Talanta 110 (2013) 58–65

Contents lists available at SciVerse ScienceDirect

Talanta

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

Pressure-driven mesofluidic platform integrating automated on-chip renewable micro-solid-phase extraction for ultrasensitive determination of waterborne inorganic mercury

Lindomar A. Portugal [a], Luis M. Laglera [b], Aristidis N. Anthemidis [c], Sérgio L.C. Ferreira [a], Manuel Miró [b,*]

[a] Universidade Federal da Bahia, Instituto de Química, 40170-290 Salvador, Bahia, Brazil
[b] FI-TRACE group, University of the Balearic Islands, Faculty of Science, Department of Chemistry, Carretera de Valldemossa km 7.5, E-07122 Palma de Mallorca, Spain
[c] Laboratory of Analytical Chemistry, Department of Chemistry, Faculty of Science, Aristotle University, Thessaloniki, Greece

ARTICLE INFO

Article history:
Received 7 December 2012
Received in revised form
25 January 2013
Accepted 5 February 2013
Available online 17 February 2013

Keywords:
Lab-on-Valve
On-chip μSPE
Automation
Bead injection
Inorganic mercury
Atomic fluorescence spectrometry

ABSTRACT

A dedicated pressure-driven mesofluidic platform incorporating on-chip sample clean-up and analyte preconcentration is herein reported for expedient determination of trace level concentrations of waterborne inorganic mercury. Capitalizing upon the Lab-on-a-Valve (LOV) concept, the mesofluidic device integrates on-chip micro-solid phase extraction (μSPE) in automatic disposable mode followed by chemical vapor generation and gas–liquid separation prior to in-line atomic fluorescence spectrometric detection. In contrast to prevailing chelating sorbents for Hg(II), bare poly(divinylbenzene-N-vinylpyrrolidone) copolymer sorptive beads were resorted to efficient uptake of Hg(II) in hydrochloric acid milieu (pH=2.3) without the need for metal derivatization nor pH adjustment of prior acidified water samples for preservation to near-neutral conditions. Experimental variables influencing the sorptive uptake and retrieval of target species and the evolvement of elemental mercury within the miniaturized integrated reaction chamber/gas–liquid separator were investigated in detail.

Using merely <10 mg of sorbent, the limits of detection and quantification at the $3s_{blank}$ and $10s_{blank}$ levels, respectively, for a sample volume of 3 mL were 12 and 42 ng L^{-1} Hg(II) with a dynamic range extending up to 5.0 μg L^{-1}. The proposed mesofluidic platform copes with the requirements of regulatory bodies (US-EPA, WHO, EU-Commission) for drinking water quality and surface waters that endorse maximum allowed concentrations of mercury spanning from 0.07 to 6.0 μg L^{-1}. Demonstrated with the analysis of aqueous samples of varying matrix complexity, the LOV approach afforded reliable results with relative recoveries of 86–107% and intermediate precision down to 9% in the renewable μSPE format.

1. Introduction

The advent of microfluidic platforms has revolutionized the field of wet chemical (bio)analytical assays [1] as a result of the likelihood of probing single cells [2] beside the manipulation, identification and separation of cells (e.g., cancer cells) [3–5], the examination of protein structure and function [6], the simplification of polymerase chain reaction-based methods [7,8], and the exploration of aptamer interactions with proteins or small molecules [9,10]. Not the least, lab-on-a-chip (LOC) microdevices are able to cope with most of the twelve basic principles of green chemistry [11] toward the minimization in consumption of sample and reactants at the low μL level, with a downscaling option to the nL level, and in the generation of wastes. The ultimate goal is the development of reagent-free chemical assays with miniaturized on-chip electrochemical sensors or optrodes or the assembly of functional platforms interfaced to mass spectrometry [1,12,13]. Recent trends geared toward the integration of overall (bio)analytical protocols on-chip including electrophoretic and μSPE approaches for purification, enrichment and digestion of target species [14,15] and the development of rugged platforms to be transferred to routine analytical laboratories or real-life scenarios [16].

The superior attributes of LOC also accrue with metal assays (e.g. Hg(II)) in a variety of fields of research endeavor. Microfluidic systems for downscaling and simplification of inorganic mercury assays involving a variety of chemistries and detection principles have attracted a great deal of attention lately. Chromogenic probes immobilized on-chip for microfluidic optrode sensing [17] and integration of atomic emission spectrometry on-chip using dielectric barrier discharge plasma allied to cold-vapor (CV) generation [18] rendered miniaturized and/or portable devices for mercury quantification at levels ≥ 10 μg L^{-1} Hg(II). Microfabricated platforms housing electrochemical detectors furnished with

* Corresponding author. Tel.: +34 971172746; fax: +34 971173426.
E-mail address: manuel.miro@uib.es (M. Miró).

