

Analytical procedure for total mercury determination in fishes and shrimps by chronopotentiometric stripping analysis at gold film electrodes after microwave digestion

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Abstract

A method for the total mercury determination in fish and shrimps employing chronopotentiometric stripping analysis on gold film electrodes is described. Fish and shrimp tissues were digested using a microwave oven equipped with closed vessels. We developed a microwave heating program which decomposed all the samples employing diluted nitric acid and hydrogen peroxide. The proposed method was validated by analyzing a certified reference material and then applied for different fish species from fresh water and seawater acquired in local markets of São Paulo city, Brazil. The Brazilian legislation establishes 0.5 and 1 mg per kilogram of fish as upper limit of mercury for omnivorous and predator species, respectively. Except for blue shark tissues, the mercury content was situated below $0.5 \mu\text{g g}^{-1}$ for all the analyzed samples. The detection limit of the proposed method was calculated as 5 ng g^{-1} of sample utilizing 5 minutes of electrodeposition (+300 mV vs. Ag/AgCl) on the gold electrode.

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1. Introduction

Beyond the natural mercury emission to the atmosphere, the anthropogenic contribution mainly as Hg(II) has increased; consequently, the mercury content in natural waters increased as well. The main part of Hg(II) rapidly attaches to particulates in suspension found in water bodies, and then is deposited in sediments where covalent molecules are formed, such as HgCl_4^{2-} and CH_3Hg^+ ; the latter forms dimethylmercury [$\text{Hg}(\text{CH}_3)_2$], a molecular vol-

atile liquid. The formation of $\text{Hg}(\text{CH}_3)_2$ in natural waters is normally favored under anaerobic conditions, where microorganisms convert Hg(II) to $\text{Hg}(\text{CH}_3)_2$. This fact occurs mainly in the first layers of sediments in rivers and lakes. Dimethylmercury is transformed under acidic or neutral conditions into the monomethylmercury and other forms, such as CH_3HgCl and CH_3HgOH . Therefore, the production of methylmercury in natural waters predominates over the formation of dimethylmercury. Methylmercury diffuses easier than Hg(II) salts into animal fatty tissues and this characteristic facilitates its bioaccumulation. Once in the body, methylmercury may bind to sulfur-containing amino acids forming soluble compounds in animal tissues. Due to these reasons, methylmercury is the most hazardous mercury form (Baird, 1995).

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Fish has a great importance for human feeding once contains high quality proteins, essential nutrients, and other important substances for a healthy diet. However, due to the environmental mercury contamination and subsequent bioaccumulation, the mercury content is higher in fish species at the top of the trophy chain (US EPA, 2005a). The main example is the methylmercury bioaccumulation in sulphhydryl groups of proteins, such as metallothionein and glutathione (Ravichandran, 2004; Sigel & Sigel, 1994). The first and most famous case of mercury contamination and accumulation in fish occurred in Minamata Bay, Japan, 50 years ago (Baird, 1995; Lacerda, 1997; Sigel & Sigel, 1994).

Lechler et al. (2000) studied in details the mercury concentration in soils, sediments, water, and fishes of Madeira River basin (situated in the Brazilian Amazon). The authors revealed that the high mercury level was originated from both natural sources and natural biogeochemical processes.

Brazilian legislation for maximum quantities of mercury in commercialized fish is $0.5 \mu\text{g g}^{-1}$ and $1.0 \mu\text{g g}^{-1}$ for omnivorous and carnivorous species, respectively (ANVISA, 1998). The results of some studies demonstrated that the content of mercury in the Brazilian commercialized shark was normally higher than $1 \mu\text{g g}^{-1}$ (Chicourel, Tenuta-Filho, Sakuma, Zenebon, & Amorim, 1995; Morales-Aizpurúa, Tenuta-Filho, Sakuma, & Zenebon, 1999). These results emphasized the need for quality control of commercialized fish in order to monitor the total mercury content, mainly of the carnivorous species which belongs of the top of the food chain.

