IRIS AperTO

UNIVERSITÀ DEGLI STUDI DI TORINO

AperTO - Archivio Istituzionale Open Access dell'Università di Torino

New pathways in organotin speciation: LC-ICP/MS by using a novel imprinted stationary phase

This is the author's manuscript

Original Citation:

Availability:

This version is available http://hdl.handle.net/2318/74786 since

Published version:

DOI:10.1016/j.chroma.2010.03.007

Terms of use:

Open Access

Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

(Article begins on next page)

25 April 2024

UNIVERSITÀ DEGLI STUDI DI TORINO

This Accepted Author Manuscript (AAM) is copyrighted and published by Elsevier. It is posted here by agreement between Elsevier and the University of Turin. Changes resulting from the publishing process - such as editing, corrections, structural formatting, and other quality control mechanisms - may not be reflected in this version of the text. The definitive version of the text was subsequently published in M.Gallego-Gallegos, M.L.Garrido, R.Muñoz Olivas, P.Baravalle, C.Baggiani, C.Cámara: "New pathways in organotin speciation: LC-ICP/MS by using a novel imprinted stationary phase" J.Chromatogr.A, 2010, 1217, 3400-7 (DOI 10.1016/j.chroma.2010.03.007).

You may download, copy and otherwise use the AAM for non-commercial purposes provided that your license is limited by the following restrictions:

(1) You may use this AAM for non-commercial purposes only under the terms of the CC-BY-NC-ND license.

(2) The integrity of the work and identification of the author, copyright owner, and publisher must be preserved in any copy.

(3) You must attribute this AAM in the following format: Creative Commons BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/deed.en), [***DOI 10.1016/j.chroma.2010.03.007***]

A new application of imprinted polymers: Speciation of organotin compounds

Mercedes Gallego-Gallegos [a], María Liva Garrido [b], Riansares Muñoz Olivas [a,*], Patricia Baravalle [c], Claudio Baggiani [c], Carmen Cámara [a]

[a] Departamento de Química Analítica, Facultad de Ciencias Químicas, Universidad Complutense de Madrid, Avda. Complutense S/N, 28040 Madrid, Spain
[b] Departamento de Química Analítica, IMRE, Universidad de La Habana, Habana, Cuba
[c] Dipartimento di Chimica Analitica, Università di Torino, Via Giuria 7, 10125 Torino, Italy

ABSTRACT

Molecular imprinting technology has been employed for the first time to prepare a specifically affinity chromatographic stationary phase for speciation purposes. Tributyltin has been chosen as the template molecule and the non-covalent approach has been applied. Three different polymerization methods have been evaluated: (i) a composite material, (ii) a polymer prepared via-Iniferter grafting; (iii) an emulsion polymer. Columns packed with different polymers have been evaluated by liquid chromatography (LC) coupled to inductively coupled plasma mass spectrometry (ICP-MS). The chromatographic conditions as well as the analytical characteristics of the developed method are discussed in this paper. Our findings have shown formation of specific cavities in the grafted Iniferter as well as in the emulsion polymers with the latter achieving resolution of four organotin compounds. Detection limits are similar to those obtained with commercial, but not specific, stationary phases (6 pg for monobutyltin, MBT; 10 pg for both tributyltin, TBT, and triphenyltin, TPhT; and 20 pg for dibutyltin, DBT). The main advantage of this proposed stationary phase is that good recovery is obtained for all species, including MBT. Baseline resolution for TBT and TPhT has also been obtained. The high selectivity of this column prevents matrix interferences. The method has been validated by analyzing two biota reference materials (ERM-CE477 mussel tissue and T-38 oyster tissue).

1. Introduction

The toxicity, bioavailability and mobility of a compound in the environment are critically dependent on the particular form in which this compound occurs. Therefore, the study of metal species and organometallic compounds is an important topic of analytical research. Speciation techniques involve a number of discrete analytical steps comprising extraction of the analytes from a solid sample, preconcentration to gain sensitivity, modification of the analytes through derivatization, separation by chromatography, and the eventual specific detection. Quantification is still a formidable task, mainly because of the high complexity of environmental matrices which contain very low concentrations of organometallic compounds. New methodologies must be developed to reduce sample manipulation and to improve detection limits.

The high toxicity of organotin compounds (OTCs), even at low concentration levels, demands development of accurate and sensitive analytical methods for their determination in environmental matrices. In particular, tributyltin (TBT) has been produced for many years with an important range of applications such as thermal and UV stabilizers for polylvinyl chloride (PVC), fungicides, pesticides, and general biocides [1]. One of the most reported uses of TBT is as an effective antifouling paint employed to prevent the settlement and growth of marine organisms on submerged structures [2,3]. For many years, the main use of triphenyltin (TPhT) has been [4,7] as an agricultural pesticide. Both TBT and TPhT are well known to be strong endocrine disrupters. Monobutyltin (MBT) and dibutyltin (DBT) have been employed as stabilizers and catalysts for silicons and other industrial processes [1]. Many of these uses, especially those related to the aquatic environment, have been internationally regulated since 1990 due to their severe impact on aquatic ecosystems. The International Marine Organization (IMO) has strictly limited the use of TBT as an antifouling agent [5]. Recently, TBT and TPhT have been included in the European list of priority pollutants establishing a maximal amount of 10 ng L^{-1} as sum of all OTCs in natural waters [6]. Despite these efforts, harmful concentrations of these compounds and their metabolites can still be detected in waters, suspended matters, sediments, and sewage sludge. The high accumulation capacity of these compounds by molluscs is well known [8,9]. Therefore still it is very important to develop simple, sensitive, selective, and rapid methods for the quantitative determination of organotin compounds in the environment.

Table 1
Analytical methods reported in the literature for organotin compounds determination based on coupling liquid chromatography to different detectors.