0039-9140/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.talanta.2013.02.013

Au/Ag planar microelectrodes [19] or the strong binding of mercury to thymine-rich oligonucleotides anchored to metal microelectrodes [20] allowed for reliable low-abundance Hg(II) measurements at the sub-$\mu g\,L^{-1}$ level.

LOC platforms have also opened up a host of prospects within the environmental analytical arena, though the sample volume is not an issue here. A vast amount of effort has been devoted to ameliorate chip/detection technology and tackle challenges to trigger new platforms for *in-situ* 24/7 unattended monitoring of environmental parameters using stand-alone deployable devices [21–23]. Although the development of a wide range of intense light-emitting diodes that can be coupled to fiber optics enabled the integration of optical detection within microfluidic devices, on-chip absorbance measurements lack sensitivity for trace level analysis as a result of the handling of sample volumes down to the low nL or pL level and the tiny channel dimensions which render optical path lengths within the nanometer range [24,25]. Likewise, the minute electroactive exposed surface of embedded electrodes in LOC platforms detrimentally affects the chip-based method sensitivity for metal assays [19]. The main obstacle for extensive acceptance of LOCs in the context of environmental trace element analysis lies in their inability to cope with the stringent requirements of current regulatory authorities for priority metal pollutants. The current EU Water Framework Directive poses a maximum allowed concentration (MAC) of mercury in surface waters of 70 $ng\,L^{-1}$ [26], which is regrettably far below the lower limit of quantitation and the bottom end of the dynamic range of the above mentioned optical and electrochemical chip devices [17–20]. The inorganic mercury guideline values in drinking water endorsed by the European Commission, US-EPA and World Health Organization (WHO); 1 $\mu g\,L^{-1}$ [27], 2 $\mu g\,L^{-1}$ [28] and 6 $\mu g\,L^{-1}$ [29], respectively, are not even met by the majority of recently reported integrated chip systems [17–19]. Further, LOCs are frequently dedicated, that is, they bear fixed architecture for predetermined chemistries as a result of which little room remains for additional sample processing steps other than those initially conceived [30].

To tackle the aforementioned shortcomings, Lab-on-Valve (LOV) mesofluidic approaches bearing channel dimensions with sizes within the low mm range have emerged as appealing alternates to the LOC counterparts [30–34]. LOV practitioners are not being dictated by a rigid module architecture, yet they are able to tune hydrodynamic parameters at will using pressure-driven programmable flow [30,33], as precisely controlled by bi-directional syringe pumps, which are also attracting much attention in the LOC field [24,35].

LOV modules housing multi-purpose flow-through cells furnished with optical fibers or miniaturized electrodes for on-chip optical or electrochemical detection, respectively, are able to admit larger sample volumes than those of LOCs, and afford versatile optical path lengths up to 10 mm by tailoring the liquid gap between both optical fibers [33,36]. Most importantly, LOV has evolved as a downscaled tool for automated sample processing of environmental samples for trace metal analysis using atomic spectrometric detection [37–40]. The constraints of LOCs for reliable μSPE as a consequence of the progressive tighter packing of the beads embedded in the channels and the subsequent build-up of backpressure are readily overcome with the flexible manipulation of beads in LOV using the so-called bead-injection (BI) mode [30,41]. In BI, bead suspensions are processed akin to solutions, trapped as microcolumns within the conduits of the mesofluidic platform, and employed to preconcentrate target species (with concomitant removal of unwanted matrix components) followed by on-line disposal after each single assay or a given number of measurements on the basis of the feasibility of regeneration of the sorptive surfaces.

Membrane-based gas-diffusion procedures and gas–liquid separators have been likewise integrated or combined with LOV devices for selective isolation of metals and metalloids as evolved gases. These approaches have been primarily linked to CV generation of mercury with detection by UV-photometry [42] or atomic fluorescence spectrometry with the aid of side-on photomultipliers [43,44] or external devices [45]. Although the limits of detection (LODs) reported are suitable at first sight for meeting the demands of regulatory bodies in terms of water quality standards, the actual limits of quantification for inorganic mercury and the concentrations determined accurately in environmental waters are at least exceeding the MAC of the EU Council Directive 2008/105/EC in one order of magnitude [42–45].

