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MULTIANALYTE VOLTAMMETRIC DETERMINATION OF TRAFFIC-LINKED METALS IN MARINE ORGANISMS EMPLOYED AS POLLUTION BIO-MONITORS.

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Abstract

A new procedure is proposed for the voltammetric determination of ultra-trace cadmium(II), copper(II), lead(II), platinum(II), palladium(II), rhodium(III) and zinc(II) by a single run of square wave adsorptive catalytic stripping voltammetry.

The voltammetric cell was based on a typical three-electrodes set-up: working electrode (stationary hanging mercury drop), reference electrode ($Ag|AgCl|KCl_{sat}$) and auxiliary electrode (platinum). The supporting electrolyte contained the formazone complex together with dimethylglyoxime and the disodium salt of ethylenediaminetetraacetic acid.

Validation of the analytical procedure was obtained by the analysis of standard reference materials (Mussel Tissue BCR-CRM 278, Oyster Tissue NIST-SRM 1566a). Comparison with spectroscopic measurements was performed. Good accuracy was achieved.

The analytical procedure was finally applied to mussels and clams sampled on the mouth of Po river, employed as bio-monitors.

Keywords: Platinum Group Metals (PGMs), Toxic Metals, Mussels, Clams, SWAdCSV, Spectroscopy.

1. Introduction

In the last few years, the huge problem relevant to the presence in the environment of toxic metals [1] has become a topical subject of great interest. These metals are strictly linked to the vehicle emissions, since the automotive catalytic converters contain platinum group metals (PGMs). PGMs allow the significant reduction of the harmful gas emission levels

from motor vehicles, like lead, nitrogen oxides, carbon monoxide, and unburned hydrocarbon. However, PGMS are contemporaneously the cause of a widespread distribution of fine particulate matter and dust originated from deterioration/abrasion of the bulk catalysts [2-13]. The consequence is a considerable increase of Pt(II), Pd(II) and Rh(III) concentrations in superficial waters, vegetation, soil surfaces, especially in sites next to roadways at high traffic density. In fact, such concentrations are generally lower than 0.1-0.2 $\mu g\ L^{-1}$ in liquid matrices (fresh and sea water) and lower than a few units of $\mu g\ kg^{-1}$ in solid matrices (soil, plant and particulate matter) [1]. However, considering their toxicity [14-25] and the lack of rules establishing the maximum tolerable concentration levels for humans (the problem is still under discussion [26]), these elements can be dangerous for health. The danger may come from direct contact with the dust, inhalation of fine particulate matter (aerodinamic diameter < 10 μm), food and water. The most involved environmental matrices are surface waters, fresh- and also sea water. Indeed, all the waterways are totally influenced by the environment, acting as collectors of all the pollution load due to human activities.

To check the pollution load of a superficial water ecosystem, single samplings can be made at specific times, and then the determinations are punctual. In our opinion, complete information can be obtained only following the path of bio-monitoring [27-42]. The scope is to ascertain the variation over time of the pollutant concentrations in organisms that live or are permanently present in the ecosystem. The use of bio-monitors to evaluate the pollutant load of an aquatic ecosystem is considered with great suspicion by the scientific community, but in our opinion no feasible alternative is available. In this regard, the literature reports only the numerous and very interesting works by Zimmerman and coworkers [12, 43-46], who first proposed and discussed the possibility of biomonitoring by means of a species of

mussel (*Dreissena polymorpha*) using spectroscopic techniques, total reflection X-ray fluorescence analysis and voltammetric techniques.

In addition to the possible use of mussels and clams in bio-monitoring campaigns, another fundamental aspect of public health concerns the huge food use of such marine species. Indeed, certain marine species accumulate toxic metals, making them enter the food chain and become hazardous to human health. In this context, mussels and clams, but also oysters, fishes, shrimps and algae, were found to sequestrate and concentrate several metals from their aqueous environment. In particular, oysters, mussels and clams, being filtering organisms, require special attention and surveys before they are placed on the market for sale: an adult organism is able to filter up to 5 L h^{-1}, depending on weight, size and water temperature.

In any case, for the determination of Pt(II), Pd(II) and Rh(III), spectroscopy is the most widely employed technique [4, 39, 47, 48]; very interesting are also the works of Essumang and coworkers, who propose the neutron activation analysis (NAA) as instrumental technique [21, 34, 49]. Also in the case of Cu(II), Pb(II), Cd(II) and Zn(II) the most widely used technique is spectroscopy in all its versions, while NAA [50-52] and voltammetry [53-56] are seldom employed. Finally, as regards the analytical procedures employed for the determination of the metals object of the present study, from sampling to instrumental techniques used, very interesting and exhaustive is the recent review by Locatelli and Melucci [57, and therein references].

The present paper proposes for the first time an innovative analytical procedure that allows to simultaneously determine seven elements, very toxic for humans – Pt(II), Pd(II), Rh(III), Cu(II), Pb(II), Cd(II) and Zn(II) – in complex matrices like mussels and clams, using a single

voltammetric scan by square wave adsorptive catalytic stripping voltammetry (SWAdCSV). The method is suitable for mussels and clams, either possible bio-monitors or also cultivated, harvested and placed on the market for human consumption. In addition, at the Authors' knowledge, this work proposes and discusses for the first time the possibility of using the adsorptive catalytic voltammetry to determine also Cu(II), Pb(II), Cd(II) and Zn(II).

2. Experimental

2.1. Apparatus

A Multipolarograph AMEL (AMEL, Milan, Italy) Mod. 433 was employed for all the voltammetric scans. A conventional three electrode measuring cell was employed: a stationary hanging mercury drop electrode (HMDE) as working electrode, an $Ag|AgCl|KCl_{satd.}$ electrode and a platinum wire as reference and auxiliary electrode, respectively.