Species	Sample	Column	Detector	Detection limit (ng Sn)	Reference
DBT, TBT	Wood	Partisil SCX-10	HG-GFAAS	0.5	[27]
DBT, TBT	Sediments	Nucleosil	GFAAS	5	[28]
TMT, TET, TPrT, TBT, TPhT	Estuarian waters	Partisil SCX-10	UV	100–800	[29]
Sn(IV), TBT, TPhT	Water	Adsorbosphere SCX	ICP-AES	450–1500	[29]
DBT, TBT	Sediments	Partisil SCX-10	ICP-MS	0.02–0.04	[29]
DBT, TBT, DPhT, TPhT	Sediments	Kromasil 100 C18	APCI-MS	2.5–5	[30]
DBT, TBT, DPhT, TPhT	Sediments	TSK gel ODS-80TM	ICP-MS	0.2–0.5	[26]
MBT, DBT, TBT	Sediments	Hypersil ODS	ICP-MS	0.002	[31]
DBT, TBT, DPhT, TPhT	Sediments	Zorbax SB C18	APCI-MS	0.02–0.07	[32]
DBT, TBT, DPhT, TPhT	Seawater Oyster	Kromasil 100 C18	FLD	0.02–0.5	[20,33]
DBT, TBT	Seawater	Cianopropil	APCI-MS	35, 25	[34]
MBT, DBT, TBT, TPhT	Mussel, Oyster	MIP	ICP-MS	0.006–0.02	This work

MBT: Monobutyltin; DBT: dibutyltin; DPhT: diphenyltin; TBT: tributyltin; TET: triethyltin; TMT: trimethyltin; TPhT: triphenyltin; TPrT: tripropyltin; APCI: atmospheric pressure chemical ionization GFAAS: graphite furnace atomic absorption spectrometry; FLD: molecular fluorescence detection; HG: hydride generation; ICP-AES: inductively coupled plasma emission spectrometry; ICP-MS: inductively coupled plasma mass spectrometry; UV: ultraviolet detection.

Speciation of these compounds is usually achieved by gas chromatography (GC), due to its excellent sensitivity and resolution [10–16]. However, the poor volatility of OTCs means a previous derivatization step is required. On the contrary, liquid chromatography (LC) does not require such step. This leads to a simpler and faster analysis, but implementation of LC is limited due to its lower resolution and lack of sensitivity [17,18]. Detection methods such as ultraviolet, atomic absorption spectroscopy and inductively coupled plasma optical emission spectrometry are not sensitive enough [19]. Fluorescence detectors satisfy sensitivity and selectivity requirements but a derivatization step is needed as OTCs are not fluorescent compounds [20]. The most suitable detector for coupling to LC is ICP-MS due to its high sensitivity, wide linear dynamic range and ability to perform isotopic analysis [21].

Several LC modes including ion-exchange silica-based columns, ion pairing chromatography and reversed phase with C8 and C18 stationary phases have been previously employed [22–26]. Detection limits for those methods are usually within the $\mu g L^{-1}$ range independent of the separation mode applied, and typical precision values are about 5% in terms of relative standard deviation. These detection limits are not usually low enough to meet environmental legislation requirements. The most prominent drawbacks in terms of compound separation are the strong interactions between mono-substituted tin species and the stationary phase due to its high residual charge as well as the poor resolution of TPhT and TBT because the retention mechanism of those compounds is also based on the electrostatic interactions with the stationary phase. Table 1 summarizes some of the main LC methods developed for OTCs [27–34].

To our knowledge, MIP technology has not previously been applied to prepare an affinity stationary phase for speciation of organometallic compounds by LC-ICPMS. A stationary phase based on molecularly imprinted technology has been designed and tested by our group. Molecularly imprinted polymers are defined as polymers formed in the presence of the molecule of interest, a so-called template molecule. The template is later extracted, leaving behind complementary nanocavities in the polymer. These polymers show a chemical affinity for the original template molecule in a manner very similar to that of natural receptors like antibodies or enzymes. This methodology, first proposed by Wulff et al. in 1972, has shown to be suitable for many analytical applications such as catalysis, solid-phase extraction, micro-extraction, and for use in sensors [35–39]. Chromatographic stationary phases with predetermined and specific molecular recognition properties can be prepared using the molecular imprinting technique [40–44]. The polymers synthesized in our group are based on the non-covalent approach [45,46]. Polymer beads formed by three different methods were synthesized: emulsion polymers [47,48], surface-grafted initiators [49] on divinylbenzene particles and a DVB-composite material [50–52]. These three synthesis methods were also performed without addition of the template to prepare equivalent non-imprinted polymers (NIPs). All resulting polymers have been used as the stationary phase in LC, and their applicability for OTC speciation in mussel tissue and oyster tissue by LC-ICPMS.

2. Experimental

2.1. Reagents and standards

Analytical grade chemicals have been used for all studies. Tributyltin chloride (>97%), dibutyltin chloride (>97%), monobutyltin chloride (>95%) and triphenyltin chloride (>95%) have been purchased from Sigma–Aldrich Quimica S.A. (Madrid, Spain). Stock solutions of organotin in methanol ($10 mg L^{-1}$) of tin have been prepared and stored at $-4\,°C$ in the dark. Working solutions have been daily made from the stock solutions in water or methanol. Polymerization reagents, methacrylic acid (MAA), ethylene glycol dimethacrylate (EGDMA), and azobisisobutyronitrile (AIBN), have been obtained from Sigma–Aldrich Quimica S.A. (Madrid, Spain). Chloromethylstyrene-*co*-divinylbenzene beads and divinylbenzene beads have been prepared in the laboratory according to literature [53]. Iniferter modifier has been Sodium *N*,*N*-diethyldithiocarbamate trihydrate (NaDEDTC), Polyvinyl alcohol (PVA), ammonium fluoride and silica particles (Pharmaprep 60, 40–63 μm mean diameter) have been provided by Merck (Darmstadt, Germany). A Milli-Q water system (Millipore Iberica S.A. Madrid, Spain) was used for purified water. Analytical grade organic solvents have been purchased from SDS (Barcelona, Spain). Hydrochloric and acetic acid have been supplied by Merck (Darmstad, Germany), and triethylamine (TEA) by Sigma–Aldrich.

2.1.1. Reference materials

Mussel tissue (ERM-CE477) was purchased from the Institute for Reference Materials and Measurements (IRMM, Geel, Belgium). Information about its certification as well as data concerning homogeneity, stability and statistical tests were already published [54]. Oyster tissue (T-38) was a candidate of CRM that was tested by IRMM but finally was not commercialized due to the high dispersion obtained by the different laboratories participating in the inter-comparison exercise. However, the proposed contents can be used as indicative values.