Despite EPA recommending stripping analysis for mercury determination (Method 7472, US EPA, 2005b) and the possibility of on site measurements due to the portable instrumentation developed for electroanalytical analysis (Brett & Brett, 1993; Wang, 1985), most environmental researches still prefer other more complicated and expensive techniques for mercury determination in biological samples, such as cold vapor atomic fluorescence spectrometry and mainly cold vapor atomic absorption spectrometry (Aduna de Paz, Alegría, Barberá, Farré, & Lagarda, 1997; Storelli, Storelli, Giacomini-Stuffler, & Marcotrigiano, 2005; Ubillús, Alegría, Barberá, Farré, & Lagarda, 2000; Voegborlo, El-Methrani, & Abedin, 1999; Hight & Cheng, 2005).

Mercury quantification methodologies utilizing electroanalytical techniques for fish analysis are still scarce and in some cases of difficult access, probably due to problems related to sample preparation. In 1981 Ahmed, Valenta, and Nurnberg (1981) presented a mercury determination procedure for tuna fish analysis using differential pulse anodic stripping voltammetry on gold electrode. The samples were treated with $\text{HNO}_3/\text{HClO}_4$ into pressurized Teflon vessels and the resultant solution was irradiated with UV light in the presence of H_2O_2 to decompose dissolved organic substances.

Golimowski and Gustavsson (1983), and Gustavsson and Golimowski (1981) determined total mercury in fish

using differential pulse anodic stripping voltammetry. These authors investigated two digestion procedures under pressure employing $\text{HNO}_3/\text{HClO}_4$ and $\text{HNO}_3/\text{H}_2\text{SO}_4$ mixtures. The samples had to be UV irradiated to complete the oxidation process. De Gregori et al. (1992) developed analytical procedures for quality control of toxic trace elements in shellfish. Differential pulse anodic stripping voltammetry, neutron activation analysis, and atomic absorption spectrometry were compared.

Our group has been working on the development of electroanalytical sensors and alternative sample treatments which result on easy electrochemical detection mediums. Recently, we developed analytical procedures for the determination of lead and copper in ethanol fuel and lubricating oil (Munoz & Angnes, 2004; Munoz, Silva, Correia, Oliveira, & Angnes, 2005; Munoz, Oliveira, & Angnes, 2006) and mercury in natural water (Augelli, Munoz, Richter, Gouveia-Junior, & Angnes, 2005; Richter, Augelli, Kume, Mioshi, & Angnes, 2000) using stripping analysis on gold electrodes.

This article describes a simpler methodology for mercury determination in fish and shrimp samples using anodic chronopotentiometric stripping analysis (CSA) on gold film electrodes combined with a prior microwave sample digestion utilizing diluted nitric acid and hydrogen peroxide. Despite employing diluted nitric acid, this microwave oven sample decomposition is efficient enough to eliminate the UV irradiation step which is commonly applied for the decomposition of organic molecules still present in resultant microwave digested samples.

2. Experimental

2.1. Chemicals and reagents

Solutions were prepared with double filtered, distilled (in a quartz apparatus), and deionized water obtained from Nanopure system (Barnstead Thermolyne, Dubuque, IA) with resistivity value not less than $18 \text{ M}\Omega\text{-cm}$. The metal stock solution (1000 mg L^{-1} , in 1%wt. nitric acid, atomic absorption spectrometry standard solution, Aldrich) was diluted as required, just before its use. Hydrochloric and nitric acids solutions were prepared by diluting the respective concentrated acids (Suprapur grade, Merck). Hydrogen peroxide (Perhidrol, 30% v/v, Pro Análisi) was purchased from Merck.

2.2. Samples obtaining

Fish and shrimp samples were bought in supermarket at São Paulo City, wrapped in plastic bags, and maintained on ice. The samples were homogenized and maintained in the laboratory at -20°C . The certified reference material “fish fresh homogenate” (MA-A-2) from IAEA (International Atomic Energy Agency) with mercury content of $0.47 \pm 0.02 \mu\text{g/g}$ was analyzed for the validation of our proposed method.