This paper reports the first fluidic platform encompassing automatic disposable BI-based μSPE and integrated gas–liquid separation for on-line CV generation and determination of inorganic mercury in environmental and drinking waters at concentration levels below those endorsed by current regulatory agencies. Copolymeric bead carriers bearing vinylpyrrolidone moieties as mercury receptor sites were explored for selective analysis of inorganic mercury in high-salt content samples. The most critical variables in on-chip sorptive BI assays of inorganic mercury and evolvement of elemental mercury were assessed by fractional factorial design. Aided by thermodynamic calculations, further insight is given into the mechanism of selective sorbent uptake of inorganic mercury in hydrochloric acid medium.

2. Experimental

2.1. Reagents

All chemicals were of analytical reagent grade and were supplied by Merck (Darmstadt, Germany). Ultra-pure water (specific resistivity of 18.2 MΩ cm) obtained from a Milli-Q system (Millipore, Bedford, USA) was employed to prepare all solutions and standards. Glassware rather than plastic containers were used throughout for standard preparation. Hydrochloric and nitric acids (*Trace*SELECT, Fluka Analytical, Seelze, Germany) were further purified by cold-finger distillation in a silica reactor. Working standard solutions of Hg(II) in a 0.005 mol L^{-1} HCl were prepared immediately before use by serial dilution of 10 mg L^{-1} Hg(II) stock solution in 0.1 mol L^{-1} HCl. The stock solution was obtained from mercury(II) chloride as per APHA-AWWA-WPCF recommendations. A 4.0% (m/v) $SnCl_2$ solution, used as in-line selective reducing agent for Hg(II) to trigger the CV reaction, was prepared fresh daily by weighing 2 g of $SnCl_2 \cdot H_2O$ ($<0.000001\%$ Hg) to which 1 mL distilled HCl was added prior to diluting to 50 mL with water. Traces of Hg(II) in the reagent were eliminated by purging the solution with a gentle stream of nitrogen for 30 min.

N-vinylpyrrolidone-divinylbenzene copolymeric beads (Oasis HLB, Waters, Mildford, MA) with average dry bead size of 30 μm, specific surface area of 800 $m^2\,g^{-1}$, total pore volume of 1.4 $cm^3\,g^{-1}$ and containing a balanced ratio of hydrophilic and lipophilic monomers were used as-purchased for mesofluidic μSPE of Hg(II) in the BI format. Working bead suspensions were prepared by moistening 200 mg of the Oasis HLB sorptive material with 2 mL of 95% (v/v) MeOH/water. The suspension of copolymeric beads was contained in 1.0 mL plastic syringe, which was mounted vertically on port 5 (see Fig. 1) of the integrated LOV platform.

2.2. Sample collection and treatment

All plastic and glass materials for sample collection and preparation of solutions were cleaned in 10% (v/v) HNO_3 for at least 24 h, and thoroughly rinsed with Milli-Q water immediately before first use.