2.2. Instrumentation

A Thermo X Series 300 (Thermo Electron Corporation, USA) ICP-MS has been employed for Sn determination. It is equipped with a

microconical nebulizer, fassel torch, double pass Scott-type spray chamber cooled by a Peltier system (−8 °C) and platinum cones. Ion monitoring at *m*/*z* 120 has been used for Sn data collection and ^{115}In selected as the void marker. An additional flow of oxygen (0.42 L min^{-1}) mixed with argon and introduced via nebulization was necessary to burn and oxidize the organic matter more efficiently.

The chromatographic system used has been a model PU-2080 Plus pump, (JASCO Corporation, Tokyo, Japan) with a stainless steel column (4.6 mm × 50 mm). Column effluent went directly into the nebulizer via a PTFE capillary tube (0.5 mm i.d.). The samples have been injected through a six-port valve (Rheodyne 9125, USA) in a 0.2 mL loop.

An ultrasonic homogenizer, model Sonoplus HD 2200 (Bandelin, Germany), equipped with a converter UW 2200, SH 213 G horn as amplifier and sonotrode MS 73 (3 mm titanium microtip) has been used for tin species extraction. The obtained extracts have been centrifuged on an Eppendorf centrifuge model 5804 (Hamburg, Germany).

The scanning electron micrographs have been recorded using an SEM Hitachi S4500 and nitrogen adsorption isotherms, for morphological studies, have been obtained using an ASAP System 2020, Micromeritics, (Norcross, GA).

2.3. Polymers preparation

All the imprinted polymers have been synthesized by employing the same template molecule (TBT), functional monomer (MMA), and crosslinker (EGDMA), but different porogens: acetonitrile for the composite material and Iniferter grafted polymers, and chloroform for the emulsion polymerization due to the necessity to employ a non-water miscible solvent.

2.3.1. Composite polymer (MIP-c)

The polymerization mixture formed by the template (TBT, 40.6 mg), functional monomer (MAA, 42 μL), crosslinker (EDGMA, 468 μL) and the initiator (AIBN, 10 mg) has been dissolved in acetonitrile (500 μL), then mixed with enough amount of porous silica beads to allow the polymerization mixture to penetrate the beads. After purging with nitrogen, the mixture has been left overnight at 60 °C to allow completion of thermal polymerisation inside the pores. The resulting beads have been suspended for 24 h in aqueous ammonium fluoride (3 mol L^{-1}) The beads have been filtered using a polypropylene filtering device (nominal porosity 0.2 μm), washed with acetone (10 mL) and dried under vacuum. Non-imprinted control polymers (NIP-c) have been prepared in the same way without addition of the template.

2.3.2. Grafted polymer via Iniferter (MIP-i)

Chloromethylstyrene–divinylbenzene beads have been modified with Iniferter groups according to literature methods [55,56] with minor modifications. Briefly, in a 100 mL round-bottomed flask, the beads (5 g) have been dispersed in ethanol (25 mL) under sonication. A solution of NaDEDTC (1.5 g) in ethanol (15 mL) has been added to the reaction vessel and the mixture stirred overnight at 40 °C. The beads have been separated from the reaction mixture by filtration, washed three times with tetrahydrofurane (THF) and acetone, and then dried under vacuum. All these operations have been performed in the dark as Iniferter modifiers are UV-light activated. Once the Iniferter modified support is clean and dry, beads (500 mg) have been suspended into the polymerisation mixture: template (TBT, 81.28 mg), monomer (MAA, 84.5 μL), crosslinker (EGDMA, 936 μL) and porogen (acetonitrile, 30 mL). The mixture has been sonicated for a few minutes, then purged with nitrogen, and the flask has been sealed and left overnight to allow photopolymerization by UV-irradiation (200 W medium-pressure Hg vapour lamp). The round-bottom flask containing the polymerization mixture has been introduced into a thermostat water bath at 15 °C. The grafted beads have been filtered and washed with acetonitrile. *NIP-i* has been prepared in the same way without addition of the template.

2.3.3. Emulsion polymers (MIP-e)

The template molecule (TBT, 163 mg) and functional monomer (MAA, 169 μL) have been dissolved in the porogen (chloroform, 8 mL). The crosslinker (EGDMA, 1900 μL) and the initiator (AIBN, 100 mg) have been added to this mixture and sonicated until the solution was transparent. Polyvinyl alcohol (PVA, 6 g) has been dissolved in hot water (150 mL) in a 250 mL flanged reactor flask fitted with magnetic stirring, and nitrogen inlet. After cooling to room temperature, the organic mixture has been added drop by drop across the septum with a syringe and left stirring overnight at 65 °C. The formed beads have been washed with hot water to remove PVA from their surfaces and then dried. *NIP-e* was prepared in the same way without addition of the template.

2.4. LC columns packing

A few milligrams of each of the different polymers has been suspended in an aqueous–methanol (2:8, v/v) solution and the resulting slurry packed into an empty stainless-steel column (50 mm × 4.6 mm i.d.). Packing has been performed by gradually filling the column with the polymer slurry and connecting the HPLC pump, operating at a high pressure by passing methanol through the column at a flow rate of 2.0 mL min^{-1} for 5 min. This procedure has been repeated several times until column was packed. The template has been washed from the stationary imprinted phase by passing of acetic acid in methanol (1:9, v/v) until a stable baseline was observed by ICPMS.

2.5. Polymers characterization

2.5.1. Scanning electron microscopy (SEM)

Micrographs have been recorded using an SEM Hitachi S4500 by placing a few milligrams of dried polymer into a metallic mould covered by a graphite adhesive tape. The mould has been coated with a thin layer of gold in order to make them conductors as the SEM technique irradiates with a beam of electrons that allow conduction of the high energy required to create magnified three dimensional images.

2.5.2. Nitrogen sorption measurements

Pore size distribution and surface areas have been assessed by Brunauer–Emmett–Teller (BET) analysis. The dried polymer (100 mg) has been used for nitrogen adsorption/desorption isotherms at 77 K. Before the experiment, the polymers need to be degassed at 313 K.

