Anodic stripping voltammetry with gold electrodes as an alternative method for the routine determination of mercury in fish. Comparison with spectroscopic approaches

Heavy metals are considered among the most alarming forms of pollution in the aquatic environment because of their toxicity and accumulation by marine organisms. Fish absorb heavy metals from the surrounding environment depending on a variety of factors such as the characteristics of the species under consideration, metal concentrations in water and exposure period, as well as abiotic factors such as temperature, salinity, pH, and seasonal changes (Ginsberg & Toal, 2009). The distribution of metals varies among fish species, since it depends on age, development status and other physiological factors. Tuna, as a top predator, being able to concentrate high amounts of heavy metals are also used for biomonitoring of environmental contamination (Has-Schon, Bogut, & Strelec, 2006).

Several metals, such as iron, copper, zinc, and manganese play an essential role in biological systems; other elements, as mercury, lead, and cadmium are toxic, even in trace amounts, and they have been included in the regulations of the European Union for contaminants in foodstuffs EC (Commission Regulation, 2008). Mercury, in particular, is a known toxicant which is present in the environment as a result of both natural processes and anthropogenic activities. Fish accumulate considerable amount of this metal in their tissues and, in fact, the primary source of mercury contamination in man is through eating fish (Inskip & Piotrowsiki, 1995).

In the last years, the consumption of fish has increased in importance due to the high protein supply, and low saturated fat and omega fatty acids content that are known to contribute to good health. Consequently, the health risk associated with consumption of fish contaminated by heavy metals is an important global concern. The techniques generally adopted for Hg determination are cold vapour atomic absorption spectroscopy (CV-AAS) (Souza-Araujo, Giarrizzo, & Lima, 2015), cold vapour atomic fluorescence spectroscopy (CV-AFS) (Fricke, Götz, Schleyer, & Püttmann, 2015) and inductively coupled plasma mass spectrometry (ICP-MS) (Sasmaz, Akgül, Yıldırım, & Sasmaz, 2016). These methods are well established, but they are affected by several drawbacks, such as lengthy analysis times, the use of expensive equipment, the lack of multi elemental analysis, the incapacity for speciation studies (Bagheri, Afkami, Saber-Tehrani & Khoshsafar, 2012) and they cannot be used for field-analysis. Furthermore, several complex steps must be performed, and these require specially trained personnel. Thus, the availability of simple, inexpensive and rapid methods suitable for the routine determination of mercury in food samples is highly desirable.

Recently, new devices for direct mercury analysis have been developed, which automatically perform both sample decomposition and Hg detection by AAS, with short analysis times and low limit of quantitation, LOQ = 0.010 mg/kg wet weight) (Squadrone, Benedetto, Brizio, Prearo, & Abete, 2015).

For ASV and CV-AAS analysis, a dissolution of the matrix was required. Aliquots of 0.5 g of Tuna Fish BCR463 and Tuna Fish ISPRA T22 were transferred into the bombs and digested without any pretreatment with a mixture of 3 ml of HNO_3 and 3 ml of H_2O_2. Aliquots of 0.25 g of CTs were treated in the same way. 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; ventilation for 25 min. The bombs were left to cool at room temperature. The resulting solutions were diluted to 15 mL with HPW. The digested solutions were freshly prepared for analysis with each technique.

For DMA the analysis was performed directly on the freeze-dried powder, since no other sample pretreatment is required.

All the experiments were performed in duplicate and blanks were simultaneously run.

2.2.2 Deposition of gold nanoparticles on the glassy carbon electrode

Modification with gold nanoparticles layer was performed following the procedure described in our previous work (Abollino, Giacomino, Malandrino, Piscionieri, & Mentasti, 2008)

The presence of AuNPs was confirmed by SEM analyses and by cyclic voltammetry (see below). The latter was performed varying the potential from 0 to 1.3 to 0 V in 0.5 M H_2SO_4.

