
	b	0.15	0.29
	c	0.43	0.47
River Sediment BCR-CRM 320	a	0.16	0.25
	b	0.13	0.23
	c	0.45	0.39
Mercury in Water NIST-SRM 1641d	a	0.81	1.07
	b	0.62	0.69
	c	3.11	3.47

In the case of spectroscopic measurements, the limits of detection (concentration: μg L^{-1}) calculated for the aqueous reference solutions were: 0.89 [Hg(II)]; 1.07 [Cu(II)].

Table 6. Mean values of Hg(II) and Cu(II) concentrations relevant to sediments (μg g^{-1}) and sea waters (μg L^{-1}) sampled in the Lagoon ecosystem of Ravenna (see text, section 4). Number of independent determinations: 5. The confidence interval is calculated at 95% probability level.

Voltammetric measurements

	*	A Hg(II)	A Cu(II)	B Hg(II)	B Cu(II)	C Hg(II)	C Cu(II)	D Hg(II)	D Cu(II)	E Hg(II)	E Cu(II)	F Hg(II)	F Cu(II)
Sediments	a	53.3±2.7	41.3±1.5	20.7±1.1	40.5±2.1	9.6±0.5	30.6±1.5	2.9±0.1	38.7±1.7	0.42±0.02	36.9±1.8	0.58±0.02	40.1±2.1
	b	51.8±2.3	42.5±1.9	19.5±0.9	42.0±1.9	10.3±0.7	28.9±1.3	2.7±0.2	40.3±2.3	0.40±0.03	35.7±2.0	0.57±0.04	39.1±1.9
	c	54.2±2.5	43.7±1.7	19.1±1.0	42.3±1.8	9.5±0.6	32.1±1.6	3.0±0.1	40.9±2.1	< LOD	35.3±1.9	0.60±0.03	38.9±2.0
Sea water	a	30.5±1.7	69.1±3.1	18.1±1.0	43.1±2.0	12.8±0.7	37.2±1.4	2.5±0.1	35.7±1.8	< LOD	28.4±1.3	< LOD	30.7±1.5
	b	31.2±1.9	70.4±3.0	17.7±0.7	42.3±2.3	13.6±0.6	38.8±1.7	2.3±0.2	36.1±1.9	< LOD	29.3±1.9	< LOD	31.2±1.4
	c	32.9±1.5	72.3±3.5	17.0±0.9	41.5±1.8	12.5±0.8	36.1±1.9	< LOD	37.2±1.6	< LOD	27.9±1.8	< LOD	31.9±1.7

* a: HClO$_4$; b: HClO$_4$-EDTA-Na$_2$; c: HClO$_4$-KSCN

Spectroscopic measurements

	A Hg(II)	A Cu(II)	B Hg(II)	B Cu(II)	C Hg(II)	C Cu(II)	D Hg(II)	D Cu(II)	E Hg(II)	E Cu(II)	F Hg(II)	F Cu(II)
Sediments	52.3±2.7	42.3±2.5	21.0±1.2	41.5±2.3	9.7±0.9	29.6±1.5	3.1±0.2	41.0±2.4	0.43±0.02	36.5±1.9	0.61±0.03	38.7±2.1
Sea water	29.7±1.5	70.1±3.4	18.5±1.1	41.1±2.1	13.9±0.8	36.9±2.0	2.7±0.3	36.8±1.8	< LOD	29.5±1.5	< LOD	32.0±1.6

concentration results in sediments and superficial water obtained in the same area during previous surveys [72,73].

5. Conclusions

- Among the three supporting electrolytes investigated, HClO$_4$ and HClO$_4$-EDTA-Na$_2$ are practically equivalent, having similar accuracy and precision data in the Hg(II) and Cu(II) determination, although the latter seems to be better at allowing for lower limits of detection. On the contrary, HClO$_4$-KSCN is decidedly worse, considering that the too high limits of detection render such supporting electrolyte inadequate for practical applications, since Hg(II) and Cu(II), concentrations present in real matrices are, in most cases, lower than the limits of detection. However it is important to highlight that this comment, regarding HClO$_4$-KSCN, is general and not just for water samples but also for sediment matrix - the difference is evident (see Table 5) - wherein the experimental conditions employed, the LODs are about 2-3 times higher.

- Finally, with regard to the HClO$_4$-Na$_2$ supporting electrolyte, the addition of EDTA-Na$_2$, was aimed at improving either the voltammetric peak resolution or the reversibility of the reduction/oxidation processes of both metals investigated. Unfortunately only the latter one has been attained, decidedly bettering the $w_{1/2}$ (see Table 3), and consequently the reversibility of the electrode processes.

- In terms of the instrumental methods employed in this study: the precision and accuracy is good and results obtained using both the techniques are comparable in all the cases. The limit of detection using the experimental conditions employed are comparable with those obtained

by spectroscopic measurements; however, in the case of the DPASV method, such limits can be decidedly improved if higher electrodeposition times are used. The two techniques would then be equivalent, although voltammetry is better when compared with atomic absorption spectroscopy. It allows for simultaneous metal determinations, exceptions for which are cases when the concentration ratios of two neighbouring metals are. too high. However, in conclusion, such a technique may be certainly a good alternative to spectroscopy,

which would need very expensive equipment like Zeeman background corrector in order to be used for determination of metals in complex matrices and with high salt content as for example sea water.

Acknowledgement

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