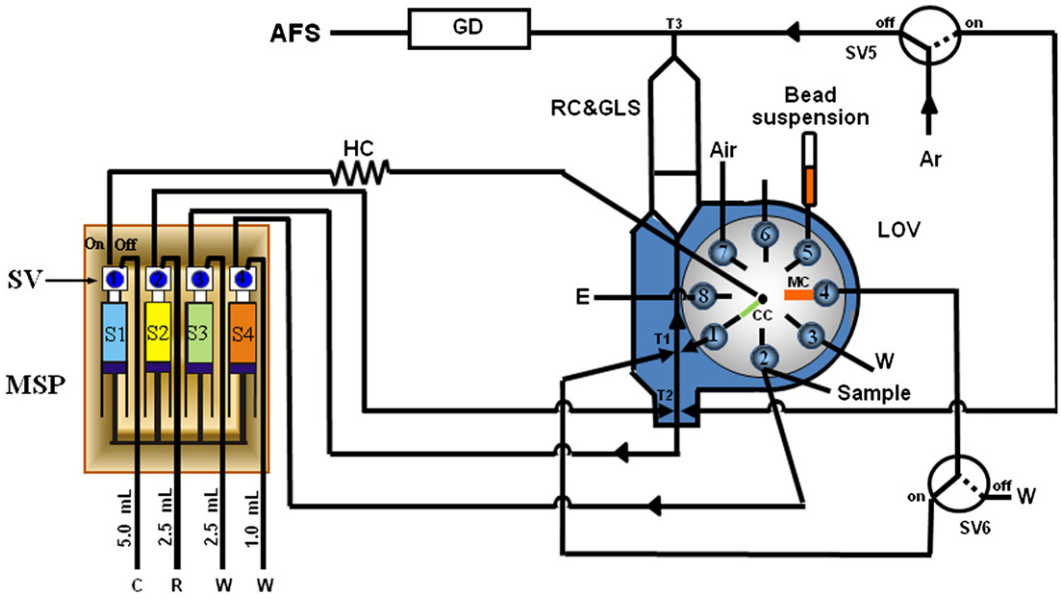

Fig. 1. Sketch of the LOV-μSPE mesofluidic platform for automatic on-chip preconcentration of Hg(II) using disposable sorptive beads followed by on-line CV generation and separation of elemental mercury. SV: solenoid valve; MSP: multi-syringe pump; S: glass syringe; LOV: Lab-on-a-Valve; MC: microcolumn; CC: central communication channel; RC: reaction chamber; GLS: gas liquid separator; GD: gas dryer; AFS: atomic fluorescence spectrometer; E: eluent; HC: holding coil; C: carrier; R: reducing reagent; T1, T2, and T3: confluence points; and W: waste.

A number of environmental waters from the Balearic Islands, Spain (*viz*., spring water, ground water, drinking water from a city water reservoir, and coastal seawaters in highly populated (hotel resort) sites) were collected in low density polyethylene bottles and immediately filtered through 0.45 μm cellulose acetate membrane filters. Samples were collected following GEOTRACES' recommendations for mercury [46]. The filtrate was stored in airtight glass bottles, acidified with distilled HCl to pH 2.3 and analyzed in less than 24 h.

2.3. Analytical instrumentation

A schematic diagram of the pressure-driven mesofluidic platform integrating μSPE and CV generation for automatic determination of low-abundance waterborne Hg(II) is illustrated in Fig. 1. The LOV platform, fabricated from polymethylmethacrylate and encompassing eight integrated channels (1.66 mm i.d./12.0 mm length), was mounted atop of an eight-port multiposition selection valve (Valco Instruments, Houston, TX). The central port of the integrated LOV sample processing unit, connected to S1, is made to address the peripheral ports of the unit (1–8), for sequential aspiration of the various constituents for BI sample preparation, via the central communication channel (CC) in the selection valve. One of the LOV channels (the one indicated as port 4 in Fig. 1) served as a microcolumn bed for trapping the disposable co-polymeric beads. To contain the sorbent within the cavities of the LOV module and prevent them from escaping, the outlet of the microcolumn bed (port 4) was furnished with a polyethylene frit of 10 μm pore size (Mo Bi Tec, Göttingen, Germany). The bead container and the eluent reservoir were attached to the peripheral ports 5 and 8, respectively, while port 3 was used for sorbent disposal. Direct analysis of samples without prior sample processing is also feasible without the need of manifold reconfiguration through port 1 in the LOV unit. A dedicated membrane-less gas–liquid expansion separator from a commercial polypropylene syringe barrel (40 mm long, 11 mm i.d.) with an internal volume of ca. 7 mL was attached in upright position to the LOV platform through home-made PVC nuts. The flow manifold houses two external three-way solenoid valves (SV5 and SV6) for delivery of the argon stream and eluate, respectively, to the gas–liquid separator via T2 and T1 confluences (see Fig. 1), respectively.

A multi-syringe bi-directional piston pump with programmable speed (MSP, Multiburete 4S, Crison, Alella, Spain) equipped with four syringes (S1, S2, S3, and S4) (Hamilton, Switzerland) connected in block to a single stepper motor was exploited as a liquid driver for pressure-driven flow. S1 with a capacity of 5.0 mL contained the carrier (0.005 mol L^{-1} HCl) and was connected to the central port of the LOV via a PTFE tubing (2.0 mm i.d., 160.0 cm long) working as a holding coil (HC). S2 with an internal volume of 2.5 mL contained the reducing solution to be brought to the gas–liquid separator through the confluence point (T2) for evolving elemental mercury. S3 and S4 with a capacity of 2.5 mL and 1.0 mL, respectively, were connected to the confluence T2 and the dual LOV channel (port 2), respectively, for rinsing the gas–liquid separator at the end of the analysis cycle and the sampling line between consecutive samples or standards, respectively. Three-way solenoid valves (V_1, V_2, V_3, V_4) (N-Research, Caldwell, NJ) were mounted atop of each syringe, enabling the communication with the liquid reservoirs in the Off position, or with the LOV platform when activated to the On position. The flow network was built from polytetrafluoroethylene (PTFE) tubing of 0.8 mm i.d., excepting the lines connecting S1–S4 with the external reservoirs which were made from 1.5 mm i.d. PTFE tubing.