2.3. Instrumentation and electrochemical cell

CSA measurements were performed with an electrochemical system (Autolab PGSTAT 20, Eco Chemie, Utrecht, The Netherlands) connected to a personal computer, utilizing a cell containing three electrodes, a Pt wire as auxiliary electrode, a homemade Ag/AgCl reference electrode (Pedrotti, Angnes, & Gutz, 1996), and a working gold electrode (Angnes, Richter, Augelli, & Kume, 2000) obtained from compact discs (Mitsui Gold Standard). Each compact disc can be utilized to construct dozens of electrodes, as the one presented in Fig. 1. The working electrode area was delimited manually, typically with sizes situated between 3 and 4 mm². However, this fact was not important in this work because in all measurements done in this work the standard addition method was applied.

More recently, an easy way to construct electrodes with very reproducible areas was developed (Lowinsohn, Richter, Angnes, & Bertotti, 2006) based on the hot transference of toner to gold surfaces.

The electrochemical cell was easily constructed utilizing 10 mL graduated polypropylene cylinders. These flasks were cut to work with 4 mL electrochemical cells (interior volume). A Teflon cover was constructed on the adequate measurements to fit firmly at the top of these flasks and to position the three electrodes into holes exactly designated onto this cover. The low price of these volumetric flasks allowed us to buy many units and replace them after each analysis. Before the use, the flasks were immersed in a nitric acid (6 mol L⁻¹) solution to clean overnight. This fact diminished the mercury contamination during repeti-

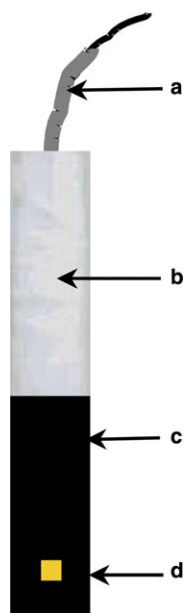


Fig. 1. Working electrode: (a) multi-stranded flexible copper wire; (b) Teflon tape (pressing the copper wire to establish the electrical contact with the gold surface); (c) nitrocellulose varnish; (d) gold working electrode (exposed area).

tive analysis. The same procedure was adopted even in case of brand new flasks.

2.4. Microwave heating system

The sample digestions were accomplished in “Microwave Sample Preparations System” model MDS-2000 (CEM Corporation, Matthew, NC, USA) provided with Teflon flasks. This system works with closed flasks and its maximum power (630 W) was applied for all procedures.

A mass of sample between 0.1 and 0.4 g was put into each Teflon vessel and then 1 mL of water plus 2 mL of HNO₃ 70% was added. The microwave program is listed in Table 1.

After the fourth step of the program, the vessels were carefully opened for the addition of 2 mL of hydrogen peroxide and closed again for the last step of the heating program. The final solutions were cooled and transferred to 10 mL calibrated flasks and their volumes completed with deionized water. The microwave vessels were cleaned with hot nitric acid solution and subsequently washed with deionized water. All sample preparation steps were performed in a laminar flow fume cupboard to avoid external contamination.

2.5. CSA measurements

Table 2 presents the CSA optimized parameters for mercury determination in the digested samples. The mercury peak potential was ca. +575 mV (vs. Ag/AgCl). Discussion about CSA parameters optimization is discussed in the next section. It was not necessary to remove the oxygen dissolved in solutions prior analysis.

Table 1
Microwave oven heating program for sample decomposition

Step	Hold time (min)	Pressure (psi)
<i>Sample + 2 mL of HNO₃ + 1 mL of H₂O</i>		
1	10	50
2	10	60
3	6	80
4	6	100
<i>Addition of 2 mL H₂O₂</i>		
5	10	80

Table 2
Parameters of stripping chronopotentiometric measurements for the analysis of the digested samples

Technique parameters	
Conditioning potential (mV)	700
Conditioning time (s)	20
Deposition potential (mV)	300
Deposition time (s)	120–300
Equilibrating time (s)	15
Potential limit (mV)	700
Stripping current (μA)	0.4

The high sensitivity of electroanalytical methods authorized to make significant dilutions of the samples (in accordance with the mercury content). Therefore, the high acid concentration decayed with solution dilutions and this increased the lifetime of the gold film electrode. Chloride ions were added into the cell (50 mmol L^{-1}) before each analysis. The accuracy for mercury determination using the proposed method was evaluated by analyses of the certified reference material.

