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New Easy Method for the Monitoring of Hg Concentration in Fish, Using a Nanostructured Gold Electrode

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Abstract: The applicability of a gold nanoparticle-modified glassy carbon sensor (AuNPs-GCS) for the determination of inorganic mercury in fresh and canned tuna fish by square wave anodic stripping voltammetry (SW-ASV) is demonstrated. Mercury content in sample Tuna Fish ISPRA T22 was determined to value the accuracy of the determination. The concentration in this sample is not certified, so, the Hg amount was determined also with atomic absorption spectroscopy (AAS): the results obtained with ASV were in good agreement and confirmed literature value reported for this sample. Then, real samples of tuna fish were analyzed. The voltammetric analyses were performed using previously optimized conditions (deposition potential 0 V, step potential 0.004 V, frequency 150 Hz and amplitude 0.003 V). Medium exchange technique permitted to eliminate possible matrix effects. The concentrations in the real samples were found to be in agreement with the common Hg levels reported in literature for commercialized tuna fish in different countries.

Key words: Gold nanoparticle modified glassy carbon sensor, square wave stripping voltammetry, mercury, tuna fish, medium exchange.

1. Introduction

There is increasing concern about the quality of foods in several parts of world. One source of risk for human health upon consumption of inadequate food is the presence of potentially toxic elements. The availability of reliable procedures for their determination in food is a pre-requisite for studying their effects on humans [1].

Natural global cycling has always been a primary contributor to the presence of chemical elements in the different environmental compartments. In the case of mercury, this process involves off-gassing from the lithosphere and hydrosphere to the atmosphere, where it is transported and deposited onto land, surface water and soil.

Heavy metals are considered the most important form of pollution of the aquatic environment because of their toxicity and accumulation by marine organisms. In particular, mercury pollution has dangerous effect on marine ecosystem and humans. It provokes substantial apprehension because it is a known toxicant. It bioaccumulates and biomagnifies in the aquatic food web and accumulates in fish with unfortunate effects on humans [2, 3].

Therefore, accurate data on mercury levels in food is invaluable in the assessment of Hg exposure risks from food consumption. Exposure to mercury by the ingestion of contaminated water and food products results in mortality, reproductive failure and other health effects in predatory wildlife and humans. The distribution of metals varies between fish species,

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depending on age, development status and other physiological factors. Fish accumulate substantial concentrations of mercury in their tissues and thus, can represent a major dietary source of this element for humans [4].

Most methods for the determination of mercury in fish, fishery products and other biological tissues rely on the use of cold-vapour atomic fluorescence spectrometry (CV-AFS) [5] or cold-vapour atomic absorption spectrometry (CV-AAS) [6]. CV-AFS is not widely available and is expensive, but has lower detection limits and better suited to analysis Hg in small tissue quantities [7].

Electroanalytical techniques appear as attractive alternatives. The availability of sensors for the fast and easy detection of Hg would allow a considerable saving of time and money for the analyses. Furthermore, enable more frequent and widespread food controls. Moreover, electrochemical sensor can be connected to a portable instrumentation that can permit to the operators to make checks on site.

Several types of electrodes have been reported for the determination of mercury by voltammetry and most of the results are well described in the review of Martín-Yerga et al. [8]. In previous papers [9, 10], a new procedure for the determination of Hg and for the speciation of Hg/CH_3Hg using a Gold Nanoparticle-Modified Glassy Carbon Sensor (AuNPs-GCS) without any further modification was described. Gold permits to enhance the pre-concentration effect during the deposition step due to its high affinity to mercury. Thanks to the large surface area of the gold nanoparticles, very low detection limit and with short deposition times, was attained.

Another great advantage offered by AuNPs-GCS is the possibility to work with a renewable active surface which permits to eliminate the problem of irreversible contamination of the gold layer. Thus, minimize memory effects and avoid frequent time-consuming and dangerous mechanical cleaning necessary with solid bulk electrodes.

AuNPs-GCS permitted to quantify mercury concentrations in the low ng/L range with high accuracy and precision. The applicability of the technique was demonstrated determining the Hg concentration in: (i) certified samples: estuarine sediment, BCR 276 and city waste incineration ash—BCR 176; (ii) in real samples spiked with a known Hg concentration—drinking water and vegetables extracts and (iii) in pharmaceuticals—an ocular lubricant gel [10].

In this paper, the attention has been focused the applicability of the AuNPs-GCS on the determination of mercury in the matrix "fish". Initially, the analytical performance of AuNPs-GCS on synthetic solutions containing known as mercury concentrations were evaluated determining linearity, repeatability, accuracy and sensitivity. Then, the reliability of the technique was tested on a sample of fish known as *Tuna* fish ISPRA T22: this sample has been adopted by Detcheva & Grobecker [11] to validate analytical procedures and its Hg content is reported in literature. To verify Hg concentration in this sample, an Atomic Absorption Spectroscopy with Graphite Furnace atomizer (AAS-GF) was also used to optimize the instrumental response for this matrix. Then, the applicability of the voltammetric technique to the determination of mercury concentration in commercial fresh and canned tuna fish was valued.