The LOV platform was coupled to a non-dispersive atomic fluorescence spectrometer (AFS, PS Analytical, Orpington, UK) for in-line detection of evolved elemental mercury. The detector was equipped with a high intensity mercury vapor lamp (Cathodeon Ltd., Cambridge, UK), a fixed 254 nm filter and a photomultiplier tube. The flow rates of the argon carrier through the reactor chamber/gas–liquid separator for sweeping the mercury gas into the AFS flow cell, and of the shield gas were affixed to 120 mL min^{-1} and 300 mL min^{-1}, respectively. Unless otherwise stated, the AFS operated at a 100-fold electronic gain and the transient signals were processed in the peak height or peak area mode. Activation of the AFS was performed using the same software controlling pumps and valves via dedicated dynamic link libraries. A shell-and-tube configured drying membrane (Perma Pure Inc, Toms River, NJ) utilizing nitrogen at 400 mL min^{-1} as a

purge gas was connected to the outlet of the gas–liquid separator to circumvent entrainment of moisture into the AFS and subsequent quenching of the atomic fluorescence intensity.

2.4. Analytical procedure for μSPE of Hg(II) in the LOV-BI mode

The operational sequence for automatic sample clean-up and sorptive preconcentration of inorganic mercury onto the LOV renewable microcolumn and further in-line CV-AFS detection is compiled in Table S1. This table lists experimental details of the analytical procedure and the LOV and solenoid valve positions along with the consumption of sample and reagents and delivery flow rates. The overall sample processing cycle involves six steps, *viz*, system preconditioning, microcolumn packing, analyte uptake, elution of pre-concentrated Hg(II) followed by vapor generation in the reaction chamber and transportation of volatile species into the AFS, and finally sorbent disposal, as described below:

(1) **Preconditioning of the flow network** (not shown in Table S1**).** Initially, the syringes S1 and S2 are filled with carrier and reducing reagent, respectively, and the HC is cleansed with 4.6 mL of eluent. On changing the sample, S4 is set to aspirate a well-defined volume of sample or standard (300 μL) past flow-through port 2 to rinse the sampling line. Metered volumes of eluent and air (250 μL each) are aspirated into HC to wash and fill the peripheral lines. The surplus is directed to waste through port 3.
The analytical operations in this step are aimed at eliminating sample cross-contamination effects and potential analyte carry-over.

(2) **Microcolumn packing within LOV conduits** (steps 1–3 in Table S1**).** A minute volume of the Oasis HLB bead suspension (70 μL) is drawn slowly into HC. Bead dispersion into the carrier solution is avoided with a prior aspirated 100 μL-air plug. The communication channel (CC) is then connected to the peripheral port 4 to flush the bead suspension toward the microcolumn bed with the aid of S1. The trapped beads inside the LOV conduit are subsequently rinsed with 200 μL of carrier.

(3) **Sample loading** (steps 4–7 in Table S1**).** S1 is set to pull 3000 μL sample from the dual-line port 2 into HC. Afterward, the flow is reversed and the sample plug plus 1000 μL of carrier solution are perfused through the packed-bead microcolumn for uptake of Hg(II) followed by the removal of weakly or non-retained matrix constituents from the bead surfaces. It should be noted that during sample loading the solenoid valve SV6 is switched to Off to prevent the introduction of sample matrix components into the gas–liquid separator.

(4) **Analyte elution** (steps 8–11 in Table S1**).** First, the tubing connecting the outlet of the microcolumn (port 4) with the gas–liquid separator via the confluence T1 is filled with air. Afterward, a 350 μL of acidic eluent is aspirated from port 8 and dispensed forward in air-segmented mode to the microcolumn for quantitative stripping of Hg(II). The eluate (SV6 is activated to On) is delivered to the reactor chamber/gas–liquid separator through the microchannel engraved in the LOV platform from the confluence T1 to the bottom end of the reactor (see Fig. 1).

(5) **Cold vapor generation and detection** (steps 12–14 in Table S1**).** Evolvement of mercury vapor is triggered with the activation of SV2 to On and introduction of 500 μL of reducing reagent into the reaction chamber. Though the reduction of inorganic mercury to elemental mercury is virtually instantaneous, a delay time of 10 s is used for reaction completion. The evolved mercury is confined within the reaction chamber because of the positive pressure of the argon carrier at T3 against the pressure inside the reactor. The reaction mixture is then purged with the argon carrier at 120 mL min^{-1} (SV5 is switched to On) and the mercury vapors feed the AFS flow-cell. At this time the detector is triggered via software and the transient fluorescence signal is recorded.