2.6. Chromatographic evaluation of the polymers

Optimization of the best mobile phase is described in the results and discussion section of this paper. The mobile phase flow rate has been limited to 0.5 mL min^{-1} because higher values provoke plasma instability and a noisy baseline due to the content of the organic solvents. The capacity factors of the column have been calculated as for any LC chromatographic column [57]. The imprinting factors (IFs) of the different stationary phases have been calculated from the ratio of the capacity factors obtained for the MIP and NIP.

Table 2
Results of BET analysis for the three polymers studied. *MIP-c*: DVB composite polymerization; *MIP-i*: grafted via Ineferter polymerization; *MIP-e*: emulsion polymerization.

Polymer	μpore volume (cm³/g)	μpore area (m²/g)	External surface area (m²/g)	Pore size diameter (nm)
MIP-c	0.1856	328	18	<5
MIP-i	0.0026	5.3	2.1	20–40
MIP-e	0.0003	0.7	0.2	20–50

2.7. OTC extraction from biota samples

The optimized procedure for OTC extraction from biota samples has been previously reported [46]. Briefly, 0.1 g of the two biota materials has been exactly weighed in an amber-glass vial (20 mL) and the extraction media (MeOH:HAc, 1:1, 5 mL) added. An ultrasonic probe has been used to sonicate the solution for 30 s at 20–25% amplitude. The resulting solution has been centrifuged at 4500 rpm for 2 min and the supernatant kept for analysis. Solvent has been removed from 2 mL and the remaining solid re-dissolved in Milli-Q water (4 mL) before injection into the LC system. This assured effective retention of the OTCs by the stationary phase.

3. Results and discussion

3.1. Scanning electron microscopy analysis

The micrographs recorded and summarized in Fig. 1 have shown formation of spherical particles for all three polymerization strategies. The diameters of the MIP beads are in the micron range regardless of the polymerization method employed. This makes them optimum for chromatographic purposes. *MIP-e* (Fig. 1A) consists of particles of size 1–25 μm whereas *MIP-i* and *MIP-c* (Fig. 1B and C) have bigger (30–60 μm) but more homogeneous particles. The broad size range found with the emulsion procedure has been attributed to some irregularities of shaking which is necessary to produce the microdrops in the water–oil emulsion. The higher particle diameter of *MIP-c* could be due to a possible polymerization out of the commercial silica beads. The micrograph of *MIP-i* has revealed a non-homogeneous deposition of the Iniferter over DVB beads generating so-called "cauliflower beads". Finally, no morphological differences have been observed between the imprinted and non-imprinted polymers of a given synthesis method.

3.2. Nitrogen sorption isotherms

The general morphology of MIPs arises from nuclei that form around the initiator and then aggregate into larger clusters forming spherical beads. The porosity and resulting surface area is formed; from irregular voids located between clusters of the microspheres (macropores, >50 nm); from the interstitial space of a given cluster of microspheres (mesopores, 2–50 nm); or even within the microspheres themselves (micropores, <2 nm). Typical values for surface area are 100–400 $m^2\,g^{-1}$. Concerning pore size distribution 2–100 nm for both macro and mesopores and 0.6–2 nm for micropores [58]. Table 2 summarizes the micropore volume ($cm^3\,g^{-1}$), micropore area ($m^2\,g^{-1}$), external surface area ($m^2\,g^{-1}$) and average pore size diameter (nm) of the three polymers studied. It should be noted that although binding and selectivity are not dependent on those values, porosity may rely on the mass transfer kinetics of compounds through the polymeric matrix [59].

3.3. Chromatographic evaluation

For an imprinted polymer used as chromatographic stationary phase, selectivity and efficiency are major concerns. These characteristics are notably determined by the nature and number of interactions between the imprinted polymeric matrix and the ana-

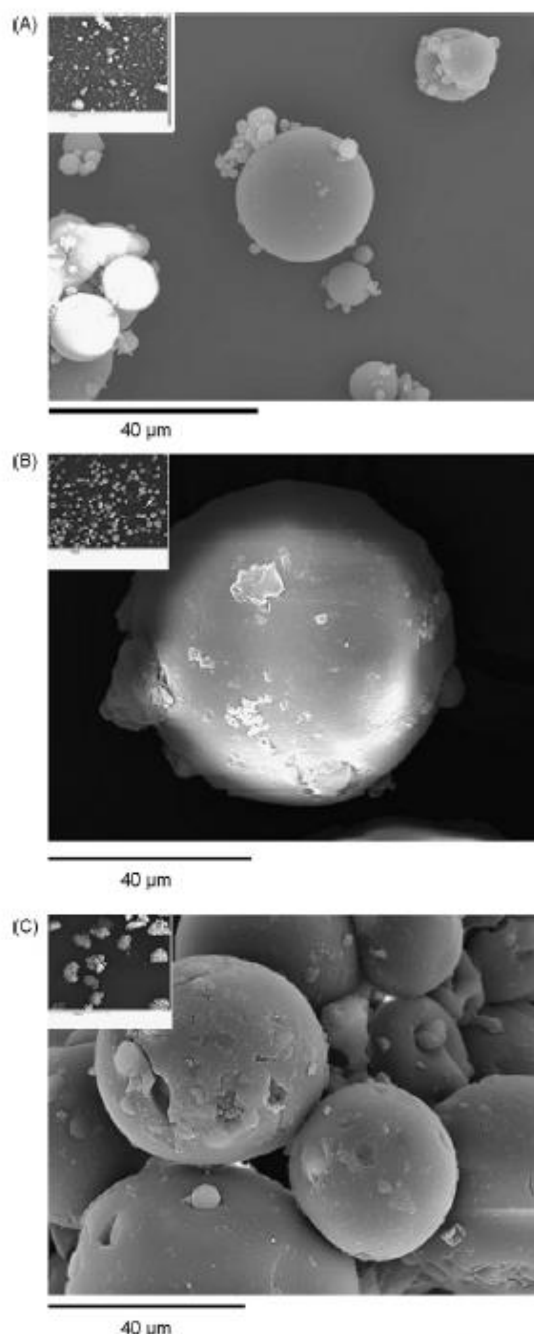

Fig. 1. Scanning electron microscopy for the three synthetized polymers: (A) *MIP-e*, (B) *MIP-i*, (C) *MIP-c*.

