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Analytical Methods

A new method for preconcentration and determination of mercury in fish, shellfish and saliva by cold vapour atomic absorption spectrometry

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ABSTRACT

The development of a method using solid phase extraction for preconcentration and determination of mercury by cold vapour atomic absorption spectrometry is described. Hg (II) ions are sorbed on a mini-column packed with Amberlite XAD-4 sorbent functionalised with 2-(2′-benzothiazolylazo)-p-cresol (BTAC). Then, a reducing solution was used for desorption and the transport of the analyte for subsequent detection. The assay presented a limit of detection of 0.011 μg L^{-1} (0.011 μg g^{-1}, for solid samples), a limit of quantification of 0.038 μg L^{-1} (0.038 μg g^{-1}, for solid samples), a precision of 0.50% (1.000 μg L^{-1} Hg solution) and an enrichment factor of 46. The proposed method was applied to the determination of mercury in human saliva (0.055–0.200 μg L^{-1}). The following seafood collected in Todos os Santos Bay, Brazil was also analysed: bass (0.169–0.195 μg g^{-1}), mullet (0.043–0.361 μg g^{-1}), shrimp (0.075–0.374 μg g^{-1}) and mussel (0.206–0.397 μg g^{-1}).

1. Introduction

Mercury and its various chemical forms are known as "species." The most important species of mercury are elemental mercury (Hg^0), inorganic (Hg^+ and Hg^{2+}) and organic mercury (methylmercury and ethylmercury). Mercury is most often present in the inorganic form Hg^{2+} throughout the Earth's crust. Dental amalgam is one of the most commonly used restorative materials for posterior teeth in dentistry. Despite widespread use, this amalgam contains mercury, the use of which has undergone some restrictions due to the toxic effects of this element (Rupp & Paffenba, 1971). Some researchers have reported an increase in mercury release from dental amalgams after dental restoration (Vimy, Takahashi, & Lorscheider, 1990). Human saliva is one of the most important fluids used as a biomarker of exposure to chemical pollutants (Wang, Du, & Zheng, 2008). Due to their toxicity and bioaccumulation, quantification of mercury levels in several matrices including human saliva is extremely important (Shao et al., 2013; Wang et al., 2008).

Fish and shellfish are often exposed to toxic substances such as mercury because the aquatic environment is highly susceptible to contamination by industrial discharge. In many regions, the seafood is the main source of income of the population (da Silva et al., 2008). However, contamination of some aquatic environments hinders the availability of marine species, thus affecting human health. Most of this contamination comes from industrial activities that rely on chemicals toxic to health, including mercury. Thus, biomonitoring of toxic elements such as mercury in matrices such as marine foods, natural waters and sediments is crucial.

Chronic exposure to mercury causes toxic effects even at trace concentrations because it can cause irreversible neurological damage. Due to these health risks, there is great interest in the development of sensitive and reliable analytical techniques to determine mercury content. Various detection techniques are currently available, such as cold vapour atomic absorption spectrometry (CV AAS), inductively coupled plasma optical emission spectrometry (ICP-OES) and electrothermal atomic absorption spectrometry (ET AAS) (Leopold, Foulkes, & Worsfold, 2010; Tuzen, Karaman, Citak, & Soylak, 2009). Despite instrumental advances, some techniques do not have adequate sensitivity and selectivity for the determination of this element in a large number of matrices. Thus, separation and enrichment of the analyte are usually required prior to analysis. Different materials and analytical strategies have been developed for this purpose (da Silva, Portugal, Serra, Ferreira, & Cerda, 2013; Leopold et al., 2010; Tuzen, Uluozlu, Karaman, & Soylak, 2009).

Solid phase extraction has been widely used in the separation and preconcentration of various species. There are many advantages to this technique, such as simplicity, reliability, reduction in analysis time, reduction or elimination of the use of organic solvents and a high potential for automation (Behbahani et al., 2013; Teixeira, Costa, Assis, Ferreira, & Korn, 2001). Some reagents may be associated with supports for solid phase extraction by two

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different methods: (1) by formation of a chemical bond between the reagent and the polymeric matrix (functionalised sorbents) (Lemos, Santos, & Nunes, 2005), and (2) by impregnation of the binder matrix, employing a solution containing the reagent (loaded sorbents) (Amorim & Bezerra, 2007; Soylak, Elci, Narin, & Dogan, 2001). The second method is simpler in practise. However, this functionalisation enables a long lifetime for the solid phase because of covalent bonds between the ligand and the support because the leaching of the ligand molecule associated with the solid support is sharply reduced (Camel, 2003).

Polyurethane foam (Abdel-Azeem, Bader, Kuss, & El-Shahat, 2013; Cassella et al., 1999), silica gel (Rudner, Pavon, Rojas, & de Torres, 1998), alumina (Ahmed, 2008) and polystyrene divinylbenzene (Amorim & Bezerra, 2007) are widely used as supports for the immobilisation of ligands. Chelating reagents are usually anchored to supports through —N=N—, —CH_2—, and —N=C— linkages (Persaud & Cantwell, 1992; Saxena, Singh, & Sambi, 1994). The anchored ligand can increase the sorption capacity of these materials, which favours the extraction of metal ions in solution and may also acquire specificity for a metal ion, reducing the time for sorption equilibrium (Camel, 2003).

This work aims to develop a sensitive, rapid and economical method for the determination of mercury in various matrices using detection by CV AAS and preconcentration employing Amberlite XAD-4 (polystyrene–divinylbenzene) resin functionalised with BTAC as a sorbent.

