Microchemical Journal 114 (2014) 106–110

Contents lists available at ScienceDirect

Microchemical Journal

journal homepage: www.elsevier.com/locate/microc

Ionic imprinted polymer based solid phase extraction for cadmium and lead pre-concentration/determination in seafood

M.C. Barciela-Alonso, V. Plata-García, A. Rouco-López, A. Moreda-Piñeiro, P. Bermejo-Barrera *

Department of Analytical Chemistry, Nutrition and Bromatology, Faculty of Chemistry, University of Santiago de Compostela, 15782 Santiago de Compostela, Spain

ARTICLE INFO

Article history:
Received 28 October 2013
Received in revised form 2 December 2013
Accepted 9 December 2013
Available online 16 December 2013

Keyword:
Ionic imprinted polymer
Solid phase extraction
Fish samples
Cadmium
Lead
ETAAS

ABSTRACT

A method for trace Cd and Pb determination in fresh fish samples by Electrothermal Atomic Absorption Spectrometry (ETAAS) has been developed. A preconcentration step was necessary due to the low levels of these elements in fresh fish samples. Solid phase extraction (SPE) using ionic imprinted polymers (IIPs) as an adsorbent was selected as the preconcentration technique. The IIPs were prepared via precipitation polymerization using 2-(diethylamino) ethyl metacrilate (DEM) as a monomer and 8-hydroxyquinoline as a complexating agent. The 2,2′-azobisisobutyronitrile (AIBN) and acetonitrile/toluene (3:1) mixture were used as initiator and porogen, respectively. A microwave assisted acid digestion procedure using nitric acid and hydrogen peroxide was applied for sample preparation.

The experimental parameters for SPE extraction in column mode, such as pH of the digested sample, sample volume, load rate, and elution rate, have been optimized. The optimum pH for quantitative Cd and Pb retention was 8.5, and the elution was completed with 2 mL of 2.0 M nitric acid. Cadmium and lead concentrations in the acid extracts were determined by ETAAS using palladium and magnesium nitrates as a chemical modifier. The limits of detection (LODs) and limits of quantification (LOQs) obtained were 0.15 and 0.52 μg L^{-1} for Cd determination, and 0.50 and 1.68 μg L^{-1} respectively for Pb determination. Taking into account the mass of sample used and the preconcentration factor (12.5), the LODs obtained were 0.21 and 0.67 ng g^{-1} for Cd and Pb respectively. These limits are adequate to determine Cd and Pb at levels lower than the maximum levels allowed in fresh marine products, by the European Union legislation. The optimized method was applied for Cd and Pb determination in different seafood samples, such as squid, hake, sardine, horse mackerel, grouper and gilthead bream, and it was possible to detect both metals in all samples.

1. Introduction

Cadmium and lead are toxic elements found in fish samples due to their presence in the aquatic environment. Consumption of food with high concentrations of these elements can produce problems for human health, such as damage to the kidneys and central nervous system; different legislations establish therefore, maximum levels of these elements in food to avoid these problems. The European Union sets maximum levels of Cd and Pb in fresh fish of 0.05 and 0.3 mg kg^{-1} respectively [1].

Several analytical methods have been used for Cd and Pb determination in fish and mollusk. These methods include a sample preparation step usually performed by microwave assisted acid digestion, or the use of the solid sample using the slurry sample preparation. The determination can be performed by Flame Atomic Absorption Spectrometry (FAAS) [2–9], Electrothermal Atomic Absorption Spectrometry (ETAAS) [10,11], Inductively Coupled Optical Emission Spectrometry (ICP-OES) [12] or Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) [13–19]. In most of the reported methods, Cd and Pb were determined in lyophilized fish samples [2,5,13,20], which offer good sensitivity. However, low sensitivity was obtained when these methods were applied to determine these elements in fresh fish samples (Cd and Pb concentrations at ng g^{-1} levels) [21–23].

In order to improve the sensitivity, several authors include a preconcentration step prior to analyte determination. Therefore, Citak et al. [5] performed a coprecipitation step with zirconium(IV) hydroxide prior to Pb, Co, Cu, Cd, Fe and Ni determination by FAAS. Cloud point extraction was used by Mohamadi et al. [3], and also by Citak and Tuzen [24], for the determination of Cd and Pb in fish samples. Recently, Mendil [2] used solid phase extraction (SPE) on modified silica gels with thiourea for Cd, Cu and Pb determination in food samples by FAAS.