The experimental conditions are reported in Table 1.

Before the measurements, the voltammetric cell was rinsed with suprapure concentrated 1:1 HNO_3 and then many times with Milli-Q water, to avoid accidental contamination. The solutions were thermostated at 20.0±0.5°C and deaerated with water-saturated pure nitrogen for 5 min prior to analysis. 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 using a Perkin-Elmer (Perkin-Elmer, Waltham, MA, USA), Mod. A-Analyst 100 Atomic Absorption Spectrometer, equipped with a deuterium background corrector, Autosampler AS-72 and

with HGA 800 graphite furnace. Single-element Lumina (Perkin-Elmer, Waltham, MA, USA) hollow-cathode lamps were used. For each element to be determined, ashing and atomization steps were optimized before measurements [58].

The instrument settings were those recommended by the Manufacturer [59].

2.2. Reagents and Reference Solutions

All acids and chemicals were suprapure grade (Merck, Germany). Acidic stock metal solutions (1000 mg/L, Merck, Germany) were respectively employed in the preparation of reference solutions at varying concentrations for each element. Water demineralized by a Milli-Q system was used for diluting.

0.1 mol L^{-1} HCl + $2.3 \cdot 10^{-4}$ mol L^{-1} dimethylglyoxime (DMG) + formazone complex [0.7 mmol L^{-1} formaldehyde + 1.5 mmol L^{-1} hydrazine in 0.1 mol L^{-1} HCl] + $8.5 \cdot 10^{-2}$ mol L^{-1} $NaBrO_3$ + $4.9 \cdot 10^{-4}$ mol L^{-1} EDTA-Na_2 was employed as the supporting electrolyte.

A stock DMG solution was prepared by dissolution of the pure substance in absolute ethanol. The formaldehyde - hydrazine (formazone complex) in 0.1 mol L^{-1} HCl solution was prepared immediately before the employment, owing to its instability.

To optimise and set up the analytical procedure, Mussel Tissue BCR-CRM 278 and Oyster Tissue NIST-SRM 1566a were employed as standard reference materials.

2.3. Sampling Area

The sampling site for mussels and clams was the Goro Bay (Province of Ferrara, Italy), a very important area devoted to the fishing and breeding of mussels and clams for food. This area suffers considerable pollution problems, due to its location in proximity of the Po River mouth, which carries a large amount of industrial and domestic waste waters towards the sea. In fact, it should again be reiterated what previously stated, *i.e.* particular attention should be

paid to surface waters matrices, fresh waters and sea water. Indeed, the environment influences all the waterways, which consequently act as huge collectors of all the pollutants present in the crossed areas. In particular, pollutants linked to vehicular traffic are conveyed to sea.

In the Goro Bay, directly connected with the Adriatic Sea, three branches of the Po river delta mouth are present: Po of Volano (site A), Canal Bianco (site B) and Po of Goro (site C). Samplings of mussels and clams were carried out in front of the points where such branches flow into the Bay itself, while an additional sampling (site D) was chosen at open Adriatic Sea, for eventual comparisons.

The sampling were carried out in the Summer 2019.

2.4. Sample Preparation

About 8 kg of *Mytilus Galloprovincialis* and of *Tapes Philippinarum* were collected in the four sampling sites (see section 2.3 “Sampling Area”), taken to the laboratory and prepared for analyses. They were opened with a plastic appliance, and the organisms were carefully extracted and placed in polyethylene containers. The containers were previously treated with suprapure HNO_3 diluted in 1:1 proportion with water, and then by repeated rinsing for 48 h with Milli-Q water in order to avoid any contamination. The samples were frozen and then lyophilised for 30 h. After this treatment, the samples were thoroughly homogenized in an agate mortar.

The sample preparation for Mussel Tissue BCR-CRM 278, Oyster Tissue NIST-SRM 1566a and for real samples of mussels and clams was the following: approximately 1.0 g, accurately weighed, was placed in a platinum crucible and dissolved in 5 mL 69 $\%_{w/w}$ HNO_3 + 3 mL 37 $\%_{w/w}$ HCl + 7 mL 98$\%_{w/w}$ H_2SO_4 at 130-150 °C. The mixture was evaporated to

dryness and, after cooling, soluble salts were dissolved in 25 mL of supporting electrolyte. The so obtained solutions were then diluted, if necessary, before spectroscopic measurements.

2.5. Total Analytical Procedure

10-mL sample aliquots were pipetted into the voltammetric cell, and deaerated for 5 min by bubbling water-saturated pure nitrogen. The samples were: i) supporting electrolyte used as aqueous reference solution; ii) solutions obtained in the mineralization step of the standard reference material; iii) real samples. Metal determinations were carried out by SWAdCSV, employing the standard addition methods (the determination coefficients were good, being better than 0.9989 for all the elements).

The voltammetric scans were carried out using the instrumental parameters listed in Table 1.

Table 2 shows the element peak potentials in the aqueous reference solutions and in the standard reference material solutions.

3. Results and Discussion

The procedure here proposed shows very high sensitivity, with detection limits at the sub-ppb level, and is based on the dual current-magnifying effect of the stripping catalytic response of the adsorbed metal complexes in the presence of bromate.