2. Experimental

2.1. Instrumentation

The absorbance measurements were performed using an atomic absorption spectrometer (Perkin Elmer model AA400, USA) equipped with hydride generator system. An electrodeless discharge lamp (EDL) was used as radiation source of Hg (253.65 nm). Electrothermal atomic absorption spectrometry was carried out using a HGA 900 (Perkin Elmer) model graphite furnace, coupled to spectrometer. The equipment was operated under the following temperature conditions: drying I: 100 °C (ramp 5 °C s^{-1}, hold 25 s); drying II: 140 °C (ramp 15 °C s^{-1}, hold 15 s); pyrolysis: 250 °C (ramp 10 °C s^{-1}, hold 20 s); atomizing: 1100 °C (ramp 0 °C s^{-1}, hold 5 s); cleaning: 2600 °C (ramp 1 °C s^{-1}, hold 5 s). Measurements of cold vapour atomic absorption spectrometry were carried out under the following conditions: slit 0.7 nm; argon flow rate 70 mL min^{-1}; carrier solution 3.0% (v/v) HCl; read time 20 s; peak height signal mode.

The pH of the solutions was monitored using a pH metre model Q400AS (Quimis, Brazil). The digestion of solid samples was performed using acid digestion bombs and microwave digestion bombs models 4749 and 4781 (Parr, USA), respectively. A peristaltic pump model 204 (Milan, Brazil) and silicone and PTFE tubes were also used in the preconcentration procedure.

2.2. Reagents

Ultrapure water obtained from a water purification system (Elga – Purelab Classic model, Bucks, UK) was used to prepare all solutions. Working solutions of mercury (μg L^{-1}) were prepared by diluting a 1000 μg mL^{-1} solution of the element (Merck, Germany) with ultrapure water. Amberlite XAD-4 (Sigma–Aldrich, Brazil) was used to prepare the functionalized sorbent (XAD-BTAC). The reagent BTAC. (Lemos & Ferreira, 2001) and the sorbent XAD-BTAC (Lemos et al., 2005) were prepared in the laboratory according to procedures described previously. A 2.0% (w/v) sodium borohydride (Sigma–Aldrich) in 0.2 mol L^{-1} NaOH solution was used in the hydride generator system. The pH of Hg solutions was adjusted with the aid of acetic acid/sodium acetate (pH 4.0–4.7), phosphate (pH 6.0), boric acid/sodium hydroxide (pH 7.5–8.5) and ammonium (pH 9.0–10.0) buffer solutions (Perrin, 1974). The certified reference materials BCR 482 Lichen, BCR 414 Plankton and ERM-CE278 Mussel tissue (Institute for Reference Materials and Measurements, Belgium) were used to study the accuracy of the method.

2.3. Sample preparation

2.3.1. Human saliva and certified reference material

Saliva samples (approximately 6.0 mL) were collected from healthy volunteers aged between 20 and 25 years. These samples were stored under refrigeration and immediately transported to the laboratory. An aliquot of 2.0 mL (or 0.100 g of certified reference material) sample was transferred to a Teflon cup of acid digestion bomb. Nitric acid (65% w/v, 4.0 mL) and hydrogen peroxide (30% v/v, 1.0 mL) were added. The digestion bomb flask was closed and placed in an oven at 75 °C for 6 h. After cooling to room temperature, the pH was adjusted with NaOH solution. The digest was transferred to a 100.0 mL volumetric flask containing 20.00 mL of the buffer solution, and then, the flask was filled to the mark with ultrapure water.

2.3.2. Seafood

The samples were collected in the municipality of São Francisco do Conde (specifically, the village of Muribeca). Muribeca is located in Todos os Santos Bay, Bahia, Brazil. The seafood used was: bass (*Centropomus undecimalis*), mullet (*Mugil brasiliensis*), shrimp (*Penaeus brasiliensis*) and mussel (*Mytella guyanensis*). Samples (0.1 g) were weighed and transferred to Teflon cups in order to microwave the acid digestion bombs. Next, 2.0 mL of 65% (w/v) nitric acid and 1.0 mL of ultrapure water were added. The acid digestion bomb was closed, and the system subjected to microwave radiation for 60 s at a power of 100 W. After cooling and adjusting the pH with an NaOH solution, the digest was transferred to a 100.0-mL flask containing 20.00 mL of buffer solution, Finally, the flask was filled to volume with ultrapure water.

2.4. General procedure for preconcentration

Solutions (100.00 mL) containing Hg (II) at pH 8.0 were passed through a minicolumn (0.30 cm internal diameter × 8.0 cm length) containing 0.20 g of XAD-BTAC at a flow rate of 5.50 mL min^{-1}. After passing the whole solution containing Hg (II) through the column, a 1.75 mol L^{-1} hydrochloric acid solution was used to desorb the metal from the minicolumn. The eluate (2.50 mL) was collected in a microtube, and the mercury content in the enriched phase was measured by CV AAS.

3. Results and discussion

3.1. Optimization of conditions for the preconcentration

A 1.0 μg L^{-1} Hg (II) solution was used in the optimization of variables. The extraction of the element by XAD-BTAC was calculated using the following equation: $E = (CV/C_oV_o) \times 100$, where C is the concentration of mercury in the eluate, V is the volume of the eluent, C_o is the initial concentration of mercury in the solution passed through the minicolumn, and V_o is the volume of the mercury solution that was passed through the minicolumn.