Ionic imprinted polymers (IIPs) have been used in recent years as adsorbent for solid phase extraction procedures. The most common applications are for ion preconcentration from environmental samples such as natural water or seawater. García-Otero et al. [25] used an IIP for on-line solid phase extraction of nickel and lead from seawater. Xubiao et al. [26] synthesized an IIP for the removal of Pb(II) ions from environmental water samples; whereas, Gawin et al. [27] prepared a Cd(II)-imprinted polymer for its application as a sorbent in SPE before

* Corresponding author. Tel.: +34 881814266; fax: +34 981 547 141.
E-mail address: pilar.bermejo@usc.es (P. Bermejo-Barrera).

0026-265X/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.microc.2013.12.008

determination of Cd(II) in groundwater and wastewater by FIA-FAAS. The use of IIPs in SPE offers several advantages: these materials can be synthesized using inexpensive reagents and present high capacity for recognizing ions and high selectivity [28].

To the best of our knowledge, there are no applications in the literature of IIPs as SPE sorbent for Cd and Pb preconcentration from digested fish samples. Therefore, the objective of this work is to develop a method using an ionic imprinted polymer as an adsorbent for SPE when isolating Cd and Pb from digested fresh fish samples. The IIP used in this work was synthesized in a previous work performed by our research group [28]. The method developed was applied for Cd and Pb determination in different fresh seafood products.

2. Experimental

2.1. Instrumentation

An Ethos Plus microwave digester (Milestone, Sorisole, Italy) equipped with a carousel provided with ten 100-mL high pressure Teflon vessels with cover, HTC adapter plate and HTC safety springs was used. The acid microwave digestion was performed operating at a maximum power of 1000 W. Measurements were performed using a PerkinElmer Model 1100B atomic absorption spectrometry equipped with an HGA-700 graphite furnace atomizer and an AS-70 autosampler (PerkinElmer, Waltham, Massachusetts, USA). A temperature-controllable incubation camera (Stuart Scientific, Surrey, UK) equipped with a low-profile roller (Stovall, Greensboro, NC, USA) was used for performing the polymerization process. A peristaltic pump (Gilson, Middleton, USA) and polyethylene tubings of 1.4 mm i.d. were used for SPE. IIPs were packed into 5 mL syringes (Brand, Wertheim, Germany) between replacement Teflon frits (Supelco, Bellefonte, PA, USA). Lab Blender Stomacher 400 (Seward Med. Ltd., London, UK) with Stomacher Closure bags 6041/CLR (Seward) was used for fish sample homogenization. An ORION 720A plus pH-meter with a glass-calomel electrode (ORION, Cambridge, UK) was used for pH measurements.

2.2. Reagents

Cadmium and lead standard solutions (1000 mg L^{-1}) as well as 8-hydroxyquinoline (8-HQ), used as non-vinylated monomer, were form Merck (Darmstadt, Germany). Vinylated monomer (2-(diethylamino) ethyl metacrilate, DEM) and divinylbenzene-80 (DVB), used as cross-liker, were from Sigma-Aldrich (Steinheim, Switzerland). The latter reagent was previously treated for removing the polymerization inhibitor by passing a few milliliters of the reagent through a mini-column containing around 0.5 g of neutral alumina (Sigma-Aldrich). 2,2′-Azobisisobutyronitrile (AIBN), used as initiator, was purchased from Fluka (Buchs, Switzerland). This reagent was purified by crystallization at −20 °C after dissolving the reagent in methanol (Merck) at 50–60 °C. After purification, it was stored at 4 °C. Nitric acid (69% (m/m)), ammonia 25% (v/v), ammonium chloride, hydrogen peroxide (33% (v/v)), and analytical grade nickel dichloride ($NiCl_2 \cdot 6H_2O$) were purchased from Panreac (Barcelona, Spain). HPLC grade acetonitrile and toluene were obtained from Scharlab (Barcelona, Spain). Palladium nitrate 10 g L^{-1} in HNO_3 (15%) (Merck) and magnesium nitrate (Merck) were used as chemical modifier for Cd and Pb determination by ETAAS. Ultrapure water of resistivity 18 MΩ cm was obtained from a Milli-Q purification device (Millipore Co.).