For all elements, the catalytic reactions occur at the surface of the working electrode when, inside the electrode double layer, the reduced form of the depolarizer is oxidized to its previous voltammetric active form by an oxidizing agent in the layer of the solution close to the electrode surface. The scheme of “catalytic systems of the first kind” described by Bobrowski and Zarebski [60] is followed:

$Me_{(ox)}$-complexing $agent_{solution} \leftrightarrow Me_{(ox)}$-complexing $agent_{surface}$ (accumulation) (1)

$Me_{(ox)}$-complexing $agent_{surface} + ne^- \leftrightarrow Me_{(red)}$-complexing $agent_{surface}$ (2)

$Me_{(red)}$-complexing $agent_{surface} + Ox + (H^+, H_2O) \rightarrow$

$\rightarrow$ $Me_{(ox)}$-complexing $agent_{surface} + Red + (H_2O, OH^-)$ (3)

where $Me_{(ox)}$-complexing $agent_{surface}$ and $Me_{(red)}$-complexing $agent_{surface}$ are oxidized and reduced form of the depolarizer on the electrode surface, Ox is the oxidizing agent, Red is the product of catalytic reduction of the oxidizing agent and where complexing agents are DMG and formazone complex for Pd(II) and Pt(II) – Rh(III), respectively.

The depolarizer $Me_{(ox)}$-complexing $agent_{surface}$ repeats the described cycle many times (equations 2 and 3), and this causes a large increase of the current signal; consequently, the sensitivity of the method is improved. In particular, the catalytic electrodic reactions of the two elements are the following: Pt(II)→Pt(0), Pd(II)→Pd(0), Rh(III)→Rh(0), Cu(II)→Cu(0), Pb(II)→Pb(0), Cd(II)→Cd(0) and Zn(II)→Zn(0) while the oxidizing agent Ox is $NaBrO_3$.

The adsorptive-catalytic nature of the electrodic processes in presence of bromate is confirmed by cyclic voltammetric measurements, following the same procedure reported in our previous works [61-63]. Indeed, all the seven elements show one cathodic peak during the negative-going scan. In the reverse direction scan, each element shows a peak decidedly higher in presence than in absence of bromate. This experimental evidences, together with the fact that the voltammetric signals increase strongly when an accumulation period precedes the cyclic potential scans, unequivocally show that both forms $Me_{(ox)}$-complexing $agent_{surface}$ and $Me_{(red)}$-complexing $agent_{surface}$ remain adsorbed on the electrode surface.

3.1. Aqueous Reference Solutions

3.1.1 Choice of the supporting electrolyte and reversibility degree of the electrodic processes

The choice of supporting electrolyte and reversibility degree of the electrodic processes are closely related to each other. Indeed, the metal electrodic processes in the employed supporting electrolyte must show a high reversibility degree. As it is well-known, the reversibility degree is linked to the half peak width of the voltammetric peak of the metal itself.

a) Choice of the supporting electrolyte

Some points regarding the choice of the supporting electrolyte must be highlighted.

Preliminarily voltammetric measurements were made in the presence of only one complexing agent, and more precisely:

- in the presence of the only DMG, Pd(II) showed a very good reversible signal, with values of half peak width ($w_{1/2}$) next to theoretical ones; Pt(II) and Rh(III) showed lower signals (*i.e.* lower sensitivity) but equally reversible signals, as in the case of the Pd(II);
- even in the presence of only the formazone complex, Pt(II), Rh(III) showed excellent reversible signals (high sensitivity), while Pd(II) showed a lower sensitivity, always maintaining a high reversibility degree.

Other important observations regarding the composition of the supporting electrolyte must finally be reported and discussed:

- the simultaneous presence of the two complexing agents (DGM and formazone complex) allowed high sensitivity for all the PGMs [Pt(II), Pd(II) and Rh(III)];
- in the presence either of DGM and formazone complex, Cu(II), Pb(II), Cd(II) and Zn(II) show reversible or quasi-reversible voltammetric peaks, on the basis of $w_{1/2}$ values (Table 3);

- the contemporary presence of $NaBrO_3$ allowed an additional current-magnifying effect of the stripping catalytic response;

- the presence of EDTA-Na_2 permitted a better separation of the voltammetric peaks, allowing to simultaneously determine Cu(II), Pb(II), Cd(II) and Zn(II), together with Pt(II), Pd(II) and Rh(III), as punctually described in section 3.1.3 “Interference Problems”.

b) Reversibility degree of the electrodic processes

In the absence of heterogeneous rate constant K_s relevant to the electrodic processes of the metals here discussed in the supporting electrolyte employed, only a qualitative indication about the reversibility of each electrode process has been evaluated by measuring the $w_{1/2}$ values.

In the case of pulse voltammetric techniques [64-68], it is well-known that totally reversible electrodic processes, for small pulse height [69] as in the present work, independently of concentration, show $w_{1/2}$ value equal to 3.53 RT/nF mV. At 25°C, $w_{1/2}$ is equal to 90.6/*n* mV, where *n* is the number of electrons involved in the electrodic process.

The $w_{1/2}$ values reported in Table 3 are obtained in the supporting electrolyte, in the solutions obtained by digestion of the standard reference material, and in the solutions obtained by digestion of the mussels and clams. They show the fairly good reversibility of the Pt(II)-Pt(0), Pd(II)-Pd(0), Rh(III)-Rh(0), Pb(II)-Pb(0) and the quasi-reversibility of the Cu(II)-Cu(0), Cd(II)-Cd(0) and Zn(II)-Zn(0) electrodic processes.

Evidently, the quasi-reversibility of the Cu(II)-Cu(0), Cd(II)-Cd(0) and Zn(II)-Zn(0) electrodic processes influences their limits of detection (LoD). These LoDs, although not completely satisfactory, are suitable for marine organism matrices, which generally do not

show excessively low Cu(II), Cd(II) and Zn(II) concentrations. Consequently, this allows to employ the here proposed analytical procedure also for the determination of these metals.