2. Materials and Methods

Digestion of samples was performed in Poly Tetra Fluoro Ethylene (PTFE) bombs with a Milestone MLS-1200 Mega microwave laboratory unit (Milestone, Sorisole, Italy).

Voltammetric analyses were performed with a PGSTAT 10 potentiostat (Eco Chemie, Utrecht, the Netherlands) coupled to a 663 VA Metrohm (Herisau, Switzerland) stand cell. It consists of an AuNPs-GCS working electrode, prepared from a commercial Metrohm GCE (section 2.2.1), a glassy carbon counter

electrode and an Ag/AgCl/KCl (3M) reference electrode. The analyzer was interfaced to a personal computer. The operational conditions were selected and voltammograms visualised and processed with the aid of GPES 4.9.

Analytical grade reagents were used. A 1,000 mg/L standard solution of mercury was prepared from $HgCl_2$ in 0.012 M HCl. More diluted Hg(II) standard solutions were prepared from the concentrated standards in the supporting electrolyte.

High purity water (HPW) obtained from a Milli-Q apparatus (Millipore, Bedford, USA) was used throughout.

One hundred mg/L stock solutions of $HAuCl_4 \cdot 3H_2O$ (Sigma > 99.9% trace metals basis) in HPW were prepared and used for the deposition of gold nanoparticles onto the carbon surface.

The characterisation of the sensor surface was performed by scanning electron microscopy (SEM), using an Inspect F with Field Emission Gun LEICA-Stereo scan 410 SEM.

An Analyst 600 Atomic Absorption Spectrometer with Graphite Furnace (AAS-GF) by Perkin Elmer was used to verify Hg concentration in Tuna Fish Ispra T22 sample.

A mixture 1:1 of 1,000 mg/L Pd and 1,000 mg/L Ru (both in HNO_3) was prepared. Then, it was diluted 1:1 with HPW (final Pd/Ru concentration: 250 mg/L) and was used as matrix modifier.

2.1 Deposition of Gold Nanoparticles on the Glassy Carbon Surface

A 100 mg/L $HAuCl_4 \cdot 3H_2O$ solution (corresponding to 50 mg/L of Au) was prepared in Milli-Q water previously filtered through a 0.45 μm cellulose acetate filter and deaerated by passing a N_2 stream. The GC substrate was polished with a suspension of 0.1 μm alumina in HPW for 1 min. Then, it was rinsed three times with ethanol and water, alternatively and dried using a nitrogen stream. Modification with gold nanocrystals was performed by dipping the GC substrate into the $HAuCl_4$ solution and applying a potential of -0.80 V for 6 min. The obtained sensor was washed with Milli–Q water and kept in 0.1 M NaOH until use.

Before proceeding with the voltammetric determinations, it was necessary to effectuate an activation step by applying a potential of 0.60 V for 60 s. While, the working sensor was stirred in 0.06 M HCl.

The presence of gold nanoparticles was confirmed by SEM analyses and by cyclic voltammetry. The latter was performed varying the potential from 0 V to 1.3 V to 0 V in 0.5 M H_2SO_4.

When required, the dissolution of the gold layer was performed by varying the potential from 0 V to 1.6 V in 6 M HCl whilst stirring the electrode [9].

2.2 Samples and Sample Pretratment

Tuna Fish ISPRA T22 was analysed to value the efficiency of the acid digestion and the accuracy of the AuNPs-CGS response for mercury quantification working with this type of matrix. Detcheva & Grobecker [11] reported its application to check the applicability of new analytical techniques for the determination of Hg in fish.

Canned tuna fish, produced in Spain, was purchased in a discount in Torino.

Fresh tuna fish was purchased in a fish shop in Torino Province.

Aliquots of 0.5 g of Tuna Fish ISPRA T-22 were transfer in the bombs and digested without any pretreatment with a mixture of 3 mL of HNO_3 and 3 mL of H_2O_2.

The real samples of tuna fish were previously dried at room temperature and homogenized. Then, aliquots of 0.25 g of obtained samples were treated with the same mixture as the ISPRA material.

The following heating program of the microwave unit was adopted: 250 W for 1 min, 0 W for 1 min, 250 W for 5 min, 400 W for 5 min, 650 W for 5 min and ventilation for 25 min. The bombs were left to

cool at room temperature [10].

The resulting solutions were diluted with HPW to 30 mL for ISPRA sample and to 15 mL for real samples. All the experiment performed in triplicate and blanks were simultaneously run.

2.3 AAS-GF Determination of Mercury

Since the sample Tuna Fish ISPRA T22 is not a "certified material", the Hg concentration in the considered package was also determined with the aid of AAS-GF. In this way, the obtained results were compared with those reported in literature and verified.