3. Results and discussions

Strong acidic mediums are normally utilized for the digestion of fish and shrimp as described above. At the same time, our previous work demonstrated that the presence of chloride ions (50 mmol L^{-1} from a 1 mol L^{-1} NaCl stock solution) in the electrolyte enhances the sensitivity of the mercury-stripping signal (Augelli et al., 2005). However, our initial preoccupation was to obtain a softer sample decomposition which must decrease the nitric acid concentration, since its high concentration in solutions containing chloride ions originates nitrosyl chloride, a powerful oxidant which attacks the gold electrode affecting electrochemical measurements. Traditional methods but now employing diluted nitric acid to decompose fish samples were explored using microwave oven. Therefore, the heating program must be carefully optimized in order to decompose fish samples, if diluted acid is employed.

The heating program detailed in Table 1 was successfully applied for both the certified reference material and commercialized samples. The use of diluted nitric acid and hydrogen peroxide in a subsequent step for the decomposition of fish and shrimp samples resulted in clear solutions, which were directly diluted in the electrochemical cell for CSA mercury determination. The aspect of the shrimp sample after the digestion process was very clear, similar to the observed in the case of the fishes. Any interference due to residual organic matrix was verified during the stripping process of this resulting solution. Nitric acid and hydrogen peroxide are commonly employed for decomposition of organic samples in pressurized vessels under microwave irradiation. The oxidant power of nitric acid is enhanced at the high pressure employed in the microwave oven in such way that it was not necessary to add sulfuric acid to increase the boiling point. Hydrogen peroxide has replaced other oxidants such as perchloric acid due to the possibility of explosions when using this acid.

Furthermore, the acidity of final digested solution was substantially decreased by dilution (20–100 times) of the sample with deionized water, once mercury in samples are present at relatively high concentrations in all samples when comparing to the high sensitivity of CSA measurements on gold electrodes.

At the same time that the presence of chloride ions in solution enhances the sensitivity of the stripping-mercury signal, the gold oxidation starts to occur at less positive

potentials. Hence, the conditional (cleaning) and limit potentials (listed in Table 2) cannot be higher than +700 mV (vs. Ag/AgCl) for solutions containing the optimized chloride concentration. The application of higher potentials removed the gold film from electrode substrate before the standard addition measurements, not allowing the final analysis. Despite these gold film electrodes are disposable, the same one can be used for more than eight hours of analysis.

Copper can also be found at considerable concentrations in biological tissues. This metal has very good response on gold electrodes and its oxidation potential (ca. +300 mV vs. Ag/AgCl) is situated near to the mercury one. The influence of copper and other metals on mercury determination was studied in a previous work (Augelli et al., 2005). The best deposition potential applied for mercury determinations was +300 mV (vs. Ag/AgCl), since less positive potentials may increase the copper signal interference. Even so, copper interference was not observed for all analyzed samples in the present study. When copper concentrations were higher than twenty times, the mercury signal started to decrease when the deposition potential was +200 mV (vs. Ag/AgCl).

Studies involving the deposition time were done to determine the best working conditions. As described in previous studies (Augelli et al., 2005; Richter et al., 2000), it was also explored the relationship between peak area and deposition time. The digested certified sample was used for the deposition time optimization evaluating times between 60 and 300 s. The peak area values increased linearly in this interval. The increase of signal was not proportional for higher deposition times than 300 s and some decrease of signal was observed after 600 s of deposition. This fact may be attributed to the saturation of the very thin gold film utilized (50–100 nm thickness) when more than 300 s of deposition time was applied. Therefore, 300 s was adopted for further experiments. The optimization of stripping current depended on the electrode area. Despite the electrode area being greatly dependent on its hand construction (Angnes et al., 2000), we produced electrodes with low relative deviation, which allows us to use the same optimized stripping current.