An estimation of the roughness of the obtained gold layer was obtained by AFM (see below).

2.2.3 Solid gold electrode pretreatment

The SGE was polished and activated following the procedure reported in our previous paper (Giacomino, Abollino, Malandrino, & Mentasti, 2008).

During all the analyses hereafter described there was no need to renew electrode surface with alumina powder.

2.2.4 Determination of mercury by SW-ASV

Aliquots of 4 or 2 ml of digested solutions were transferred into the voltammetric cell and diluted to 20 ml with 0.06 M HCl or 60 mM NaCl, in the case of SGE or AuNPs-GCE respectively.

The following procedures were the same for the two electrodes.

After 120 s of deposition at 0 V, a voltammetric scan was performed, adopting the parameters, previously optimised in our laboratory (Giacomino, Abollino, Malandrino, & Mentasti, 2008).

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 and the sample solution cell was replaced by a solution of 0.06 M HCl or 0.06 M NaCl in which the stripping step was then performed (Abollino, Giacomino,Malandrino, Piscionieri, & Mentasti, 2008).

After each determination the electrode 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 (Metrohm, Application Bulletin No 96/4e). The presence of EDTA, probably thanks to its complexing properties, favoured this removal.

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. We obtained well defined peaks by subtracting the blank signal from the voltammograms of the sample solutions.

Each sample was analysed in duplicate.

2.2.5 Determination of mercury by CV-AAS and DMA80

DMA-80 was used to determine the concentration of total mercury, as reported in U.S. EPA Method 7473 (EPA, Method 7473, 2007). By injecting increasing volumes of standard into quartz sample boats, calibration graphs of 0–20 ng and 20–500 ng of mercury were created using the 0.1 and 1 mg/l standards, respectively. The analyses were carried out using the following parameter: drying temperature/time: 90 s to 200°C; decomposition ramp: 120 s to 750°C; decomposition hold: 90 s at 750°C; catalyst temperature: 600°C; purge time: 60 s; 12 s at 900°C; recording time: 60 s; oxygen flow: 120 mL/min Aliquots of freeze-dried tuna (about 0.1 g) were transferred into the sample boats and directly analysed.

For CV-AAS 1 ml of sample solution was diluted to 15 ml with 1,5% HNO_3 directly in the MHS vessel. A few drops of $KMnO_4$ solution were added to monitor the reaction of reduction (the colour of the solution changes as a consequence of the reduction $MnO_4^- \rightarrow MnO_2$). The method of external calibration was adopted for the evaluation of mercury content in the sample solutions. Blank

solution and five standards were prepared for the instrument calibration (40, 50, 70, 100 and 200 ng of Hg).