The determination of Hg by AAS-GF was performed following the method suggested by Krata, Jedral & Bulska [12] as a starting point and optimizing it by changing the operating parameters to obtain the best response in terms of repeatability—shape of the signal and stability. The following furnace program was adopted: (1) T = 110 °C, ramp time (R_t) = 5 s, hold time (H_t) = 10 s; (2) T = 130 °C, R_t = 10 s, H_t = 30 s; (3) T = 1,100 °C, R_t = 10 s, H_t = 20 s; (4) T = 110 °C, R_t = 5 s, H_t = 10 s and (5) T = 130 °C, R_t = 10 s, H_t = 30 s.

The technique was previously tested on synthetic solutions containing a known mercury concentration (50 μg/L) prepared in the blank (HNO_3/H_2O_2/HPW) obtained by digestion of the reagents as described in par 2.2.

2.4 SW-ASV Determination of Mercury

ISPRA sample: 0.5 mL of obtained solution was diluted to 20 mL with 60 mM HCl in the voltammetric cell. Mercury concentration was determined directly or following medium exchange technique.

Real samples: 0.5 mL of obtained solution was diluted to 20 mL with 60 mM HCl directly in the voltammetric cell. Mercury concentration was determined using medium exchange technique.

After 120 s of deposition at 0 V, a voltammetric scan was performed working in SW-ASV and adopting the following parameters: frequency = 150 Hz, initial potential = 0 V, final potential = 0.8 V, step potential = 0.0040 V; amplitude = 0.03 V; scan rate = 0.6075 V/s and stirring rate = 2,000 r.p.m.

After recording the voltammogram of the sample solutions, aliquots of Hg were added and the corresponding signals were recorded. The standard addition method was adopted for the evaluation of the concentration of mercury in all investigated samples. For concentration, lower than 3 μg/L well defined peaks were obtained by subtracting the blank signal from the voltammograms of the sample solutions [9].

Each sample was analysed in triplicate.

Unless otherwise stated, the medium exchange technique was adopted for the analysis. After the electrodeposition step from the sample solutions, the potential was maintained at 0 V with the optional function "Hold" of the instrument. Then, the sample solution cell was replaced by a solution of 0.06 M HCl in which the stripping step was then performed.

After each determination the sensor was maintained in a mixture of 0.2 M $HClO_4$/3 mM NaCl/1 mM NaEDTA·for 30 s at 0.80 V to remove residues of mercury from its surface.

3 Results and Discussion

3.1 Gold Nanostructured Active Surface

The formation of the gold nanoparticles on the surface of GC substrate is associated to a colour change from black (GC) to red-orange (AuNPs).

The status of the gold nanoparticles deposition was valued with SEM analysis and monitored with cyclic voltammetry.

From SEM image (Fig. 1), it is possible to see the presence of a homogeneous gold layer composed of particles with an average diameter of approximately 100 ± 20 nm. A very good repeatability in the morphology of the nanostructured layer was obtained starting from different GCEs and different brands of Au salts.

A faster and more easily available method to

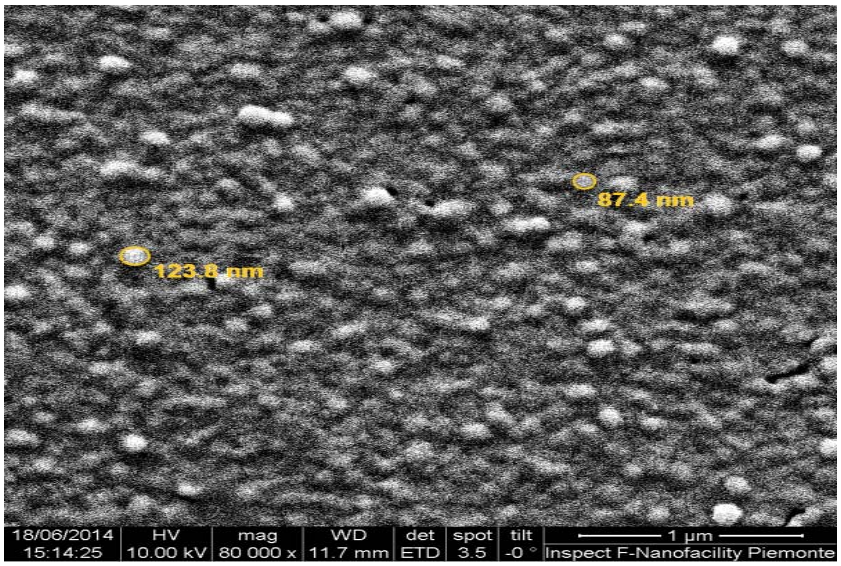

Fig. 1 SEM image of gold nanoparticles layer electrochemically deposited (This work has been performed at NanoFacilities Piemonte, INRiM, a laboratory supported by Compagnia di San Paolo).