Another important parameter to be evaluated is the repeatability of signals. Fig. 2 presents 10 (of 30) repetitive chronopotentiograms of the digested tahira sample, after dilution of 20 times. The thirty chronopotentiograms pre-

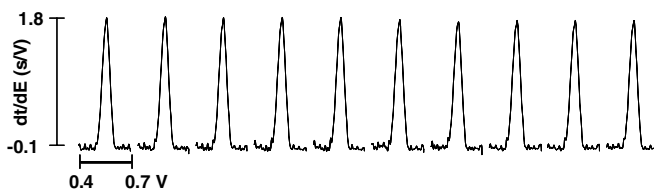


Fig. 2. Ten chronopotentiograms of the digested tahira (*Hoplias malabaricus*) sample after 20 time dilution. Peak area deviation: 0.084 ± 0.001 s (RSD = 1.2%).

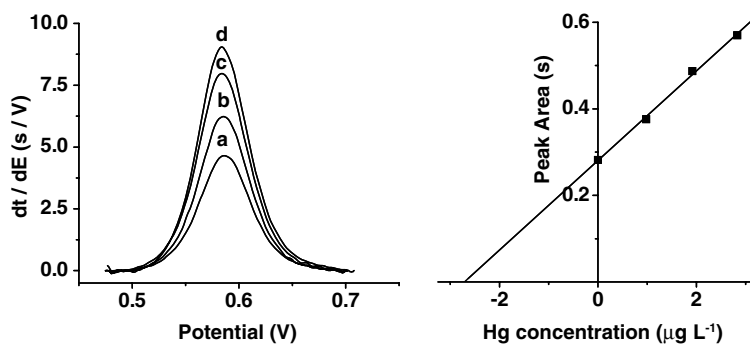


Fig. 3. Chronopotentiograms of the digested blue shark (*Prionace glauca*) sample after 40 time dilution (a) plus increments of $1 \mu\text{g L}^{-1}$ of mercury (b–d).

sented an average peak area of 0.0210 ± 0.0003 s (RSD = 1.2%; $n = 30$).

The analysis of a certified sample was performed to verify the reliability of the proposed method. The digestion of this sample was done utilizing the same process used for fish and shrimp samples. The medium value for the digested certified sample ($n = 7$) was $0.55 \pm 0.06 \text{ mg kg}^{-1}$, in agreement with the nominal value of $0.47 \pm 0.02 \text{ mg kg}^{-1}$. Good reproducibility (RSD = 11%) was attested and the calibration curves showed good linearity (>0.99).

Fig. 3 shows chronopotentiograms for the digested blue shark (*Prionace glauca*) sample diluted 40 times (a) plus increments of $1 \mu\text{g L}^{-1}$ of mercury (b–d). The correlation coefficient was 0.9989. The medium value obtained was $1.9 \pm 0.1 \text{ mg kg}^{-1}$, almost twice higher than the Brazilian legislation value established for predator fish (1 mg kg^{-1}) (ANVISA, 1998). This result shows the importance of alternative ways for mercury quantification in fishes. Actually, this kind of control is not systematic in Brazil and we hope to be contributing for this purpose.

The same methodology was applied for other eight different species commonly available at local markets of São Paulo city. The respective chronopotentiograms were very similar to the ones shown in Fig. 2, as well as the calibration curves, with correlation coefficients no less than 0.99. Table 3 shows the total mercury content and the respective scientific names of the analyzed species.

Except for blue shark, all results indicated that mercury levels found in the analyzed samples were compatible with the Brazilian legislation. Standard deviations were similar

Table 3
Total mercury concentration obtained in the digested samples ($n = 4$)

Species Common names (scientific names)	mg kg ⁻¹
Blue Shark (<i>Prionace glauca</i>)	1.9 ± 0.1
Sea-Bob Shrimp (<i>Xiphopenaeus kroyeri</i>)	0.38 ± 0.01
Meagre (<i>Argyrosomus Regius</i>)	0.14 ± 0.01
Tahira (<i>Hoplias malabaricus</i>)	0.31 ± 0.02
Sardine (<i>Sardinella aurita</i>)	0.053 ± 0.003
Nile Tilapia (<i>Oreochromis niloticus</i>)	0.15 ± 0.01
Couch's Sea-Bream (<i>Pagrus pagrus</i>)	0.12 ± 0.01
Gafftopsail Catfish (<i>Bagre marinus</i>)	0.35 ± 0.02
Anchovy (<i>Anchoviella spp</i>)	0.050 ± 0.005