3 Results and discussion

3.1 ASV analysis

The performances of both the SGE and the AuNPs-GCE for the determination of Hg in aqueous solutions were shown in our previous works (Abollino, Giacomino,Malandrino, Piscionieri, & Mentasti, 2008; Giacomino, Abollino, Malandrino, & Mentasti, 2008), which report the repeatability, linearity, accuracy, detection limit of the procedure and the interferences of other cations and of anions. 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. In synthetic solution, the limit of detection (estimated as LOD= 3σB/slope) for SGE was 0.40 μg/l and sensitivity was 1.71 μA/μgl^{-1}; the relative error for the determination of 1 μg/l of Hg was -1%. The LOD and sensitivity for AuNPs-GCE were 0.15 ng/l and 3.5 μA/μgl^{-1} respectively. A concentration as low as 10 ngl^{-1} could be quantified with a relative error of - 0.8%. The interference of several metal ions (As(V), Bi(III), Cd(II), Co(II), Cr(III), Cu(II), Fe(II), Mn(II), Ni(II), Pb(II), and Se(IV)) and some anions (ClO_4^-, SO_4^{2-}, S^{2-}, PO_4^{3-}, $HCOO^-$, BO_3^{3-}, IO_3^-, CH_3COO^-, CO_3^{2-}, ClO_2^-, F^-, I^-, NO_3^- and NO_2^-) on the mercury stripping signal was investigated using both the electrodes in our previous works (Abollino, Giacomino, Malandrino, Piscionieri, & Mentasti, 2008; Abollino, Giacomino, Ginepro, Malandrino & Zelano, 2012). The voltammogram of a solution with 5 μg/l of Hg was recorded in the presence of each ion (added into the vessel in 1:1; 1:10 and 1:100 concentration ratios with respect to the analyte). For all considered cations, no interference was observed on the Hg quantification and the linearity of the calibration curve of Hg was maintained, in good agreement with literature data reported in literature (Augelli, Munoz, Richter, Gouveia & Angnes, 2005; Okcu, Ertas¸ Gokcel & Tural, 2005). Among the considered anions, no interference was observed with the exception of Br^-, I^- and S^{2-}. These ions are known to adsorb strongly on gold surfaces, and among the halogen ions iodides are those with the highest affinity for gold. They have been found to shift analyte signal to more positive potentials as reported by Tamer et al. (Tamer,U., Oymak T., & Ertas N., 2007). The presence of Br^- interfered with the quantification of Hg for [Hg]< 3μg/l or in presence of a ratio Hg:Br ≥ 1:100. The presence of I^- and S^{2-} caused a decrease of the background current, a sharp decrease of the mercury signal and a loss of linearity, which hindered the quantification of the analyte. To minimize the excessive adsorption effect a negative potential of -

0.80 V was applied for 30 s between the deposition step and the stripping step. At this potential I^-, S^{2-} and Br^- and the other cited anions do not adsorb on the AuNPs-GCS surface. This procedure permits to have well defined voltammetric peaks and a stable voltammetric baseline (Salaun P., & van den Berg C.M.G. 2006).

Considering the low analyte concentrations expected in sample solutions, the medium exchange technique was adopted to eliminate the effect of components in the sample matrix that might cause interferences in the stripping step (Detcheva & Grobecker, 2006). Using this method, we observed an improvement of Hg recovery (about + 8%) in the reference materials for both the electrodes.

3.2 Experiments with SGE

Using SGE, the stripping step was conducted in a solution of either 60 mM HCl or 60 mM NaCl. In our previous work, the measurements in HCl showed higher sensitivity in comparison with NaCl; however, in the case of real sample solutions containing HNO_3 we chose to work in NaCl to avoid the formation of traces of aqua regia which could damage the Au surface. In the present study, we have checked the response of the SGE using both the supporting electrolytes.

All the results are reported in Table 2. We can observe as, using NaCl instead of HCl, the recoveries increase from 89.6% to 94.4 % for BCR 643 and from 80.2 % to 99.0 % for Ispra T22

As to CT solutions, we found higher concentrations using NaCl in comparison with HCl. This is due to the fact that, using HCl, the baselines of the voltammograms related to each sample (i.e. blank, sample solution before and after standard additions) were not perfectly overlapped: this behavior caused a systematic underestimation of the mercury concentration in all CTs. Using NaCl, the baselines for each sample are exactly overlapped.

The LOQ in the considered matrix (computed as the minimum amount determined with a good accuracy) was 0.7 μg/l Hg, corresponding to 0.4 mg/kg in the dried sample.

Using SGE, no detectable mercury peaks were observed for samples 3, 4 and 8.

3.3 Experiments with AuNPs-GCE

All the analyses were repeated using AuNPs-GCE adopting the best condition found for SGE, that is NaCl 60 mM as supporting electrolyte (Table 2). In this conditions, we obtained recoveries of 96.8 % and 99.8% for BCR 643 and Ispra T22 respectively. Using AuNPs-GCE, it was possible to quantify mercury also in the CTs characterised by the lowest analyte concentrations. The LOQ in the investigated matrix is 0.1 μg/l Hg, corresponding to 0.06 mg/kg in the dried sample. These results confirm that the large surface area of the deposited gold nanoparticles permits an improvement of sensitivity in comparison with a traditional solid surface.