3.1.2. Optimisation of the Instrumental and Chemical Voltammetric Parameter Conditions

3.1.2.1. Instrumental Voltammetric Parameters

Certainly, the most important instrumental parameter to which particular attention has been paid is the electroadsorption time t_{ads}.

Electroadsorption Time (t_{ads})

An electroadsorption step, at potentials more anodic than the peak potentials of all elements, shows to enhance the voltammetric signals and consequently the adsorptive catalytic current of all the elements. In all cases, the peak area linearly increases [$A_p = K\ t_d$] at least up to 13-15 min. Thus, the choice of the optimum value is strictly linked to the concentration of elements being determined.

3.1.2.2 Chemical Conditions

a) DMG Concentration

In acidic medium, all the elements show well-defined adsorption voltammetric peaks employing DMG as complexing agent [70], even if with different reversibility degree (see section 3.1.1 “Choice of the supporting electrolyte and reversibility degree of the electrodic processes”). To determine the best DGM concentration, the same procedure developed in previous works by the same authors [71-73] was followed.

b) Formaldehyde and Hydrazine Concentrations

Formaldehyde and hydrazine react with formation of formazone; this species forms complexes in the presence of Pt(II) and Rh(III), in acidic medium [74]. As reported in the literature [74-76], formazone presents the problem to be stable only for a few hours and,

consequently, it is advisable its formation *in situ* by adding formaldehyde and hydrazine directly to the sample in the voltammetric cell prior to the de-aeration.

For this reason, it is important to optimise the concentration of the two compounds in order to obtain the best voltammetric response for all the elements. In the present case, 0.7 mmol L^{-1} formaldehyde + 1.5 mmol L^{-1} hydrazine in 0.1 mol L^{-1} HCl have shown to be the optimum compromise values.

c) Bromate Concentration

All elements show a pseudo-isotherm relationship peak-area *vs.* bromate concentration [62, 63]; an increasing trend with the increase in bromate concentration, until reaching practically constant values, is observed.

In the employed experimental conditions, $8.5 \cdot 10^{-2}$ mol L^{-1} seems the optimum BrO_3^- concentration compromise for all the elements.

d) EDTA-Na_2 Concentration

To determine the best EDTA-Na_2 concentration, the same procedure developed in previous work by the same authors [62] was followed. Additions were performed at increasing concentrations of EDTA-Na_2 to obtain a satisfactory separation of all the seven peaks of the metals, in order to consequently allow for their quantification.

It should also be noted that concentrations higher than $4.9 \cdot 10^{-4}$ mol L^{-1} did not show a significant improvement of the position of the voltammetric peaks, so this concentration seemed to be the best compromise.

3.1.3. Interference Problems

a) Interference from Cu (II), Pb(II), Cd(II) and Zn(II)

In the absence of EDTA-Na_2 in the supporting electrolyte (see Table 2 and Figure 1), the pairs of interfering elements are Pd(II)-Pb(II), Cd(II)-Pt(II) and Rh(III)-Zn(II).

Unfortunately, under the experimental conditions employed and in absence of EDTA-Na_2, Pb(II), Cd(II) and Zn(II) show well defined reversible or quasi-reversible voltammetric peaks, very close to those of Pd(II), Pt(II) and Rh(III) (see Table 2).

Considering that Pb(II), Cd(II) and Zn(II), together with Cu(II), are always present in all environmental matrices, and in particular in marine filtering organisms like mussels and clams, evidently this involves a great interference problem: it that does not allow the simultaneous voltammetric determination of all the elements considered in the present study.

Our methodological procedure, already proposed by the same authors [62, 77-79], shows that, by adding EDTA-Na_2, the position of the voltammetric peaks is modified and shifted towards more cathodic values. Consequently, the peaks are enough separated, and the new peak positions (Table 2) allow their resolution and quantitative determination as well (Figure 2).

b) Possible but Improbable Interferences from Co(II) and Ni(II)

In basic media, both elements show very reversible voltammetric peaks at about –1.1 V *vs.* Ag|AgCl|$KCl_{satd.}$ [Ni(II)] and –1.2 V *vs.* Ag|AgCl|$KCl_{satd.}$ [Co(II)]. Consequently, these elements may potentially interfere with Rh(III) and Zn(II), which present the voltammetric signals in the same potential range (–1.019 and –1.196 V *vs.* Ag|AgCl|$KCl_{satd.}$, see Table 2).

In fact, in acidic media [80, and therein references], as in the present case, Ni(II) and Co(II) show voltammetric peaks practically at the same potential values: –1.077 and –1.169 V *vs.* Ag|AgCl|$KCl_{satd.}$) for Ni(II) and Co(II), respectively. However, they show a very low

reversibility degree of the electrodic processes, and consequently a very low analytical sensitivity (LoDs of the order of 900-950 μg L^{-1}, corresponding to about 23-25 μg g^{-1}).

Considering that in mussels and clams, Ni(II) and Co(II) do not exceed the concentration values of about 0.5-1.0 μg g^{-1}, and often much less, perhaps it should be better to consider these metals simply as potential, although highly unlikely, interfering species.

3.2. Standard Reference Materials

The method set up in aqueous reference solutions was applied to standard reference materials, Mussel Tissue BCR-CRM 278 (from Institute for Reference Materials and Measurements, European Commission, Joint Research Centre, Geel, Belgium), and Oyster Tissue NIST-SRM 1566a (from National Institute of Standards and Technology, Gaithersburg, MD, USA). The scope was to confirm and verify the applicability of the analytical procedure, and to evaluate its trueness and precision (Table 4).