to that found by spectrometric methods. Usual cold vapor atomic absorption spectrometry determinations (Chicourel et al., 1995) presented an approximate uncertainty of 10%, similar to the results presented in this study ($<11\%$). Moreover, the certified sample analysis ($0.55 \pm 0.06 \text{ mg kg}^{-1}$) was in good agreement with the certified value ($0.47 \pm 0.02 \text{ mg kg}^{-1}$). The optimization of reagents and microwave oven conditions to carry out the sample decomposition was fundamental to allow the successful use of CSA for mercury determination. The procedure here developed for fish and shrimp analysis opens an interesting possibility for the analysis of many other types of seafood.

4. Conclusions

The proposed methodology for mercury determination in fish and shrimp samples using CSA and microwave digestion shows many advantages. The utilization of diluted nitric acid at the first step and the addition of hydrogen peroxide at the second step (when part of the nitric acid was consumed oxidizing the organic material of samples) is more favorable than the utilization of nitric acid plus hydrochloric acid, once this mixture generates nitrosyl chloride which attacks the gold electrodes and slightly attacks the microwave-oven Teflon vessels or even when nitric acid plus perchloric acid are utilized, because the digestion is not fully completed in this last case (Ahmed et al., 1981). Despite the digestion process involving a five step microwave program, the process is completed in less than one hour. Moreover, the digestion process evolved the utilization of diluted nitric acid which produces a less acidic final solution which is an important advantage even for spectrometric detection.

Calibration curves showing good linearity (>0.99), high sensitivity and selectivity are the main advantages of the CSA technique presented in this paper. The analysis of the certified material sample showed good accuracy ($102\% \pm 15$) of the method comparable to the usual spectrometric methods. Good reproducibility was attested by low standard deviations ($<11\%$) comparable to the spectroscopic ones. This analysis also certified that there were no losses of mercury during the microwave sample digestion in the conditions adopted.