As expected, we obtained similar values of concentration for all the considered samples with both electrodes (see section 3.7 for statistical comparison).

3.4 Effect of supporting electrolyte

In our previous study we demonstrated that the performance of HCl and NaCl as supporting electrolytes with AuNPs-GCE is the same. This finding is in agreement with EPA Method 7472, based on ASV with a gold film electrode (GFE), which states that the solutions can be rendered electrically conductive by adding HCl or NaCl indifferently. These reagents permit to obtain the best results, since the formation of complexes between Hg and chloride ions enhances the sensitivity of the stripping signal (Metrohm, Application Bulletin No 96/4e). On the other hand, the two electrolytes have a different behaviour with SGE, as shown above, probably because of the formation of traces of aqua regia, and consequently of nitrosyl chloride, which attacks the gold surface forming a passivating layer. In the case of AuNPs-GCE (Abollino, Giacomino, Malandrino, Piscionieri, & Mentasti, 2008) or GFE (EPA, Method 7472, 1996) this effect is not appreciable due to the renovation of active surface The fact that NaCl provides more stable signals with SGE confirms the observations reported by Bonfil (Bonfil, Brand, & Kirowa-Eisner, 2000), who suggested a mixture of NaCl and HNO_3 as the best supporting electrolyte for the determination of metals using this kind of electrode.

Figure 1 shows the voltammograms obtained with SGE using HCl (a) and NaCl (b) as supporting electrolytes for the analysis of BCR 643; the baseline considered for the evaluation of peaks height is also reported. As we can see, in the case of NaCl the baseline is well defined by the points in which the voltammograms cross, while in the case of HCl a shift of the baseline on the left of the peaks seems to cause an underestimation of the mercury concentration. For comparison Fig.1c reports the voltammograms recorded at AuNPs-GCE for the same sample: as specified in "Procedure" section in the case of SGE the sample was diluted in the electrolyte with a ratio 1:5, while in the case of AuNPs-GCE the ratio is voltammetric cell is 1:10; even if the concentration in cell is lower, the voltammograms reported in Fig.1 show the higher sensitivity obtained by the nanostructured electrode. The coefficient of correlation obtained was greater than 0.9950 for all the considered samples. Figure 1d reports the voltammograms obtained analysing CT3 with AuNPs using NaCl as electrolyte. In all CT-voltammograms it is possible to see another peak at 0.38 V, caused by the presence of traces of Cu (Abollino, Giacomino, Malandrino, Piscionieri, & Mentasti, 2008), present in tuna as a micronutrient; a fraction of this metal might derive from the metallic package, as found by Buculei et al. (Buculei, Amariei, Oroian, Gutt, Gaceu, & Birca, 2014).

3.5 Monitoring of the electrodes surface

The deposition of gold nanoparticles is well detectable through a colour change of the glassy carbon surface from black to red-orange. The status of the gold nanoparticles layer was valued with SEM From SEM images (Fig. S1, Supplementary material), it is possible to see the presence of a homogeneous gold layer composed of particles, having an average diameter of approximately 100 ± 25 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 drawback of SEM technique as method to monitor the surface is that once a metal nanoparticles-modified electrode is analysed with SEM, the metal particles are charged by the beam and they could coalesce and form an "Au film", so another method has to be used for the daily monitoring of the goodness of the active surface.

A fast and easy method to do this is cyclic voltammetry (CV). Fig. 2a and 2b show voltammograms recorded in 0.5 M H_2SO_4 with SGE and AuNPs-GCE respectively.