It is important to highlight that, in order to test accuracy of the analytical procedure, the Pt(II), Pd(II) and Rh(III) concentrations listed in Table 4 have been spiked in the above Reference Materials. This may seem an anomalous procedure, but, in our opinion, it resulted to be the only way. In fact, Standard Reference Materials for mussels and clams containing certified concentrations of Pt(II), Pd(II) and Rh(III) were not available; under these conditions, the trueness and precision data may be doubtful and prudentially considered and/or discussed.

In the employed experimental conditions, precision as repeatability [81], expressed as relative standard deviation (s_r%) on five independent determinations, was satisfactory: it was lower than 5 % in all cases. Trueness, expressed as relative error (e%) was generally in the order of 6 %.

Figures 1 and 2 show the metal square wave voltammograms in Mussel Tissue BCR-CRM 278 Standard Reference Material in absence and in presence of EDTA-Na_2, respectively.

3.3. Limits of Detection

In the aqueous reference solution and in the solutions obtained by digestion of the standard reference materials and of mussels and clams sampled in the Goro Bay, the LODs for both techniques (Table 5) were obtained by the equation LOD=$K \cdot s_{y/x}/b$ [82]. The parameters $s_{y/x}$ 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 [81].

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

Finally, considering the extremely low concentrations of platinum, palladium and rhodium in the matrices considered in the present study, it should be pointed out that the instrumental voltammetric datum used for the quantitative determination was the peak area (A_p) instead of the height of the peak (*i.e.* the peak current i_p). This allows to obtain decidedly lower LODs, even one or two orders of magnitude or more, as amply demonstrated by studies carried out in our laboratories and reported in literature [83, and therein references].

3.4 Validation of the Voltammetric Analytical Procedure by Spectroscopic Measurements

To better validate the proposed voltammetric analytical procedure, electrothermal atomic absorption spectroscopy (ET-AAS) was chosen as comparison technique, because of its well-established and tested robustness [84]. The concentrations of both elements, either in the aqueous reference solutions or in the solutions obtained by digestion of the standard

reference material and of mussels and clams sampled in the Goro Bay, have been also determined by ET-AAS.

The experimental confirmation of such a validation can be deduced from the results reported in Tables 4 and 6: the agreement between the voltammetric and spectroscopic data is certainly good (differences lower than 7% for all the elements).

4. Practical Application

Once set up in aqueous reference solutions and validated by analysis of standard reference materials, the method for the voltammetric determination of the investigated metals was transferred to mussels and clams sampled in three sites inside the Goro Bay and one site at open sea in front of Po river mouth (see section 2.3 "Sampling Area").

The experimental results, reported in Table 6, show that the proposed analytical procedure is certainly applicable and transferable without problems to mussels and clams, and, in general, to all other filtering marine organisms.

As an example, Figure 3 reports the voltammogram relevant to the determination of all metals in mussels sampled in site B.

5. Conclusions

A new analytical procedure for the simultaneous voltammetric determination in mussel and clam matrices of Pt(II), Pd(II), Rh(III), Pb(II) together with toxic metals Cu(II), Pb(II), Cd(II) and Zn(II) has been described for the first time. The proposed procedure is simple, analytically well performing in terms of accuracy, selectivity, sensitivity, and it is suitable for multicomponent metal determinations in complex matrices. Therefore, voltammetry

resulted to be a good alternative to spectroscopic techniques. Substantially, the comparison shows that, as for the sample preparation before the instrumental measurements and the possibility of applying the method to real matrices, the two techniques are equivalent. Evidently, the difference regards the equipment cost to obtain comparable limits of detection. In fact, in the case of metal determinations at very low concentration in complex matrices, spectroscopic methods need too expensive equipment like Inductively Coupled Plasma (ICP) or better Inductively Coupled Plasma/Mass Spectrometry (ICP/MS) ─ they also allow a multi-component analysis ─, since ET-AAS, many times, shows to have inadequate limits of detection [85].

The possible use of mussels and clams as bio-monitors (*Mytilus Galloprovincialis* and of *Tapes Philippinarum* in the present case) has been demonstrated in the case of a long sampling plan. It is important to highlight that, as regards the concentrations of Pt(II), Pd(II) and Rh(III) in mussel and clam matrices, a comparison of the results reported in the present paper with those relevant to different sampling sites may be very difficult, owing to the availability of few relevant data in the literature.

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Conflict of interest

The authors declare no conflicts of interest.