All glassware and plasticware were rigorously cleaned and kept into 10% (v/v) nitric acid for at least 48 h. The material was then rinsed three times with ultrapure water before used.

2.3. Seafood samples

Seafood samples (gilthead bream, *Sparus aurata*; horse mackerel, *Trachurus trachurus*; sardine, *Sardina pilchardus*; grouper, *Epinephelus marginatus*; squid, *Loligo vulgaris*; and hake, *Merluccius merluccius*) were obtained from a local supermarket. Composite samples by pooling together the soft tissues or flesh of all specimens were prepared by mechanical blending after removing the bones and entrails. The homogenized fish samples were stored at 4 °C until analysis.

2.4. Microwave acid digestion

Portions of 3.0000 g of homogenized fresh fish samples were directly weighed into digestion vessels and mixed with 2 mL of ultrapure water, 4 mL of 69% (m/m) HNO_3 and 2 mL of 33% (m/m) H_2O_2. Vessels were then placed into the microwave oven, where one sample vessel was temperature and pressure monitored during the operation. The temperature program used lasted 23.5 min and consisted of four steps: (1) The temperature was increased from room temperature (24 °C) to 90 °C in 2.5 min; (2) a temperature ramp of 8.3 °C min^{-1} was applied up to 140 °C (6.0 min); (3) the temperature was increased from 140 °C to 200 °C in 5 min; and (4) the temperature was maintained at 200 °C during 10 min. After a cooling down stage (venting time of 40 min), each acid digest was recovered, the digestion vessel was rinsed with small volumes of ultrapure water, and the washing water was combined with the acid digest before adjusting to 25 mL with ultrapure water.

Several blanks were prepared in the same way as the sample but without the introduction of the sample. The samples analyzed in this work were digested in triplicate.

2.5. Synthesis of ionic imprinted polymer

The ionic imprinted polymer was synthesized following the method proposed by Otero-Romaní et al. [28]. Therefore, 0.0668 g of $NiCl_2 \cdot 6H_2O$, 226 μL of DEM and 0.653 g of 8-HQ were mixed in 25 mL of porogen (3:1 acetonitrile:toluene). This solution was stirred for 5 min and then filtered. Finally, 1 mL of DVB (cross-linker) and 82.6 mg of AIBN (initiator) were added, the glass tubes purged with nitrogen and immediately sealed just before thermal induction of precipitation polymerization. The temperature was ramped from room temperature to 60 °C over 2 h, and then maintained at 60 °C for a further 24 h under a low stirring rate (33 rpm). The polymer obtained was then vacuum-filtered, washed with acetonitrile, and oven-dried overnight at 40 °C. The masses of polymer obtained after polymerization were around 0.624 g and the efficiency of the polymerization process was 62%. The same procedure, except the presence of $NiCl_2 \cdot 6H_2O$, was applied for preparing non-imprinted polymers (NIPs).

2.6. Preparation of IIP cartridges

5 mL syringes were filled with 0.20 g of IIP or NIP absorbents. The IIP or NIP material was between two Teflon frits. The IIP syringes were then connected to a peristaltic pump via polyethylene tubings, and the template [nickel(II) ions] was removed from the polymer particles by extensive washing with 2.0 M nitric acid (5.0 mL aliquots). An efficient removal of the template from IIP particles was achieved after passing 50 mL of washing solution. This was verified by ETAAS measurements of nickel in the elution solutions from the cartridges. The NIP was subjected to the same washing pretreatment described above.

2.7. IIP-solid phase extraction procedure

For the SPE procedure, the IIP was first conditioned by passing 25 mL of buffer solution (NH_3/NH_4Cl) at pH 8.5 ± 0.5. Afterwards, 25 mL of digested fish sample adjusted at pH 8.5 was passed through the syringe at a flow rate of 2.35 mL min^{-1}. The syringe was then rinsed with 25 mL of Milli-Q® water and the retained ions (Pb and Cd) were subsequently eluted with 2 mL of 2 M nitric acid. The whole process was performed using a flow rate of 2.35 mL min^{-1}. Blanks of the procedure