The shapes of the voltammograms reported as "line 1" are well known in literature and identify a solid (Fig 2a) or nanostructured (Fig 2b) gold surface (Salaun, Planer-Friedrich, & van den Berg, 2007). The anodic peak at 1.25 V is due to oxide formation, probably consisting of hydrated oxides or $Au(OH)n_{ads}$. In the CV voltammogram for AuNPs-GCE, we can observe a "shoulder" before the oxidation peak, typical of a nanostructured gold surface, caused by the formation of different multi-oxide species for the gold nanoparticles (Priano et al., 2008). The cathodic peak at +0.90 V, present in both the voltammograms, can be attributed to the reduction of the gold oxide formed during the anodic cycle. The charge recorded under the reduction peak is generally used to characterize and monitor the electroactive sensor area, since it is proportional to the amount of deposited Au (Dai, Nekrassova, Hyde, & Compton, 2004). As expected, a higher signal is obtained in the case of AuNPs-GCE in comparison with SGE, due to the presence of nanoparticles that increase the electrode surface.

The voltammogram reported as "Line 2" in Fig.2a represents the CV recorded after a prolonged use of the electrode surface, that causes a worsening in the analytical response. In the case of SGE, the CV reported in Fig. 2a shows additional peaks in comparison to CV obtained from an optimal surface. These peaks are due to the electrochemistry of gold, since are caused by the formation of multilayers of oxides in which gold can exist in different oxidation states (Au0/AuI/AuIII) (Giacomino, Abollino, Lazzara, Malandrino, & Mentasti, 2011); the presence of these layers causes a worsening of the electrode performance. After the treatments with NaOH, ethanol and water a

cyclic voltammogram like that reported in Fig. 2a-line1 is again obtained. We suppose that in the presence of NaOH the surface is covered with a layer of hydroxides, that replace completely or in part the hydration layer; ethanol removes this deposited layer and water removes the traces of ethanol.
Regarding AuNPs-GCE, in Fig. 2b-line2 we can observe i) a shift of the anodic peak to more positive potential contemporary to a reduction of the peak height and to the disappearance of the shoulder and ii) a drastic reduction of the cathodic peak due to a loss of available active surface. In this condition the electrode can be used as "Au film electrode", but it presents a lower sensitivity. With this kind of electrode the treatment of the surface is usually not performed, as it is more convenient to dissolve the gold layer and deposit a new one.

Daily, we recorded ten CV-scans in H_2SO_4 to check the repeatability of the electrode response and, consequently, the goodness of the electrode surface. Many researchers use this CV-treatment as an activation step for solid electrodes: for our experience, we suggest to make, after the treatment in H_2SO_4, a further activation step by applying a potential of 0.60 V for 60 s in 0.06 M HCl before mercury determination, in order to maintain the electrode surface active and reproducible and consequently to enhance the quality and reproducibility of the mercury signal.

An estimation of the roughness of the obtained gold layer was obtained by AFM. Unfortunately, AFM instruments are not suitable to analyse this type of electrode, because of its size (height = 5 cm). For this reason this evaluation was performed with the aid of a glassy carbon plate (height = 3 mm), onto which the same procedure of surface cleaning and deposition adopted for GCE was followed. Obviously, the conditions in which the deposition occurs were different because i) during the deposition the electrode is rotated, while it is not possible to move the GC plate: so, in this case, the solution was maintained in agitation with a magnetic stirrer; and ii) the sizes of the GC surfaces are different: the electrode surface can be estimated as ≈ 3,14 mm^2 (as calculated from the radius of the active surface = 1 mm ± 0.01, provided by the producer), while the plate has an area ≈ 2,5 cm^2 (manually estimated by measuring the total surface in contact with Au solution). In any case, AFM analysis (Fig. S2, Supplementary material) confirmed the presence of a homogeneous gold layer: in particular the profile reported in Fig.S2-c shows the regularity of the obtained surface.