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Figures

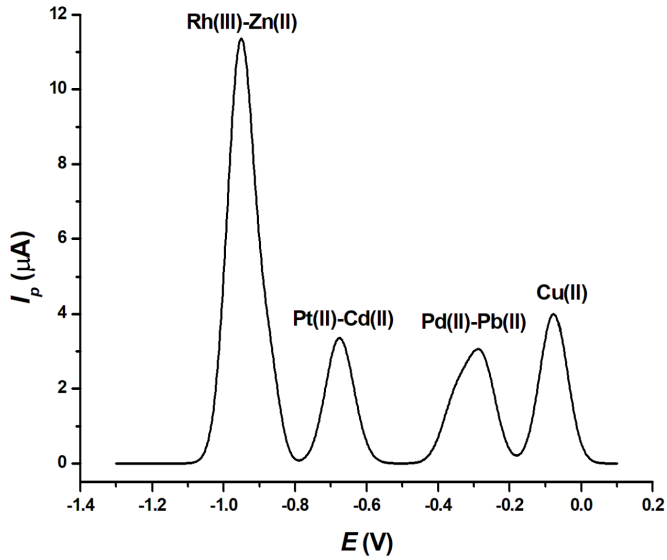

Fig. 1

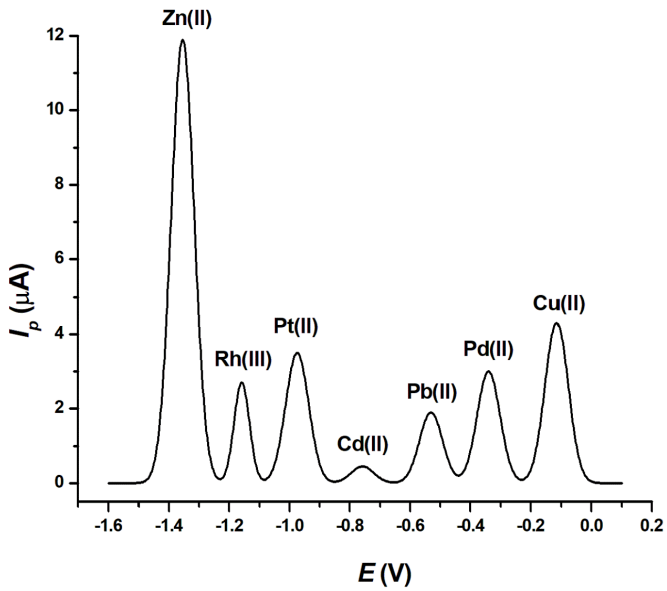

Fig. 2

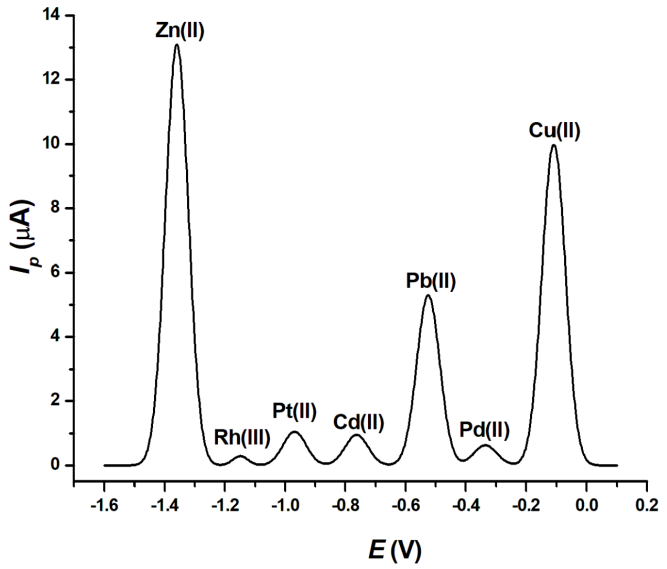

Fig. 3

Captions to Figures

Figure 1.

Square wave adsorptive catalytic stripping voltammogram of Cu(II), Pd(II), Pb(II), Cd(II), Pt(II), Rh(III) and Z(II) in the Mussel Tissue BCR-CRM 278 Standard Reference Materials in absence of EDTA-Na_2. Experimental conditions: see Table 1. Concentrations ($\mu g\ g^{-1}$): see Table 4.

Figure 2.

Square wave adsorptive catalytic stripping voltammogram of Cu(II), Pd(II), Pb(II), Cd(II), Pt(II), Rh(III) and Z(II) in the Mussel Tissue BCR-CRM 278 Standard Reference Materials in presence of EDTA-Na_2. Experimental conditions: see Table 1. Concentrations ($\mu g\ g^{-1}$): see Table 4.

Figure 3.

Square wave adsorptive catalytic stripping voltammogram of Cu(II), Pd(II), Pb(II), Cd(II), Pt(II), Rh(III) and Z(II) in mussels sampled in site B (see section 4 “Practical Application”). Experimental conditions: see Table 1. Concentrations ($\mu g\ g^{-1}$): see Table 6.

Tables

Table 1. Instrumental parameters for the metals determination by SWAdCSV. Supporting electrolytes: 0.1 mol L^{-1} HCl + $2.3 \cdot 10^{-4}$ mol L^{-1} DMG + formazone complex [0.7 mmol L^{-1} formaldehyde + 1.5 mmol L^{-1} hydrazine in 0.1 mol L^{-1} HCl] + $8.5 \cdot 10^{-2}$ mol L^{-1} $NaBrO_3$ + $4.9 \cdot 10^{-4}$ mol L^{-1} EDTA-Na_2.

E_i	-0.025	
E_a	-0.025	
E_f	-1.150	in absence of EDTA-Na_2
E_f	-1.350	in presence of EDTA-Na_2
t_a	360	
t_r	10	
dE/dt	100	
ΔE	50	
τ	0.010	
ν	0.100	
η	10	
r	600	

E_i: initial potential (V/ Ag│AgCl│KCl(sat)); E_a: adsorption potential (V/ Ag│AgCl│KCl(sat)); E_f: final potential (V/ Ag│AgCl│KCl(sat)); t_a: electroadsorption time (s); t_r: delay time before the potential sweep (s); dE/dt: potential scan rate (mV/s); ΔE: step amplitude (mV); τ: sampling time (s); ν: wave period (s); η: wave increment (mV); r: stirring rate (r.p.m.).

Table 2. Experimental peak potentials ($-E_p$, V, Ag│AgCl│$KCl_{sat.}$) in the aqueous reference solutions and in the standard reference material. Solutions in absence of EDTA-Na_2. Number of independent determinations: 5.