(6) **Automatic bead disposal and rinsing of LOV channels and reaction chamber** (steps 15–20 in Table S1**).**
The sorptive beads trapped within the LOV platform are readily discarded on-line (via port 3) after being moistened with carrier. Hence, the LOV manifold is ready to initiate a new analysis cycle with a fresh portion of beads, thus overcoming the deterioration of the analytical performance of the LOC counterparts with permanently packed bead microcolumns due to the progressive deactivation of the sorbent material and/or the potential losses of reactive sites.
The contents of the reaction chamber are pulled into S3, which is working at the end of each analytical run as a waste container. Negligible cross-contamination effects were observed in consecutive assays as the reaction chamber is thoroughly rinsed with 4.0 mL of carrier prior to initiate the ensuing sample analysis.
The overall analysis cycle involving sample injection, analyte preconcentration, elution and quantification by AFS lasts ca. 11 min.

2.5. Experimental design

A two-level fractional factorial design (2^{4-1}) [47,48] was selected to screen the relevance of the nitric acid concentration, the hydrochloric acid concentration (both as μSPE eluents), the concentration of reducing agent and the elution flow rate in μSPE upon the atomic fluorescence intensity of evolved elemental mercury preceded by sorptive preconcentration in the mesofluidic unit (see Table 1). Because of variable confounding in fractional designs the relevance of factor interactions are not to be investigated. Triplicate measurements of the center point were also added to ensure that the variability found is on account of the factor effect rather than the random error. Though the experimental measurements were merely repeated at the center point, the calculated uncertainty was utilized as estimate of the variability in the entire domain selected. Experimental domains of each factor were defined attending preliminary assays and results reported elsewhere [45]. Table 1 compiles the coded and real values (in parentheses) for the fractional factorial design.

Evaluation of the significance of main factors' influence was explored using ANOVA. The standardized factor effects are readily

Table 1
Variables selected and experimental domain of the fractional factorial design for determination of Hg(II) in a microfluidic LOV-μSPE platform.

Variable	Minimum value	Maximum value	Center point
HCl concentration (mol L^{-1})	−(1.0)	+(3.0)	0 (2.0)
HNO_3 concentration (mol L^{-1})	−(1.0)	+(3.0)	0 (2.0)
Tin(II) chloride concentration (m/v,%) in 2% (v/v) HCl	−(1.0)	+(4.0)	0 (2.5)
Elution flow rate (mL min^{-1})	−(0.3)	+(1.0)	0 (0.65)

[Hg(II)]: 1 μg L^{-1}. The eluent is composed of both HCl and HNO_3.

visualized in Pareto charts. These charts are histograms where the standardized effects are displayed in descending order and each bar length equates the value of a calculated Student's t [49]. A given factor effect is deemed statistically significant whenever its t-value is equal or larger than the t-critical value at a 0.05 significance level (represented by the vertical line on the chart), corresponding in our case to 3.18 for three degrees of freedom.

3. Results and discussion

3.1. Configuration of the mesofluidic platform for trace level analysis of inorganic mercury

The prevailing procedure for selective CV generation of elemental mercury from inorganic mercury involves the reaction of target species with tin chloride in acidic medium [50]. Reduction involving sodium/potassium tetrahydroborate is herein not examined because of the potential quenching of the atomic fluorescence signals as a result of reagent hydrolysis [43,44] and partial reduction of organic mercury species. Initial tests using surrogate seawater with chemical composition as detailed elsewhere [51] and doped seawater or ground water for direct CV assays of inorganic mercury within the LOV platform in the absence of sorptive preconcentration however rendered insufficient limits of quantification and biased results with recoveries down to 70%. Coupling on-line sorptive preconcentration to CV for improved detectability with concomitant sample clean-up is usually accomplished using iminodiacetate chelating resins or sorbents functionalized with mercapto moieties [52]. These sorbents necessarily call for accurate sample buffering at pH $\geq$ 4–5 (though some new chelators, e.g., nitriloacetic acid-Superflow resin do not pose problems for metal uptake at pH ca. 2–3), and might provide inappropriate extraction yields in harsh environmental samples (e.g. brines or hard waters) because of interfering effects from alkaline-earth metals and/or metal precipitation.

To surmount the above drawbacks, a polydivinylbenzene-co-N-vinylpyrrolidone copolymer that has been reported to uptake selectively inorganic mercury in acidic medium [45] has been herein explored in the BI format.

To investigate the operational sequence for LOV-BI, preliminary experiments involving the loading of mercury laden solutions onto the LOV microcolumn with further reductive elution using tin chloride in mild acidic media as eluent were undertaken with the aim of concomitant mercury elution and CV generation. However, the limit of quantification of the LOV-BI system did not suffice for the reliable determination of trace levels of mercury with elution recoveries down to 40%. Inorganic mercury is thus strongly bound to the functional moieties of the sorbent accounting for the fact that both acidic and oxidizing reagents are proven necessary for quantitative elution. Hereto, a post-column eluate treatment modality was adopted in this work. A well-defined plug of the vapor forming reagent was added to the eluate in the reaction chamber for generation of mercury vapor followed by on-line gas–liquid separation. The highly reproducible mixing of streams and controllable timing in pressure-driven LOV manifolds enables the handling and *in-situ* mixing of thermodynamically unstable chemicals (here the oxidizing eluate and tin(II) chloride in the reaction/separation vessel) via kinetic discrimination of the reactions involved.