3.6 Atomic Absorption Spectroscopy Analysis

In the last decade, direct mercury analysers demonstrated to be a reliable alternative to wet chemistry techniques. The DMA automatically performs thermal decomposition, catalytic

reduction, amalgamation, desorption and AAS detection to rapidly treat and analyse solid or liquid samples for mercury, with an output result for mercury content in about 5 min (per sample) with no pre-treatments required and no waste generation. For the considered matrix, the limit of quantitation (LOQ) for detection of mercury by means of this method is 0.037 mg/kg (wet weight). The obtained calibration curve, $y = -3.06\ 10^{-7}x^2+1.10x\ 10^{-3}x–5.5x10^{-3}$ shows a good linearity, $R^2 = 0.9998$ (Squadrone, Benedetto, Brizio, Prearo, & Abete, 2015).

The results are reported in Table 3: as we can observe, a quantitative recovery was obtained analysing BCR material. For this reason, and taking into account that the use of DMA for mercury determination is well-established, we considered the results obtained using this technique as "reference values" to evaluate the data obtained with the other methods. Moreover, since the process does not require the conversion of mercury to mercuric ions, both solid and liquid matrices can be analysed without the need for acid digestion or other sample preparation steps. Therefore, the comparison with DMA results permitted us to quantify the possible losses through volatilization or incomplete digestion which may take place with the other investigated analytical techniques (Haynes, Gragg, Johnson, Robinson, & Orazio, 2006).

Table 3 shows also the results obtained using conventional CV-AAS, preceded by sample digestion in microwave oven. The method has a good linearity, as shown by the calibration curve obtained ($y = 0.0012x – 0.0011$, $R^2 = 0.9981$).

The concentrations measured in the certified materials show a loss of mercury of about - 20 ± 5 % that can be ascribed to a partial volatilization of the metal during the digestion step and/or during the conversion to elemental Hg and transfer to the measurement cell. Then, the analysis of the CTs was carried out: the quantification of Hg concentration was possible in all the samples with the only exception of CT3, in which the signal was too low. The results obtained with CV-AAS were surely negatively influenced by the absence of the gold trap required for determination of Hg at ultratrace level by this technique.

3.7 Comparison among the techniques

We compared the results obtained with the considered techniques to understand the real performance of ASV determination, in particular using AuNPs-GCE.

Considering the DMA results ([Hg_{DMA}]) as "true", the concentrations in CTs found using SGE with NaCl, AuNPs-GCE and CV-AAS were expressed as percentages of recovery (Table 4) with respect to [Hg_{DMA}].

As it can be seen, the values range between 79% and 112%. The median values increase in the order CVAAS < SGE < AuNPs-GCE.

Figure 3 reports scatter plots comparing $[Hg]_{DMA}$ with the concentrations measured with SGE in NaCl (3a), AuNPs (3b) and CV-AAS (3c), the equations of the lines so obtained and the corresponding values of R^2. For SGE only the samples containing higher concentrations (six CTs) were considered, while for CV-AAS it was possible to compare the results obtained for all the samples with the exception of sample CT3. The comparison indicate a good correlation between the mercury amounts measured in the samples with different techniques. The results obtained by ASV demonstrated the highest correlation with $[Hg]_{DMA}$; in particular using AuNPs-GCE a correlation of 0.9889 was obtained considering the whole data set.

A statistical comparison among all the results obtained was also made using ANOVA (level of probability = 95%) considering the concentrations found using the different techniques for each sample. For all the considered samples, the Hg concentrations measured with DMA were not significantly different from those found by ASV using AuNPs-GCE. As to the other techniques, among the quantifiable samples, no significant differences were observed from $[Hg]_{DMA}$ with the only exception of CT3 in the case of CV-AA.

It is very important to underline the key role of analysing freshly prepared sample solutions (maximum one week), since losses of 10-30 % were observed in solutions analysed two weeks after the digestion. Good repeatability was observed among sample solutions prepared from different aliquots of the same lyophilized tuna demonstrating the homogeneity of the sample.

Finally, Table 5 summarizes the analytical performance found in this study for each adopted technique.