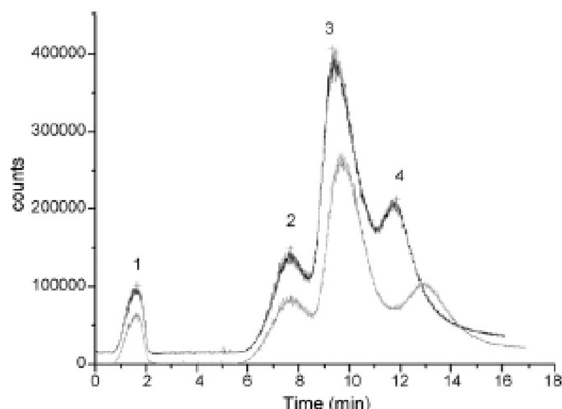

Fig. 2. Chromatograms obtained with *MIP-c* (black line) and *NIP-c* (grey line) as stationary phases. Monitored isotope ^{120}Sn; loading 20 ng of each compound in water; injection loop: 0.2 mL; mobile phase flow: 0.5 mL min^{-1}. Mobile phase gradient applied: (1) 0–1 min 50% Milli-Q water, 50% MeOH; (2) 1–3 min move to 100% MeOH; (3) 3–5 min 100% MeOH; (4) 5–9 min move to HAc 10% in MeOH; (5) 9–20 min HAc 10% in MeOH. Peaks: 1. Column void volume (t_0) with coelution of all OTCs; 2. TBT; 3. TPhT + DBT; 4. MBT

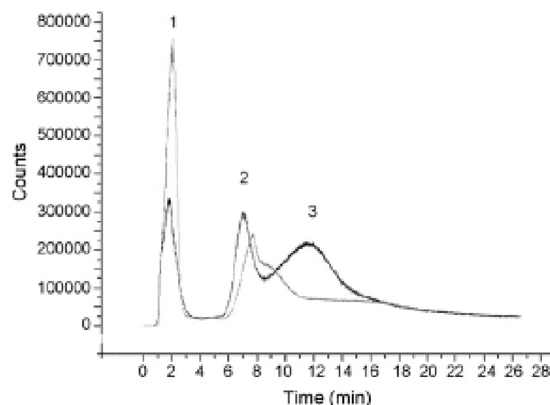

Fig. 3. Chromatograms obtained with *MIP-t* (black line) and *NIP-t* (grey line) as stationary phases. Monitored isotope ^{120}Sn; loading 20 ng of each compound in water; injection loop: 0.2 mL; mobile phase flow: 0.5 mL min^{-1}. Mobile phase gradient applied: (1) 0–2 min 100% MeOH; (2) 2–6 min: move to HAc 10% + 0.5% TEA in MeOH; (3) 6–30 min HAc 10% + 0.5% TEA in MeOH. Peaks: 1. Non-specific bonding of all OTCs; 2. TBT; 3. TPhT + DBT

lyte. The situation with molecular imprints is rather complex as the analyte molecule can interact with the stationary phase in three modes: (a) partitioned in the stagnant phase situated in the inner pores of the particles; (b) non-specifically bound to functional groups of the polymer; (c) specifically bound into the imprinted cavities [58]. Optimization of the mobile phase plays an important role in order to achieve analytes separation taking the inherent advantages of this kind of stationary phase. Six columns have been packed as explained in Section 2 (three with MIPs and three with NIPs). Optimization of the mobile phase has been performed in order to check the imprinting effect of the synthesized materials as well as to achieve satisfactory separation of the organotin compounds. An OTC standard mixture has been directly loaded in water because our previous works have demonstrated quantitative loading in this medium which is very unusual for imprinting technology [60,61]. In all cases, OTC retention has found to be greatly affected by the water content in the mobile phase: the higher the water content the stronger the OTC retention. Selective elution of the compounds required has been observed working on a gradient mode starting with mild acidic conditions and gradually increasing to the complete elution of the most retained compound.

3.3.1. *Results obtained from the different polymers*

3.3.1.1. MIP-c. When pure methanol has been used as the mobile phase the OTCs eluted quickly without separation. This behaviour has previously been explained by Kohri et al. [62] who developed an SPE method to determine OTCs in fish tissue using a commercial resin of DVB/MAA. A gradient elution of acetic acid (up to 10%) in water was required to selectively and completely elute the compounds. The procedures have been followed using *NIP-c*, and very similar chromatograms have been obtained (Fig. 2). This suggests that there is no imprinting effect or at least not enough for our chromatographic purposes. This lack of selectivity has led us to rationalise that the synthesized material presented many non-specific interactions with the analytes and consequently is not convenient for our purposes.

3.3.1.2. MIP-t. Several solvents have been tested as mobile phases, including water and acetonitrile with addition of different acids: acetic (up to 10%) and hydrochloric (up to 0.1% due to the strict limitations of our LC system). An excessively high OTC retention has been found for low polar solvents. In such cases, a modifier is required to reduce the non-specific binding and thus improve separation [41]. Our acidic stationary phase has required an amine modifier to compete with the analytes for binding sites. Triethylamine (TEA) has been used with maximum resolution at 0.5–1% TEA. Results and chromatographic conditions have been showed in Fig. 3. The first peak shows weak retention (t_R = 1.78 min) and corresponds to MBT with a small contribution from the other OTCs. For the non-imprinted polymer, this is the major peak. The differences in results for *NIP-t* and *MIP-t* show clear IFs. However, the selectivity with this chromatographic phase is not enough for our separation purposes.