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References

- Aduna de Paz, L., Alegría, A., Barberá, R., Farré, R., & Lagarda, M. J. (1997). Determination of mercury in dry-fish samples by microwave digestion and flow injection analysis system cold vapor atomic absorption spectrometry. *Food Chemistry*, *58*, 169–172.
- Ahmed, R., Valenta, P., & Nurnberg, H. W. (1981). Voltammetric determination of mercury levels in tuna fish. *Microchimica Acta*, *1*, 171–184.
- Angnes, L., Richter, E. M., Augelli, M. A., & Kume, G. H. (2000). Gold electrodes from recordable CDs. *Analytical Chemistry*, *72*, 5503–5506.
- ANVISA, Agência Nacional de Vigilância Sanitária Portaria, Portaria 685, 27 de agosto de 1998, <http://e-legis.bvs.br/leisref/public/search.php>, Last access on July 2005.
- Augelli, M. A., Munoz, R. A. A., Richter, E. M., Gouveia-Junior, A., & Angnes, L. (2005). Chronopotentiometric stripping analysis using gold electrodes, as efficient technique for mercury quantification in natural waters. *Electroanalysis*, *17*, 755–761.
- Baird, C. (1995). *Environmental chemistry*. New York: WH Freeman Pub.
- Brett, C. M. A., & Brett, A. M. O. (1993). *Electrochemistry: principles, methods, and applications*. Oxford: Oxford Science Pub..
- Chicourel, E. L., Tenuta-Filho, A., Sakuma, A. M., Zenebon, O., & Amorim, A. R. (1995). Mercúrio total em cação comercializado em São Paulo – SP, Brazil. *Ciência Tecnol. Alimentaries*, *15*, 144–149.
- De Gregori, A., Delgado, D., Pinochet, H., Gras, N., Thieck, M., Munoz, L., et al. (1992). Toxic trace elements in Chilean seafoods: development of analytical quality control procedures. *Science of the Total Environment*, *111*, 201–218.
- Golimowski, J., & Gustavsson, I. (1983). Determination of mercury in freeze-dried muscle samples of pike, cod and perch using an ASV-technique. *Science of the Total Environment*, *31*, 89–98.
- Gustavsson, I., & Golimowski, J. (1981). Analysis of mercury in fish muscle samples by ASV-technique. *Science of the Total Environment*, *22*, 85–87.
- Hight, S. C., & Cheng, J. (2005). Determination of total mercury in seafood by cold vapor-atomic absorption spectroscopy (CVAAS) after microwave decomposition. *Food Chemistry*, *91*, 557–570.
- Lacerda, L. D. (1997). *Mínimata Livre de Mercúrio*. *Ciência Hoje*, *23*, 24–31.
- Lechler, P. J., Miller, J. R., Lacerda, L. D., Vinson, D., Bonzongo, J. C., Lyons, W. B., et al. (2000). Elevated mercury concentrations in soils, sediments, water and fish of the Madeira river basin, Brazilian Amazon: a function of natural enrichments. *Science of the Total Environment*, *260*, 87–96.
- Lowinsohn, D., Richter, E. M., Angnes, L., & Bertotti, M. (2006). Disposable gold electrodes with reproducible area using recordable CDs and toner masks. *Electroanalysis*, *18*, 89–94.
- Morales-Aizpurúa, I. C., Tenuta-Filho, A., Sakuma, A. M., & Zenebon, O. (1999). Mercúrio total em cação comercializado em São Paulo-SP, Brasil. *Ciência e Tecnologia de Alimentos*, *19*, 429–432.
- Munoz, R. A. A., & Angnes, L. (2004). Simultaneous determination of copper and lead in ethanol fuel by anodic stripping voltammetry. *Microchemical Journal*, *77*, 157–162.
- Munoz, R. A. A., Silva, C. S., Correia, P. R. M., Oliveira, P. V., & Angnes, L. (2005). Potentiometric stripping analysis for simultaneous determination of copper and lead in lubricating oils after total digestion in focused microwave-assisted oven. *Microchimica Acta*, *149*, 199–204.
- Munoz, R. A. A., Oliveira, P. V., & Angnes, L. (2006). Combination of ultrasonic extraction and stripping analysis: an effective and reliable way for the determination of Cu and Pb in lubricating oils. *Talanta*, *68*, 850–856.
- Pedrotti, J. J., Angnes, L., & Gutz, I. G. R. (1996). Miniaturized reference electrodes with microporous polymer junctions. *Electroanalysis*, *8*, 673–676.
- Ravichandran, M. (2004). Interactions between mercury and dissolved organic matter – a review. *Chemosphere*, *55*, 319–331.
- Richter, E. M., Augelli, M. A., Kume, G. H., Mioshi, R. N., & Angnes, L. (2000). Gold electrodes from recordable CDs for mercury quantification by flow injection analysis. *Fresenius Journal of Analytical Chemistry*, *366*, 444–448.
- Sigel, A., & Sigel, H. (1994). *Metal ions in biological systems – mercury and its effects on environment and biology* (Vol. 34). New York: Marcel Dekker, Inc..
- Storelli, M. M., Storelli, A., Giacomini-Stuffler, R., & Marcotrigiano, G. O. (2005). Mercury speciation in the muscle of two commercially important fish, hake (*Merluccius merluccius*) and striped mullet (*Mullus barbatus*) from the Mediterranean sea: estimated weekly intake. *Food Chemistry*, *89*, 295–300.
- Ubillús, F., Alegría, A., Barberá, R., Farré, R., & Lagarda, M. J. (2000). Methylmercury and inorganic determination in fish by cold vapour generation atomic absorption spectrometry. *Food Chemistry*, *71*, 529–533.
- US EPA. <http://www.epa.gov/waterscience/fishadvice/factsheet.html#general>, Last access on July 2005 (a).
- US EPA, Method 7472. http://www.epa.gov/mercury/information.htm#tech_docs, (last access on July 2005 (b)).
- Voegborlo, R. B., El-Methrani, A. M., & Abedin, M. Z. (1999). Mercury, cadmium and lead content of canned tuna fish. *Food Chemistry*, *67*, 341–345.
- Wang, J. (1985). *Stripping analysis*. Deerfield Beach, FL: VCH.