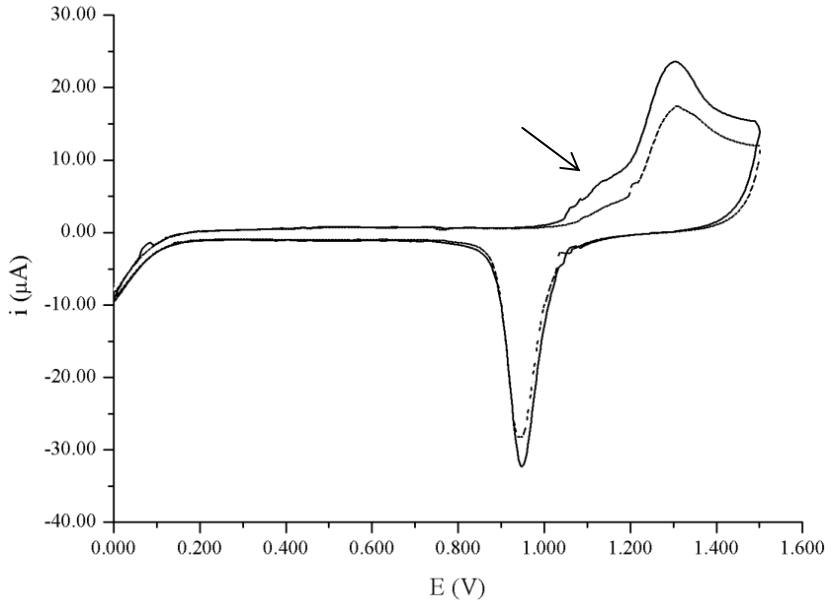

Fig. 2 CV voltammogramms recorded in 0.5 M H_2SO_4 for a nanostructured gold sensor (−) and for a solid gold sensor (···).

monitor the nanostructured surface is CV. Fig. 2 reports the voltammograms recorded in 0.5 M H_2SO_4 with an AuNPs-GCS and for comparison, with a commercial solid gold sensor.

The shape reported in Fig. 2 for the solid gold electrode is well known in literature and identifies a clean gold surface. The anodic peak at +1.25 V is due to oxide formation at gold sensor. The nature of the species formed on the active surface is not well known. The most popular hypothesis is the formation of hydrated oxides or the formation of $Au(OH)n_{ads}$ [13]. The CV voltammogram obtained with the AuNPs-GCS presents the typical peaks of gold electrodes and a "shoulder" (Fig. 2) before the oxidation peak. Such shoulder is typical for a nanostructured gold surface and it is due to the formation of different multi-oxide species formed on the gold nanoparticles [14, 15].

The cathodic peak at +0.90 V, present with both

sensors can be attributed to the reduction of the gold oxide formed during the anodic cycle. The recorded charge under the reduction peak is generally used for the characterization and monitoring of the electroactive sensor area. In particular, the intensity of the signal is proportional to the amount of deposited Au [16].

As expected, the higher signal obtained by the AuNPs-GCS in comparison with the solid sensor is due to the greater surface area which due to the presence of nanoparticles.

3.2 SW-ASV Determination of Hg in Synthetic Solutions

The performance of the AuNPs-GCS was shown in a previous works [9, 10]. Briefly, the height of Hg peak increased with increasing deposition time a value of 120 s was found to be suitable for concentrations down to 50 μg/L. The repeatability, the linearity, the accuracy, the detection limit of the procedure and the interferences of other cations and anions were evaluated. In particular, in the optimized experimental conditions, very low concentrations of mercury could be quantified with good accuracy. For instance, the concentration measured for a 10 ng/L Hg solution was 9.92 ± 0.05 ng/L. The detection limit was estimated as 0.15 ng/L.

In Fig. 3, the voltammograms obtained during the quantification of 20-60 ng/L of Hg in 60 mM HCl was shown.

3.3 Determination of Hg in Tuna Fish ISPRA T22 Sample by AAS-GF

For the determination of Hg in sample ISPRA T22 by AAS-GF the procedure suggested by Krata et al [12]: (Step1: T = 130 °C, Ramp time = 5 s, Hold time = 10 s; Step 2: T = 150 °C, Ramp time = 10 s, Hold time = 30 s; Step 3: T = 130 °C, Ramp time = 5 s, Hold time = 10 s; Step 4 T = 20 °C, Ramp time = 10 s, Hold time = 10 s; Step 5:1,000 °C, Ramp time = 0 s, Hold time = 4 s and Step 6: T = 2400 °C, Ramp time = 1 s, Hold time = 3 s) was adopted after the following