3.3.1.3. MIP-e. Methanol has been found to be the best solvent for *MIP-e* due to its high polarity. The separation achieved by applying a gradient with increasing concentration of acetic acid in methanol is shown in Fig. 4. Imprinting factors and chromatographic parameters have been calculated for both the *MIP-e* and *NIP-e* in Table 3. The highest IFs have been obtained for TPhT and TBT, while those

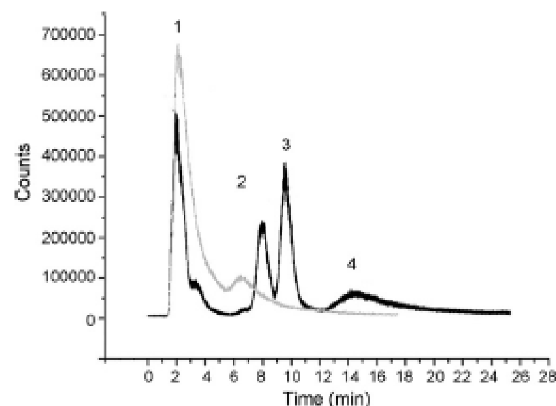

Fig. 4. Chromatograms obtained with *MIP-e* (black line) and *NIP-e* (grey line) as stationary phases. Monitored isotope ^{120}Sn; loading 20 ng of each compound in water; injection loop: 0.2 mL; mobile phase flow: 0.5 mL min^{-1}. Mobile phase gradient applied: (1) 0–2 min 100% MeOH; (2) 2–6 min move to HAc 10% in MeOH; (3) 6–30 min HAc 10% in MeOH. Peaks: 1. MBT; 2. TBT; 3. TPhT; 4. DBT

Table 3
Chromatographic parameters for the retention of the studied organotin compounds by MIP-e. t_R, retention time; k', capacity factor; IF, imprinted factor; α, selectivity; Rs, resolution; s, simmetry factor.

Compound	t_R MIP (min)	t_R NIP (min)	k'MIP	k'NIP	IF	α	Rs	s
MBT	3.26	1.31	1.53	0.1	15.3			1.000
TBT	7.38	1.31	4.72	0.1	47.2	3.08	1.16	0.933
TPhT	12.29	1.31	8.53	0.1	85.3	1.81	1.23	0.929
DBT	19.15	5.79	13.84	3.49	3.96	1.62	1.36	1.000

for MBT are a factor of 10 lower and even less for DBT. The low value obtained for DBT is due to its high retention by the *NIP-e*. Resolution obtained between peaks is $\geq$1 in all cases, and baseline resolution is obtained for all of the four compounds. MBT is the least retained OTC due to its high ionic character and high solubility in methanol which result in attractive electrostatic interactions with the mobile phase and weak interactions with the stationary phase. The peaks at 7.4 and 12.3 min correspond to TBT and TPhT respectively. Both are well retained in the *MIP-e* but not in the *NIP-e* suggesting strong imprinting effects. The close elution of both compounds suggests that both electrostatic and cavity size effects are the main reasons for specificity of the *MIP-e*. This agrees with the observations of a previously reported MISPE device [45]. The slightly higher affinity of TBT for the mobile phase could explain why it elutes before TPhT. Finally, DBT has shown the longest retention time but it is also the only compound retained by the non-imprinted polymer. This behaviour has been previously reported and it has been suggested that retention is due to a strong ionic mechanism. Comparison of the IFs for DBT and TBT, 3.96 and 47.2 respectively, indicate a better selectivity of the MIP for TBT. Thus, the apparent better recognition of DBT can be attributed mainly to non-specific interactions with the bulk of the polymer particles. These binding behaviours are not uncommon in molecular imprinting [63]. This fact can be attributed to the heterogeneity of the binding site structures obtained in non-covalent imprinting, where selectivity can be referred to a panel of related molecular structures. Still we think that cavities have been formed looking at Fig. 4 where comparison between MIP and NIP indicates clear differences in the polymeric matrix and behaviour of the OTC compounds.

Other polymers employing DBT and TPhT as templates respectively were synthesized in order to evaluate any modification in the retention/elution of the four OTCs. The first one took a gel consistence unable to use as stationary phase for LC. The second, based on TPhT, was not able to separate the trisubstituted species at any mobile phase composition compatible with ICP/MS.

Once the separation of OTCs using a *MIP-e*-filled column has been achieved, two new batches of *MIP-e* were synthesized, packed into columns and tested to assure its usability and reproducibility as a selective material for OTC speciation. Good reproducibility of retention times and sensitivity was found considering that column package as well as particle sieving have been made in the laboratory without specific technology (Fig. 5). The *MIP-e*-filled column has lasted over 100 injections each.

3.4. Analytical characteristics of the method

Analytical quality parameters of the optimized method have been determined. The linear working range with a sample loop of 0.2 mL has been established from detection limit to 100 ng (with linear coefficients obtained at least 0.99 for all compounds). The relative standard deviation is acceptable at 5–11% except for MBT which gave higher values due its prompt elution close to the column void volume. Detection limits have been calculated as usual, defined as the concentration corresponding to a signal three times the noise level of the background, resulting in 6 pg for MBT, 10 pg for TBT and TPhT, and 20 pg for DBT. These LC detection limits are among the most sensitive reported in literature.

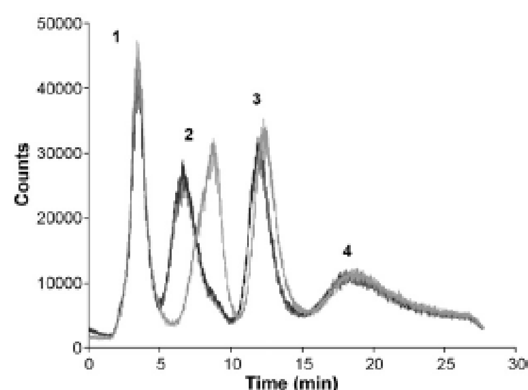

Fig. 5. Overlapping of two chromatograms obtained with two different polymerization batches of *MIP-e*. Same chromatographic conditions applied as Fig. 4. Peaks: 1. MBT; 2. TBT; 3. TPhT; 4. DBT

3.5. Application of MIP-e for the speciation analysis of OTC in biota samples

Fig. 6 has represented the chromatograms after treating ERM-CE-477 mussel tissue and T-38 oyster following the experimental procedure detailed in Section 2. Results showed in Table 4 are based on four independent replicates of each material treated the same day but analysed in different days Numerical results have showed