	Cu(II)	Pd(II)	Pb(II)	Cd(II)	Pt(II)	Rh(III)	Zn(II)
Aqueous Reference Solutions *	0.043±0.015	0.223±0.015	0.309±0.010	0.590±0.010	0.687±0.010	0.865±0.015	0.943±0.015
Mussel Tissue BCR-CRM 278	0.039±0.010	0.217±0.010	0.315±0.010	0.596±0.010	0.677±0.015	0.873±0.010	0.949±0.015
Oyster Tissue NIST-SRM 1566a	0.050±0.010	0.215±0.015	0.301±0.015	0.600±0.015	0.691±0.015	0.855±0.015	0.935±0.015

* 0.1 mol L^{-1} HCl + $2.3\cdot10^{-4}$ mol L^{-1} DMG + formazone complex [0.7 mmol L^{-1} formaldehyde + 1.5 mmol L^{-1} hydrazine in 0.1 mol L^{-1} HCl] + $8.5\cdot10^{-2}$ mol L^{-1} $NaBrO_3$.

Solutions in presence of EDTA-Na_2. Number of independent determinations: 5.

	Cu(II)	Pd(II)	Pb(II)	Cd(II)	Pt(II)	Rh(III)	Zn(II)
Aqueous Reference Solutions **	0.081±0.015	0.277±0.010	0.469±0.010	0.657±0.010	0.849±0.015	1.019±0.010	1.196±0.015
Mussel Tissue BCR-CRM 278	0.077±0.010	0.285±0.015	0.465±0.015	0.651±0.015	0.855±0.015	1.023±0.015	1.191±0.015
Oyster Tissue NIST-SRM 1566a	0.071±0.015	0.269±0.015	0.476±0.015	0.663±0.010	0.843±0.010	1.027±0.015	1.203±0.010

** 0.1 mol L^{-1} HCl + $2.3\text{x}10^{-4}$ mol L^{-1} DMG + formazone complex [0.7 mmol L^{-1} formaldehyde + 1.5 mmol L^{-1} hydrazine in 0.1 mol L^{-1} HCl] + $8.5\cdot10^{-2}$ mol L^{-1} $NaBrO_3$ + $4.9\cdot10^{-4}$ mol L^{-1} EDTA-Na_2.

Table 3. Element half peak width (mV) in the aqueous reference solutions, in solutions obtained by digestion of standard reference materials and of mussels and clams sampled in the Goro Bay.

Element	**Cu(II)**	**Pd(II)**	**Pb(II)**	**Cd(II)**	**Pt(II)**	**Rh(III)**	**Zn(II)**
Aqueous Reference Solutions *	55	48	54	53	47	32	59
Mussel Tissue BCR-CRM 278	57	47	56	55	48	34	63
Oyster Tissue NIST-SRM 1566a	56	49	55	54	48	35	65
Mytilus Galloprovincialis	57	49	54	56	49	35	64
Tapes Philippinarum	55	48	56	55	48	34	65

* 0.1 mol L^{-1} HCl + $2.3 \cdot 10^{-4}$ mol L^{-1} DMG + formazone complex [0.7 mmol L^{-1} formaldehyde + 1.5 mmol L^{-1} hydrazine in 0.1 mol L^{-1} HCl] + $8.5 \cdot 10^{-2}$ mol L^{-1} $NaBrO_3$ + $4.9 \cdot 10^{-4}$ mol L^{-1} EDTA-Na_2.

Table 4. Accuracy of the analytical procedure. The determined values are the mean of 5 independent determinations ± confidence interval at 99 % confidence level. Concentration: μg g^{-1}. Experimental conditions: see Table 1.

Voltammetric measurements.

	Element	Certified value *	Determined value	*e*(%)	s_r (%)
Mussel Tissue BCR-CRM 278	Cu(II)	9.60±0.16	9.11±0.53	-5.1	5.2
	Pd(II)	4.69	4.43±0.29	-5.5	5.8
	Pb(II)	1.91±0.04	1.81±0.12	-5.2	5.3
	Cd(II)	0.34±0.02	0.36±0.03	+5.9	5.1
	Pt(II)	4.69	4.97±0.31	+6.0	5.9
	Rh(III)	4.69	4.39±0.34	-6.4	5.7
	Zn(II)	76±2	72±5	-5.3	5.5
Oyster Tissue NIST-SRM 1566a	Cu(II)	66.3	62.7±3.9	-5.4	5.1
	Pd(II)	4.69	4.96±0.30	+5.8	5.5
	Pb(II)	0.371	0.349±0.027	-5.9	5.0
	Cd(II)	4.15	4.40±0.31	+6.0	5.3
	Pt(II)	4.69	4.44±0.28	-5.3	5.6
	Rh(III)	4.69	4.41±0.35	-6.0	5.7
	Zn(II)	830	869±43	+4.7	5.4

Spectroscopic measurements.

	Element	Certified value *	Determined value	*e*(%)	s_r(%)
Mussel Tissue BCR-CRM 278	Cu(II)	9.60±0.16	9.05±0.59	-5.7	5.0
	Pd(II)	4.69	5.00±0.35	+6.6	6.1
	Pb(II)	1.91±0.04	2.03±0.15	+6.3	5.7
	Cd(II)	0.34±0.02	0.37±0.04	+8.8	5.5
	Pt(II)	4.69	4.45±0.27	-5.1	5.3
	Rh(III)	4.69	5.03±0.37	+7.2	5.8
	Zn(II)	76±2	71±6	-6.6	5.9
Oyster Tissue NIST-SRM 1566a	Cu(II)	66.3	62.0±4.5	-6.5	5.3
	Pd(II)	4.69	4.42±0.31	-5.8	5.7
	Pb(II)	0.371	0.396±0.028	+6.7	5.6
	Cd(II)	4.15	3.89±0.30	-6.3	5.5
	Pt(II)	4.69	5.01±0.35	+6.8	5.9
	Rh(III)	4.69	5.02±0.37	+7.0	6.3
	Zn(II)	830	877±51	+5.7	5.8

* In the case of Pt(II), Pd(II) and Rh(III) the concentration listed in the certified value column have been added to both the Standard Reference Material.