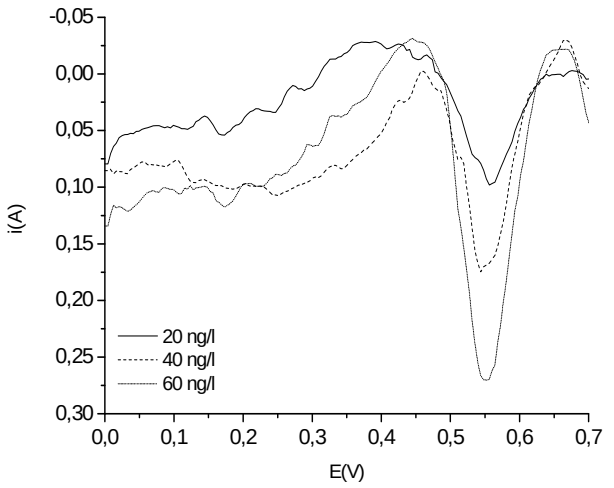

Fig. 3 SW-ASV voltammograms recorded for 20, 40 and 60 ng/L of Hg in 60 mM HCl by ASV after blank subtraction.

modifications (i) the temperature of atomization was increased from 1,100 °C to 1,300 °C which permits to observe a more define peak; (ii) the hold time during the atomization step was extended to increase the sensitivity and (iii) the pyrolysis temperature was decreased from 450 °C to 250 °C using a suitable matrix modifier (250 mg/L Pd/Ru). The obtained concentration was: Hg = 4.39 ± 0.18 mg/kg, which in agreement with the concentration reported in literature (4.43 ± 0.34 mg/kg), corresponding to a recovery of 99.1%.

3.4 Determination of Hg in Tuna Fish ISPRA T22 Sample by AuNPs-GCS

The applicability of the AuNPs-GCS to the analysis of different types of samples of tuna fish was firstly tested on Tuna Fish ISPRA T22. Table 1 reports the equations of the standard addition curves obtained in two independent analyses, with and without medium exchange, the sensitivity and the recovery, computed considering the literature value as the “true” one.

For ASV analysis, aliquots of the solution obtained after sample mineralization were diluted in the supporting electrolyte (60 mM HCl). In fact, the presence of chloride ions permits to enhance the sensitivity of the mercury signal [17].

Table 1 SW-ASV determination of Hg in Tuna Fish (ISPRA T-22).

Technique	Hg found (% recovery)	Calibration curve /R^2	Sensitivity (μA/μg)
Without medium exchange	4.07 ± 0.52 mg/kg (91.8%)	y = 0.78 ± 0.04 x + 1.37 ± 0.01 (μA) R^2 = 0.999	0.78
With medium exchange	4.42 ± 0.22 mg/kg (99.8%)	y = 0.81 ± 0.04 x + 1.47 ± 0.14 (μA) R^2 = 0.999	0.81

First of all, the accuracy of the determination of the mercury in the correspondent blank spiked with 0.500 μg/L was tested which obtain a recovery of 99.2%.

As reported in Table 1, a recovery of 91.8 % was obtained analysing ISPRA sample without medium exchange. Probably, some residual components of the sample matrix interfered with the analysis. This difficulty was overcomed by performing the voltammetric analysis using the medium exchange technique. The stripping step was carried out in a solution of 60 mM HCl instead of 60 mM NaCl as reported in our previous work. In fact, in the past, NaCl was used to avoid the formation of aqua regia in cell since some authors supposed that it could damage the Au surface [18]. However, in this manner, a lower sensitivity was obtained. In this study, the possibility to use HCl as supporting electrolyte was valued and it does not cause any problem to Au surface. Using medium exchange technique, the result obtained was in good agreement with the known concentration (recovery 99.8 %) reported by Detcheva and Grobecker who used this and other reference materials to validate a spectroscopic method [11]. The concentration of mercury found by SW-ASV was also in excellent agreement with that obtained by AAS-GF.

3.5 Determination of Hg in the Real Samples by AuNPs-GCS

Since the greater error observed in the quantification of the analyte content in ISPRA sample without the application of medium exchange technique, the other tuna samples were analysed only by using medium exchange.

The voltammograms obtained from the real samples are shown in Fig. 4 and the final Hg concentrations are reported in Table 2, together with the equations of the standard addition curves and the sensitivity observed.

As shown in the voltammograms, the resulting signals are well defined. The blank signal was perfectly overlapped with those of the samples except of course below the mercury peaks. So, it was possible to subtract it and obtain a good baseline to search the peaks.