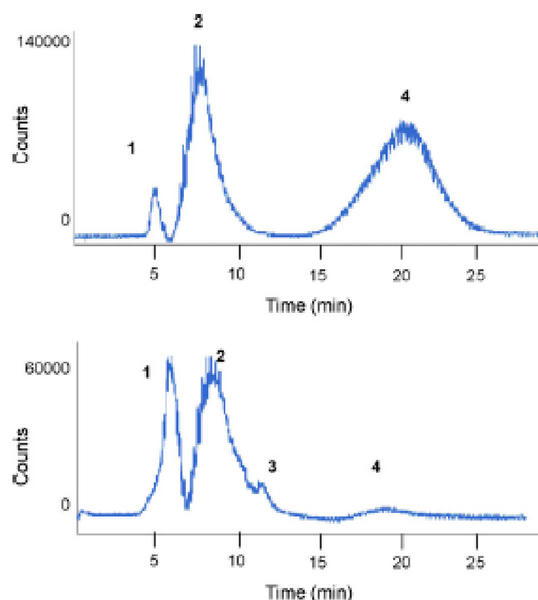

Fig. 6. Chromatograms obtained for the determination of OTC's in two biota samples. (a) ERM-CE477 Mussel tissue; (b) T-38 Oyster tissue.

Table 4
Results of organotin determination in biota samples by LC-IPMS using *MIP-e* as affinity stationary phase.

OTC	Mussel ERM-CE477 (mg kg−1)	Certified value (mg kg−1)[a]	Oyster T-38 (mg kg−1)[b]	Certified value (mg kg−1)
MBT	0.96 ± 0.05	1.05 ± 0.28	0.11 ± 0.03	0.10 ± 0.05
DBT	0.79 ± 0.05	0.78 ± 0.12	0.08 ± 0.03	0.08 ± 0.03
TBT	1.06 ± 0.06	0.90 ± 0.19	0.18 ± 0.05	0.18 ± 0.04
TPhT	nd	–	nd	–

[a] Values based on the Sn content (calculated from the certification report of ERM-CE477).
[b] Indicative values. This material was not commercialized by IRMM.

an excellent agreement with the certified values in both samples and for the four compounds. These results highlight the possibility to determine organotin compounds by LC-ICP/MS with no need of derivatization step like the one required for gas chromatography. In addition, the lack of matrix interference observed when MIP is employed as stationary phase avoids the tedious standard addition method or an additional extract cleaning step. Then, this chromatographic phase based on the polymer imprinting technology can be a good alternative to the usually employed gas chromatography for mollusc and other biota samples analysis.

4. Conclusions

This work presents a new approach for speciation of organotin compounds based on an imprinted polymer stationary phase. Synthesis of polymer beads with satisfactory shape and size for chromatographic purposes has been achieved using different polymerization methods. Imprinting effects have been shown for polymers prepared by both the grafting Iniferter and emulsion methods. Satisfactory resolution of all four OTCs studied (MBT, DBT, TBT and TPhT) has been achieved in 20 min using the emulsion polymer, and several batches of this polymers have shown excellent reproducibility in both synthesis and packaging. The main drawback of this chromatographic method is the necessity to apply a chromatographic gradient. Analytical parameters are among the best published for methods that employ commercial or non-specific stationary phases and our polymer has shown to be free of matrix interferences. Finally, the method has been validated using two biota reference materials (ERM-CE477 mussel and T-38 oyster). This work has consolidated the imprinting technique by opening new pathways for speciation of organometallic compounds.

Acknowledgements

The authors would like to thank the Dirección General de Universidades e Investigación de la Consejería de Educación de la Comunidad de Madrid y de la Universidad Complutense de Madrid for economical support through projects CAM-S505/AGR/0312 and CCG08-UCM/PPQ-3898. The authors gratefully acknowledge the Mercedes Gallego-Gallegos Ph.D. fellowship from the Spanish Ministry of Education. Professor Mª José Torralba is acknowledged for BET data, and the Electronic Service from Universidad Complutense de Madrid for SEM analysis.