3.2. Investigation of analytical parameters and sorptive mechanism

Experimental design including sorption/elution variables in BI as well as conditions for vapor generation in the integrated platform was undertaken to ascertain the most significant parameters upon the AFS readouts and elucidate the interaction of divinylbenzene-co-N-vinylpyrrolidone polymeric beads toward inorganic mercury in acidic medium. The analytical signals recorded for the overall experiments in the fractional factorial design are listed in Table 2 while the Pareto chart is illustrated in Fig. 2.

According to ANOVA results the concentrations of acids in the eluent and the eluate flow rate were statistically significant within the experimental domain at the 0.05 significance level, while the concentration of reducing agent proved to be not significant. Pareto charts revealed that both the nitric and the hydrochloric acid concentrations are the factors having a more significant influence on the AFS signals. The higher the concentration of both acids in the eluent phase the greater the amount of mercury retrieved. Because of the elevated distribution coefficient of inorganic mercury onto the co-polymeric beads the elution flow rate needs to be decreased to the lowest possible value. According to the results of the Pareto graph (see Fig. 2), the composition of eluent is set to 3 mol L^{-1} HNO_3 plus 3 mol L^{-1} HCl with an elution flow rate of 0.3 mL min^{-1}. The concentration of reducing agent was fixed to the highest level in the experimental domain (4.0% (w/v)) because of partial decomposition within the reaction chamber under oxidizing elution conditions.

To elucidate the mechanism of uptake and stripping of Hg(II) by and from Oasis HLB on the basis of the above observations, we harnessed to thermodynamic calculations in combination with a series of additional experiments for mercury complexation and sorption as described below. In hydrochloric acid medium (pH = 2.3) the thermodynamically prevailing form of inorganic mercury is mercury(II) chloride amounting to 94% of the overall inorganic complexes [53]. The addition of nitrate and sulfate renders little changes in mercury speciation as a result of the greater affinity of mercury for chloride than sulfate or nitrate. The mechanism of uptake most likely involves the coordination of neutral mercury (II) chloride to the amide mordanting groups of the vinylpyrrolidone

Table 2
Design matrix and response data of the fractional factorial design for LOV-μSPE determination of Hg(II).

Assay	HCl (mol L^{-1})	HNO_3 (mol L^{-1})	$SnCl_2$ (m/v,%)	Elution flow rate (mL min^{-})	AFS signal
1	−	−	−	−	12.0
2	+	−	−	+	12.5
3	−	+	−	+	17.5
4	+	+	−	−	30.8
5	−	−	+	+	10.9
6	+	−	+	−	24.2
7	−	+	+	−	23.3
8	+	+	+	+	24.8
9	0	0	0	0	21.3
10	0	0	0	0	21.0
11	0	0	0	0	22.0

[Hg(II)]: 1 μg L^{-1}.

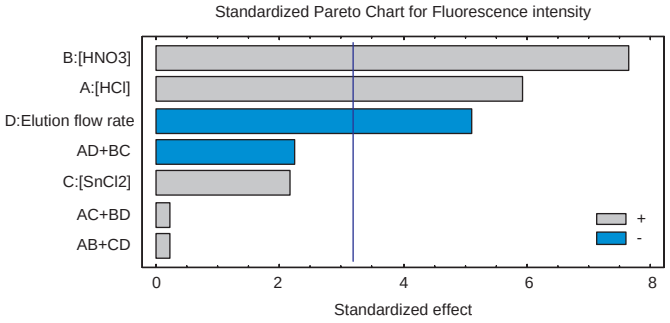

Fig. 2. Pareto chart of the fractional factorial design for determination of Hg(II) using the LOV-μSPE platform.