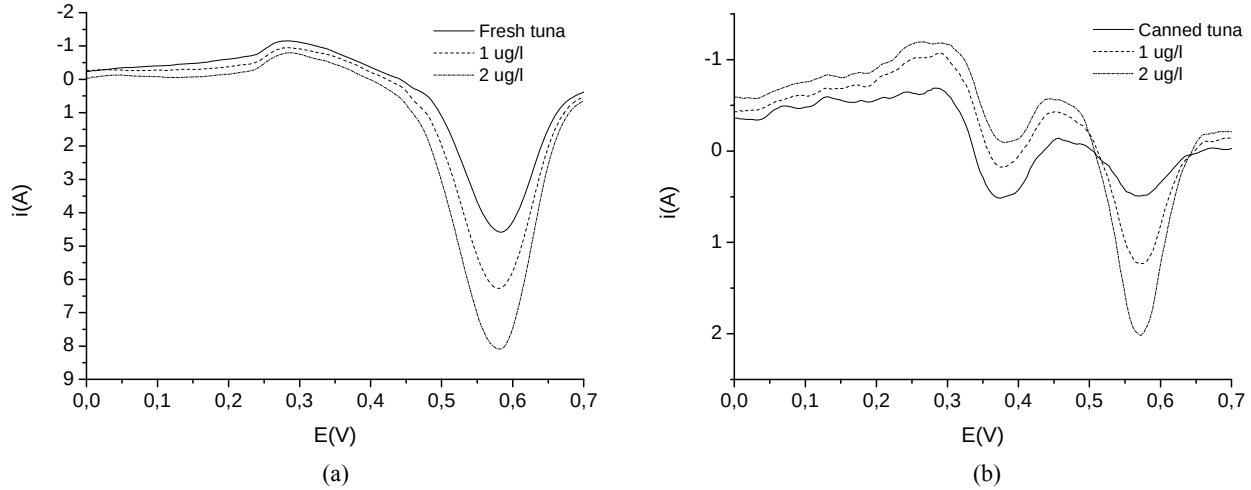

Fig. 4 SW-ASV voltammograms recorded for from fresh (a) and canned (b) tuna fish samples by ASV after blank subtraction.

Table 2 SW-ASV determination of Hg in real samples.

Tuna fush	Hg found	Calibration curve/R^2	Sensitiviy (μA/μg)
Canned	0.18 ± 0.005 mg/kg	y = 0.54 ± 0.03 x + 0.31 (μA) R^2 = 0.989	0.54
Fresh	0.52 ± 0.07 mg/kg	y = 0.51 ± 0.03 x 1.57 (μA) R^2 = 0.982	0.51

Table 3 Ranges of Hg concentration in canned tuna and in muscle of fresh fish reported in literature [1, 21 and 25-34].

Canned tuna fish	
Country	**Hg (mg/Kg)**
Jordan	0.06-0.57
Brazil	0.025-0.968
USA	0.01-0.51
USA	0.053-0.739
Turkey	< 0.0002-1.14
Iran	0.01-0.401
Spain	0.222
Poland	0.067
Italy	0.04-1.79
Libya	0.2-0.66
Portugal	0.08-1.0
Persian Gulf	0.043-0.253

The canned tuna contained lower concentration of Hg in comparison with the fresh one. This can be due to the fact that for the preparation of canned tuna, younger fishes are used. In this way, the animals had shorter life time to bioaccumulate Hg in their tissues in comparison with the fish sold as fresh tuna. In the voltammogram of the canned tuna, it is possible to see that another peak at +0.38 V does not present in the case of fresh fish. In a previous study [9], the effect of different ions in solution and among the considered element was investigated and valued: Cu was the only one to give a signal in the considered potential range at +0.38 V which did not interfere with the mercury quantification. The presence of trace of copper in sample solution can derive from the composition of the metallic package as found by Buculei et al. [19].

The European Legislation set a maximum level for heavy metals in fishery products and meat of fish. For Hg, the present limit is 1 mg/kg. The examined samples contained Hg concentrations lower than the admissible value [20].

For canned tuna, the obtained results were compared with literature data [21]. Table 3 shows the concentration ranges generally measured in canned tuna fish commercialised in different countries.

The Hg content in the sample analyzed in this work falls within the commonly reported values. In particular, in bolder face the value of Hg reported for canned tuna sold in Spain is reported, that is very similar to the result obtained in this study for a sample produced in the same country.

For fresh tuna, the results were compared with Hg concentrations recently reported in literature by Perugini et al. [22] and by Olmedo et al. [23]: the former determined the level of Hg in four fish species caught in the Adriatic sea (as the considered sample) and the latter determined mercury concentration in different fish species among tuna. The results are collected in Table 4. Hg level determined in the sample analyzed in the work is in agreement with those reported in literature.

The amount of Hg that can be accumulated in the tissues of fish is strongly correlated to the size of the speciments. For example, generally Mediterrean yellowfin tuna (Albacore species) contains higher levels of Hg in comparison with some oceanic tuna

Table 4 Ranges of Hg concentration in canned tuna and in muscle of fresh fish reported in literature [22, 23].

Fresh fish	
Species	**Hg (mg/kg)**
Red mullet (n = 14)	0.48 ± 0.09
European hake (n = 14)	0.59 ± 0.14
Blue whiting (n = 14)	0.38 ± 0.10
Atlantic mack (n = 13)	0.36 ± 0.09
Tuna (n = not reported)	0.47 (min: 0.298, max: 0.779)

species (as Skipjack), because that the former reach greater size and eat bigger fishes [24].