References

[1] K. Fent, M.D. Müller, Environ. Sci. Technol. 25 (1991) 489.
[2] S.M. Evans, C. Sheppard (Ed.), Marine antifoulants, Seas at the Millenium: An Environmental Evaluation, vol. III: Global Issues and Processes, Elsevier Science Ltd., Oxford, 2000 (Chapter 124, p. 247).
[3] A.B.A. Boxall, S.D. Comber, A.U. Conrad, J. Howcroft, N. Zaman, Marine Pollut. Bull. 40 (2000) 898.
[4] M. Hoch, Appl. Geochem. 16 (2001) 719.
[5] IMO, International Marine Organization, Anti-fouling systems, 2002, http://www.imo.org.
[6] Directive 76/464/CE, Directive 2000/60/CE.
[7] Report EPA 816-F-06-016, United States EPA, 2003, http://www.epa.gov/safewater/consumer.
[8] K. Azumi, S. Nakamura, S.I. Kitamura, S.J. Jung, K. Kanehira, H. Iwata, S. Tanabe, S. Suzuki, Fish. Sci. 73 (2007) 263.
[9] O. Madoka, A. Takaomi, M. Sayaka, H. Hiroya, M. Reiji, M. Nobuyuki, Water Air Soil Pollut. 178 (2007) 255.
[10] L. Yang, Z. Mester, R.E. Sturgeon, Anal. Chem. 74 (2002) 2968.
[11] M. Leermakers, J. Nuyttens, W. Baeyens, Anal. Bioanal. Chem. 381–386 (2005) 1272.
[12] K.B. Thurbide, B.W. Cooke, A. Walter, J. Chromatogr. A 1029 (2004) 193.
[13] E. Millán, J. Pawliszyn, J. Chromatogr. A 873 (2000) 63.
[14] S. Aguirre, O.F.X. Donard, J. Anal. At. Spectr. 16 (2001) 1429.
[15] J. Ruiz-Encinar, P. Rodríguez González, J.I. García Alonso, A. Sanz Medel, TrAC Trends Anal. Chem. 22 (2003) 2.
[16] P. Rodríguez González, A. Rodríguez Cea, J.I. García Alonso, A. Sanz Medel, J. Anal. At. Spectr. 381 (2005) 2.
[17] L. Ebdon, S.J. Hill, C. Rivas, TrAC Trends Anal. Chem. 17 (1998) 277.
[18] E. González-Toledo, R. Compañó, M. Granados, M.D. Prat, TrAC Trends Anal. Chem. 22 (2003) 26.
[19] X. Wang, L. Din, H. Zhang, J. Cheng, A. Yu, H. Zhang, L. Lin, Y. Li, J. Chromatogr. B 843 (2006) 268.
[20] E. González-Toledo, R. Compañó, M. Granados, M.D. Prat, J. Chromatogr. A 878 (2000) 69.
[21] T.L. Jones-Lepp, G.M. Monplaisir, TrAC Trends Anal. Chem. 24–27 (2005) 590.
[22] S.J. Hill, L.P. Pitts, A. Fisher, TrAC Trends Anal. Chem. 19 (2000) 120.
[23] L. Yang, J.W.H. Lam, J. Anal. At. Spectrom. 16 (2001) 724.
[24] C. Rivas, L. Ebdon, S.J. Hill, J. Anal. At. Spectrom. 11 (1996) 1147.
[25] K.L. Ackley, K.L. Sutton, J.A. Caruso, J. Anal. At. Spectrom. 15 (2000) 1069.
[26] S. Chiron, S. Roy, R. Cottier, R. Jeanot, J. Chromatogr. A 879 (2000) 137.
[27] O. Nygren, C.A. Nilsson, W. Frech, Anal. Chem. 60 (1988) 2204.
[28] A. Astruc, R. Lavigne, V. Desauziers, R. Pinel, M. Astruc, Appl. Organomet. Chem. 3 (1989) 267.
[29] C.F. Harrington, G. Eigendorf, W.R. Cullen, Appl. Organomet. Chem. 10 (1996) 339.
[30] S. White, T. Catterick, B. Fairman, K. Webb, J. Chromatogr. A 794 (1998) 211.
[31] W.S. Chao, S.J. Jiang, J. Anal. At. Spectrom. 13 (1998) 1337.
[32] E. Rosenberg, V. Kmetov, M. Grasserbauer, Fresenius J. Anal. Chem. 366 (2000) 400.
[33] E. González-Toledo, A. Ortuno, R. Campano, M. Granados, M.D. Prat, Chromatographia 55 (2002) 19.
[34] K. Békri, R. Saint-Louis, E. Pelletier, Anal. Chim. Acta 578 (2006) 203.
[35] C. Alexander, L. Davidson, W. Hayes, Tetrahedron 59 (2003) 2025.
[36] E.P.C. Lai, S.G. Wu, Anal. Chim. Acta 481 (2003) 165.
[37] E. Turiel, J.L. Tadeo, P.A.G. Cormack, A. Martín-Esteban, Analyst 130 (2005) 1061.
[38] N.T. Greene, K.D. Shimizu, S.L. Morgan, Chem. Commun. 10 (2004) 1172.
[39] E. Turiel, A. Martín-Esteban, J. Sep. Sci. 32 (2009) 3278.
[40] B. Sellergren, J. Chromatogr. A 906 (2001) 227.
[41] R.J. Ansell, Adv. Drug Deliv. Rev. 57 (2005) 1809.
[42] E. Caro, R.M. Marce, F. Borrull, P.A.G. Cormack, D.C. Sherrington, Trends Anal. Chem. 25 (2006) 143.
[43] C. Baggiani, L. Anfossi, C. Giovanolli, Anal. Chim. Acta 591 (2007) 29.
[44] F.G. Tamayo, E. Turiel, A. Martín-Esteban, J. Chromatogr. A 1152 (2007) 32.
[45] M. Gallego-Gallegos, R. Muñoz Olivas, A. Martín-Esteban, C. Cámara, Anal. Chim. Acta 531 (2005) 33.
[46] M. Gallego-Gallegos, M. Liva, R. Muñoz-Olivas, C. Cámara, J. Chromatogr. A 1114 (2006) 82.
[47] K.Y. Yu, K. Tsukaghoshi, M. Maeda, M. Takagi, Anal. Sci. 8 (1992) 701.
[48] G.Y. Sun, Q.H. Shi, Y. Sun, J. Chromatogr. A 1061 (2004) 159.
[49] F.G. Tamayo, A. Martín-Esteban, J. Chromatogr. A 1098 (2005) 116.
[50] B. de Boer, H.K. Simon, M.P.L. Werts, E.W. van der Vegte, G. Hadziioannou, Macromolecules 33 (2000) 349.
[51] C. He, F. Liu, K. Li, H. Liu, Anal. Lett. 39 (2006) 275.
[52] S. Lu, G. Cheng, X. Pang, J. Appl. Polym. Sci. 89 (2003) 3790.
[53] J.S. Downey, R.S. Frank, W.H. Li, H.D.H. Stover, Macromolecules 32 (1999) 2838.
[54] R. Morabito, H. Muntau, W. Cofino, Ph. Quevauviller, J. Environ. Monit. 1 (1999) 75.
[55] B. Sellergren, B. Ruckert, A.J. Hall, Adv. Mater. 14 (2002) 1204.
[56] B. Ruckert, A.J. Hall, B. Sellergren, J. Mater. Chem. 12 (2002) 2275.

[57] R. Cela, R.A. Lorenzo, M.C. Casais, Síntesis (Ed.), Técnicas de separación en química analítica, 2002.
[58] D.A. Spivak, Adv. Drug Deliv. Rev. 57 (2005) 1779.
[59] H. Kima, K. Kaczmarskia, G. Guiochon, Chem. Eng. Sci. 61 (2006) 1122.
[60] M. Gallego-Gallegos, R. Muñoz Olivas, C. Cámara, J. Environ. Manage. 90 (2009) S69.
[61] M. Gallego-Gallegos, R. Muñoz-Olivas, C. Cámara, J. Anal. At. Spectrom. 24–25 (2009) 595.
[62] M. Kohri, K. Sato, Y. Inoue, K. Ide, H. Okochi, Bunseki Kagaku 44 (1995) 537.
[63] E. Yilmaz, R.H. Schmidt, K. Mosbach, in: Y. Ramström (Ed.), Molecularly Imprinted Materials. Science and Technology, Marcel Dekker, 2005, p. 25.