Table 5. Limits of detection (LOD_s) determined in the aqueous reference solution (µg L^{-1}), in the solutions obtained by digestion of Mussel Tissue BCR-CRM 278 and Oyster Tissue NIST-SRM 1566a standard reference materials, and in the solutions obtained by digestion of real samples (calculated in µg L^{-1} and expressed in µg g^{-1}). The determined values are the mean of 5 independent determinations; confidence level: 95 %.

	Aqueous Reference Solutions		Mussel Tissue BCR-CRM 278	Oyster Tissue NIST-SRM 1566a	*Mytilus Galloprovincialis*	*Tapes Philippinarum*
Technique → Element ↓	Voltammetry	Spectroscopy *	Voltammetry	Voltammetry	Voltammetry	Voltammetry
Cu(II)	11.3	3.69	0.283	0.277	0.140	0.140
Pd(II)	0.37	2.73	0.009	0.011	0.026	0.026
Pb(II)	2.3	2.59	0.057	0.065	0.095	0.095
Cd(II)	2.7	2.43	0.083	0.079	0.020	0.020
Pt(II)	0.25	2.01	0.006	0.007	3.79	3.79
Rh(III)	0.49	1.77	0.012	0.015	1.12	1.12
Zn(II)	23.1	3.95	0.596	0.577	0.010	0.010

* Considering a sample weight exactly equal to 1.0 g (see section 2.4 “Sample Preparation”), the limits of detection determined in the aqueous reference solution, expressed in µg g^{-1}, were

Table 6. Mean values of the metal concentration (calculated in μg L^{-1} and expressed in μg g^{-1}) relevant to mussels and clams sampled in the Goro Bay (see text, section 2.3). Number of independent determinations: 5. The confidence interval is calculated at 95% probability level.

Voltammetry

Sampling Site → Element ↓	A		B		C		D	
	Mussels	Clams	Mussels	Clams	Mussels	Clams	Mussels	Clams
Cu(II)	49.0 ± 2.3	40.2 ± 2.7	43.1 ± 2.5	35.5 ± 2.0	55.6 ± 3.0	36.9 ± 1.9	19.7 ± 1.7	11.3 ± 0.9
Pd(II)	0.70 ± 0.06	0.53 ± 0.03	0.41 ± 0.03	0.47 ± 0.04	0.43 ± 0.04	0.39 ± 0.03	0.023 ± 0.003	< LOD
Pb(II)	7.7 ± 0.7	5.3 ± 0.4	8.5 ± 0.7	6.1 ± 0.5	6.9 ± 0.5	5.9 ± 0.5	1.3 ± 0.2	0.87 ± 0.07
Cd(II)	2.3 ± 0.3	1.9 ± 0.3	2.9 ± 0.3	1.7 ± 0.2	1.6 ± 0.4	2.0 ± 0.3	0.47 ± 0.05	0.23 ± 0.03
Pt(II)	0.96 ± 0.07	0.85 ± 0.08	1.03 ± 0.08	0.73 ± 0.06	1.19 ± 0.09	0.90 ± 0.8	0.049 ± 0.003	0.021 ± 0.003
Rh(III)	0.11 ± 0.02	0.91 ± 0.06	0.085 ± 0.005	0.069 ± 0.005	0.053 ± 0.004	0.077 ± 0.006	< LOD	< LOD
Zn(II)	125.3 ± 8.1	96.0 ± 6.3	117.9 ± 7.1	103.1± 6.7	131.5± 7.0	111.0 ± 6.3	69.0 ± 4.0	58.6 ± 3.5

Spectroscopy

Sampling Site → Element ↓	A		B		C		D	
	Mussels	Clams	Mussels	Clams	Mussels	Clams	Mussels	Clams
Cu(II)	51.9 ± 2.5	42.5± 2.9	41.0 ± 2.7	34.0 ± 2.3	58.3 ± 3.1	38.5 ± 2.1	21.0 ± 1.9	12.0 ± 1.0
Pd(II)	0.74 ± 0.07	0.51 ± 0.02	0.43 ± 0.02	0.49 ± 0.05	0.46 ± 0.05	0.41 ± 0.04	0.025 ± 0.002	< LOD
Pb(II)	8.3 ± 0.8	5.0 ± 0.5	8.0 ± 0.8	6.4 ± 0.6	7.3 ± 0.6	6.3 ± 0.6	1.5 ± 0.3	0.82 ± 0.09
Cd(II)	2.1 ± 0.3	1.7 ± 0.2	2.7 ± 0.03	1.8 ± 0.2	1.9 ± 0.3	2.2 ± 0.03	0.50 ± 0.06	0.25 ± 0.02
Pt(II)	0.90 ± 0.08	0.79 ± 0.09	1.09 ± 0.09	0.69 ± 0.011	1.26 ± 0.10	0.96 ± 0.09	0.046 ± 0.004	0.023 ± 0.004
Rh(III)	0.12 ± 0.03	0.96 ± 0.07	0.089 ± 0.006	0.065 ± 0.006	0.050 ± 0.003	0.081 ± 0.007	< LOD	< LOD
Zn(II)	132.9 ± 8.5	101.7 ± 6.5	111.3 ± 7.5	109.0± 6.9	125.0 ± 7.1	105.3 ± 6.7	72.5 ± 4.1	55.7 ± 3.9