4. Conclusions

In this work, the applicability of the nanostructured electrochemical sensor for the determination of Hg in fish products was demonstrated.

The presence of the gold nanoparticles allows to quantify low mercury concentrations with accuracy and precision and the great sensitivity of the technique permits to dilute greatly the sample solutions.

The use of the medium exchange technique allows to overcome some possible interference effects caused by components present in the sample solution, in particular, when real samples are analysed.

Working with a renewable surface, it is possible to dissolve the gold layer and deposit a new one eliminating the memory effect and possible contamination of the sensor surface.

The findings of this study can be used for the application of electrochemistry in the field of food control and human health protection. In particular, (i) the considerable lowering of the budget required for the instrumentation and the ease of the application would also increase the number of laboratories that could undertake this analysis and (ii) the possibility of connecting this sensor to a portable instrumentation could be permitted to make checks on site.

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Reference

[1] Emami, Khansari, F., Ghazi-Khansari, M., and Abdollahi, M. 2005. "Heavy Metals Content of Canned Tuna Fish." *Food Chemistry* 93: 293-296.

[2] Narvaes, D. M. 2002. *Human Exposure to Mercury in Fish in Mining Areas in the Philippines*. Morocco: FAO/WHO Global Forum of Food Safety Regulation.

[3] El-Methnani, R. B., and Abedin, A. M. 1999. "Mercury, Cadmium and Lead Content of Canned Tuna Fish." *Food Chemistry* 67: 341-345.

[4] Voegborlo, R. B., El-Methnani, A. M., and Abedin, M. Z. 1999. "Mercury, Cadmium and Lead Content of Canned Tuna Fish." *Food Chemistry* 67: 341-345.

[5] Anderson, K. A. 2000. *Mercury Analysis in Environmental Samples by Cold Vapour Techniques*. Chichester: John Wiley & Sons Ltd.

[6] Baker, R. F., Blanchfield, P. J., and Paterson, M. J. 2004. "Evaluation of Nonlethal Methods for the Analysis of Mercury in Fish Tissue." *Transactions of the American Fisheries Society* 133: 568-576.

[7] Li-Jun, S., Wu-Er, G., and Qing-De, S. 2006. "Determination of Total and Inorganic Mercury in Fish Samples With On-Line Oxidation Coupled to Atomic Fluorescence Spectrometry." *Analytica Chimica Acta* 562: 128-133.

[8] Martín-Yerga, D., González-García, M. B., and Costa-García, A. 2013. "Electrochemical Determination of Mercury: A Review." *Talanta* 116: 1091-1104.

[9] Abollino, O., Giacomino, A., Malandrino, M., Piscionieri, G., and Mentasti, E. 2008. "Determination of Mercury by Anodic Stripping Voltammetry with a Gold Nanoparticle-Modified Glassy Carbon Electrode." *Electroanalysis* 20: 75-83.

[10] Abollino, O., Giacomino, A., Ginepro, M., Malandrino, M., and Zelano, I. 2012. "Analytical Applications of a Nanoparticle-Based Sensor for the Determination of Mercury." *Electroanalysis* 24: 724-734.

[11] Detcheva, A., and Grobecker, K. H. 2006. "Analytical Note Determination of Hg, Cd, Mn, Pb and Sn in Seafood by Solid Sampling Zeeman Atomic Absorption Spectrometry." *Spectrochimica Acta Part B* 61: 454-459.

[12] Krata, A., Jedral, W., and Bulska, E. 2007. "On the Uniforming of the Atomization Process for Inorganic and Organic Mercury in Graphite Furnace Atomic Absorption Spectrometry." *Spectrochimica Acta* 62: 269-272.

[13] Hamelin, A. 1996. "Cyclic Voltammetry at Gold Single-Crystal Surfaces—Behaviour at Low-Index Faces." *Journal of Electroanalytical Chemistry* 407: 1-11.

[14] Priano, G., Gonzalez, G., Gunther, M., and Battaglini, F. 2008. "Disposable Gold Electrode Array for Simultaueous Electrochemical Studies." *Electroanalysis* 20: 91-97.

[15] Feeney, R., and Kounaves, S. P. 2002. "Voltammetric Measurement of Arsenic in Natural Waters." *Talanta* 58: 28-31.

[16] Dai, X., Nekrassova, O., Hyde, M. E., and Compton, R. G. 2004. "Anodic Stripping Voltammetry of Arsenic(Iii) Using Gold Nanoparticle-Modified Electrodes." *Analytical Chemistry* 76: 5924-5929.

[17] Okcu, F., Ertas, F. N., Gokcel, I., and Tural, H. 2005. "Anodic Stripping Voltammetric Behavior of Mercury in Chloride Medium and Its Determination at a Gold Film Electrode." *Turkish Journal of Chemistry* 29: 355-366.