Both voltammetric and spectroscopic approaches can be considered suitable techniques to monitor Hg concentration in fish, since they allowed to reach accurate Hg quantification at concentration values lower than the maximum admissible level of this element in the considered matrix (1 mg/kg_{ww}) (Commission Regulation (EC) No. 629/2008).

Considering the two investigated spectroscopic techniques, we can conclude that DMA performs better than conventional CV-AAS and can be considered a fast, accurate and reliable alternative to wet chemistry techniques. Its main advantage is the high sample throughput, as no sample

preparation is required The technique permits to determine Hg with high accuracy and precision. Its main drawback is that it is applicable only to the determination of this analyte.

Electrochemical techniques are sensitive, relatively inexpensive and they also enable the determination of a number of inorganics and organics at trace or ultratrace concentrations. The drawbacks are the longer time required in comparison with DMA, in particular for the wet digestion step, with possible loss of analyte by vaporisation, and the production of waste solutions. In the case of in-cell concentrations higher than 0.7 μg/l both SGE and AuNPs-GCE provide very accurate determinations. The presence of the nanostructured active surface in the latter permits to quantify lower concentrations with a LOQ comparable to that of DMA. Another great advantage of the electrochemical approach is the possibility to carry out speciation studies: in the case of mercury, it is possible to distinguish between inorganic Hg and methylmercury, the most harmful specie for humans (Abollino, Giacomino, Malandrino, Marro, & Mentasti, 2009)

3.8 Evaluation of Hg in examined canned tunas

Since the main purpose of our study is to verify the applicability of AuNPs-GCE for the quantification of Hg in fish in routine controls, we compared the concentrations obtained with the European Legislation limit for this analyte: the maximum admissible level for mercury in fishery products and fish tissues is 0.5 mg/kg_{ww} for most fish species, and 1 mg/kg_{ww} for tuna and some others species (Commission Regulation (EC) No. 629/2008). During the lyophilisation step, the CTs lost about 30 ± 5 % of weight, so we calculated the final concentrations in wet fish. All the examined samples contained Hg concentrations lower than the admissible value (Table S1, Supplementary material).

4 Conclusions

In this work the suitability of ASV with gold electrodes for the determination of mercury in fish was demonstrated.

Under the optimized conditions, for both the electrochemical approaches, the oxidative current exhibited a linear dependence on Hg(II) ion concentration in wide dynamic ranges of 0.2-100 μg/l (R^2= 0.9891) and 0.010-100 μg/l (R^2 = 0.9922) with detection limits of 0.02 and 0.001 μg/l for SGE and AuNPs-GCE respectively.

The main advantage of the AuNPs-GCE with respect to SGE is the improvement of the analytical performance, thanks to the increase of active surface of the gold nanoparticles. In particular the proposed sensor shows high sensitivity (even with short deposition time, 120 s), good selectivity, long-term stability, great repeatability and a limit of quantification of 0.1 μg/l for Hg in fish matrix. The comparison between DMA and conventional CV-AAS shows that the former has better performances in terms of accuracy, precision and analysis times, owing to the integration of sample pretreatment and analyte detection. The results obtained by DMA and ASV with AuNPs-GCE are not significantly different, confirming that the latter is an effective alternative to other more common techniques for the determination of mercury in fish.

The choice of the most suitable analytical method for the determination of Hg is influenced by the number of samples, by the available time and by the number of considered analytes: for a fast determination of Hg in a lot of samples, DMA seems to be the best solution; ASV is particularly suitable for a relatively low sample number in which also speciation studies or the measurement of other (inorganic or organic) species is required, The proposed technique has the main advantages of electrochemical methods i.e. versatility, sensitivity, low costs of instrument purchase and management, availability in most analytical laboratories and the possibility of using portable devices for on-site and in situ measurements. The ease of the procedures requested for the electrode modification could offer a simple, inexpensive and rapid methods for the routine determination of mercury in food samples. The good accuracy and precision demonstrated by ASV with AuNPs-GCE show as this technique can be fruitfully used in ring tests.

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