[18] Augelli, M. A., Munoz, R. A. A., Richter, E. M., Cantagallo, M. I., and Angnes, L. 2007. "Analytical Procedure for Total Mercury Determination in Fishes and Shrimps by Chronopotentiometric Stripping Analysis at Gold Film Electrodes after Microwave Digestion." *Food Chemistry* 101: 579-584.

[19] Buculei, A., Amariei, S., Oroian, M., Gutt, G., Gaceu, L., and Birca, A. 2014. "Metals Migration between Product and Metallic Package in Canned Meat LWT." *Food Science and Technology* 58: 364-374.

[20] European Ufficial Gazzette. 2011. *Regulation* (*EU*) *No 420/2011 of the European Parliament and of the Council of April 29th 2011 on* "*The Definition of Maximum Values of Some Contaminants in Food Products*". European: Amending Regulation (EC) No 1881/2006 of the European Parliament.

[21] Ababneh, F. A., and Al-Momani, I. F. 2013. "Levels of Mercury, Cadmium, Lead and Other Selected Elements in Canned Tuna Fish Commercialised in Jordan." *International Journal of Environmental Analytical Chemistry* 93: 755-766.

[22] Perugini, M., Visciano, P., Manera, M., Zaccaroni, A., Olivieri, V., and Amorena, M. 2004. "Heavy Metal (As, Cd, Hg, Pb, Cu, Zn and Se) Concentrations in Muscle and Bone of Four Commercial Fish Caught in the Central Adriatic Sea, Italy." *Environment Monitoring and Assessment* 186: 2205-2213.

[23] Olmedo, P., Pla, A., Hernández, A. F., Barbier, F., Ayouni, L., and Gil, F. 2013. "Determination of Toxic Elements (Mercury, Cadmium, Lead, Tin and Arsenic) in Fish and Shellfish Samples." *Risk Assessment for the Consumers* 59: 63-72.

[24] Natural Resource Defense Council. 2014. "Mercury Contamination in Fish. A Guide to Staying Healthy and Fighting Back." Accessed January 28, 2014. http://www.nrdc.org/health/effects/mercury/guide.asp.

[25] Morgano, M. A., Rabonato, L. C., Milani, R. F., Miyagusku, L., and Balian, S. C. 2011. "Assessment of Trace Elements in Fishes of Japanese Foods Marketed in Sao Paulo (Brazil)." *Food Control* 22: 778-785.

[26] Burger, J., and Gochfeld, M. 2006. "Mercury in Fish Available in Supermarkets in Illinois: Are There Regional Differences." *The Science of The Total Environment* 367: 1010-1016.

[27] Ikem, A., and Egiebor, N. O. 2005. "Assessment of Trace Elements in Canned Fishes (Mackerel, Tuna, Salmon, Sardines and Herrings) Marketed in Georgia and Alabama (United States of America)." *Journal of Food Composition and Analysis* 18: 771-787.

[28] Mol, S. 2011. "Levels of Selected Trace Metals in Canned Tuna Fish Produced in Turkey." *Journal of Food Composition and Analysis* 24: 66-69.

[29] Rahimi, E., Hajisalehi, M., Kazemeini, H. R., Chakeri, A., Khodabakhsh, A., and Derakhshesh, M. et al. 2010. "Analysis and Determination of Mercury, Cadmium and Lead in Canned Tuna Fish Markered in Iran." *African Journal of Biotechnology* 9: 4938-4941.

[30] Martorell, I., Perello, G., Martı-Cid, R., Llobet, J. M., Castell, V., and Domingo, J. L. 2011. "Human Exposure to Arsenic, Cadmium, Mercury and Lead from Foods in Catalonia, Spain: Temporal Trend." *Biological Trace Elements Research* 142: 309-422.

[31] Usydus, Z., Szlinder-Richert, J., Polak-Juszczak, L., Kanderska, J., Adamczyk, M., and Malesa-Ciecwierz, M. et al. 2008. "Food of Marine Origin—between Benefits and Potential Risks. Part I—Canned Fish on the Polish Market." *Food Chemistry* 111: 556-363.

[32] Storelli , M. M., Barone, G., Cuttone, G., Giungato, D., and Garofalo, R. 2010. "Occurrence of Toxic Metals (Hg, Cd and Pb) in Fresh and Canned Tuna: Public Health Implications." *Food Chemistry Toxicology* 48: 3167-3170.

[33] Sirot, V., Guerin, T., Volatier, J. L., and Leblanc, J. C. 2009. "Dietary Exposure and Biomarkers of Arsenic in Consumers of Fish and Shellfish from France." *Science of Total Environment* 407: 1875-1885.

[34] Lourenc, H. M., Asfonso, C., Martin, M. F., Lino, A. R., and Nunes, M. L. 2004. "Level of Toxic Metals in Canned Seafood." *Aquatic Journal of Food Products Technology* 13: 117-123.