receptors generating square-planar chelates. In fact, the analytical signal dropped by 32% when percolating mercury (II) standard solutions through the copolymeric sorbent in HNO_3 (pH 2.3) in lieu of the HCl medium. Interactions of Hg(II) with divinylbenzene moieties have been also described in the literature [54]. Yet, the metal binding is reported to be strongly influenced by the ionic strength of the sample matrix. In our case, the sorption of the target analyte onto poly(divinylbenzene-co-N-vinylpyrrolidone) is immune to the concentration of electrolytes in the medium provided that a 0.005 mol L^{-1} HCl concentration is ensured, thereby revealing the assistance of the polar monomer in the selective binding of Hg(II). The unrivaled specific surface area (800 m^2 g^{-1}) and pore volume (1.4 cm^3 g^{-1}) of the sorbent material is contributing to the expedient uptake of Hg(II) as well. The need of highly acidic eluents for quantitative stripping of mercury (see Pareto chart) might be explained on the basis of the low pKa of cyclic amide moieties (pKa~ -0.5 for the carbonyl group) and the shift in inorganic mercury speciation to anionic tri- and tetrachloromercurry complexes at pH below 0.5 [53]. The lack of quantitative Hg(II) elution in 6 mol L^{-1} HCl however indicates that other sorption mechanisms are concomitantly occurring. Oxymercuration is the reaction of alkenes (here the unsaturated impurity of the copolymer) with Hg(II) working as a Lewis acid on the basis of which strong binding of traces of inorganic mercury with the sorbent material might be expected. The progressive loss of active sites (alkenes) in the course of oxymercuration is offset in our work with automatic disposal of spent beads followed by replenishment of the microcolumn bed in the LOV module.

3.3. Analytical performance and analysis of real samples

Under the selected physicochemical variables detailed in the foregoing section, the analytical performance of the BI-LOV method for preconcentration of inorganic mercury with further AFS detection was studied in terms of analyte breakthrough, dynamic range, absolute recovery, enrichment factor, repeatability, intermediate reproducibility, limits of detection and quantification and measurement trueness in the analysis of water bodies of varied matrix complexity.

A linear dependence of fluorescence intensity (peak area) against sample volume is expected in sorptive SPE procedures unless analyte breakthrough occurs. Despite the channels of the LOV are able to accommodate tiny amounts of co-polymeric sorbent material (in our case, 8.9 $\pm$ 1.9 mg Oasis HLB) the increase of sample volume from 0.1 up to 6.0 mL yielded no appreciable analyte breakthrough/pre-elution.

Quantification of inorganic mercury was undertaken by exploiting a six-level external calibration plot with the following standard concentrations (in duplicate): 0.05; 0.1; 0.2; 0.3; 0.4; and 0.5 μg L^{-1} Hg(II) (I_f (peak height)=99.26 [Hg(II); μg L^{-1}]+0.19) with a correlation coefficient (r) of 0.992 using an AFS electronic gain of 100-fold. The dynamic range might be however readily extended to 5.0 μg L^{-1} according to the assay needs by tuning the electronic gain of the detector to avoid surpassing the photomultiplier tube saturation limit.

The extraction efficiency of the LOV integrated microcolumn was calculated as the ratio between the AFS readout of 0.5 μg L^{-1} Hg(II) in the proposed LOV procedure and that obtained from direct injection into the microscale reaction chamber/gas–liquid separator of 350 μL of standard solution in 3 mol L^{-1} HNO_3+3 mol L^{-1} HCl containing an equivalent mass of mercury (1.5 ng Hg(II)). Experimental results revealed that the LOV approach under the selected experimental variables affords the quantitative uptake and retrieval of inorganic mercury with recoveries of 105 $\pm$ 3% (n=3). The enrichment factor was calculated as the ratio of the linear range sensitivity of the calibration curve using LOV-BI and that without preconcentration obtained by injection of 350 μL of standard solutions (equating the eluate volume) into the LOV reactor chamber. As a consequence of analyte pre-elution at sample volumes exceeding 6 mL, the maximum enrichment factor was estimated to be 17. The proposed method however features better enrichment factors than previously reported microfluidic flow systems furnished with permanent sorptive columns containing 30 mg beads for in-line SPE [45] because greater eluent volumes were deemed necessary for quantitative stripping of Hg(II) (1.5 mL eluent in contrast to 350 μL in this present work).

The intermediate inter-day precision of the LOV-BI method calculated as the relative standard deviation of the sensitivity of 5 calibration plots was 9.0%. The limits of detection (LOD) and quantification (LOQ) were calculated at the $3s_{blank}$ and $10s_{blank}$ level (n=10), respectively, and amounted to 12 ng L^{-1} and 42 ng L^{-1}, respectively. Therefore, the automatic procedure fully meets the requirements endorsed by the US-EPA [28], WHO [29] and EU Council Directive 98/83/EC [27] for determination of inorganic mercury in drinking waters wherein the MAC are set to 2.0, 6.0 and 1.0 μg L^{-1}, respectively. Several LOC microdevices reported lately are unable to quantify concentrations of Hg(II) below 6.0 μg L^{-1} [17–19]. More stringent MAC of mercury (*viz.* 70 ng L^{-1}) is however endorsed by the current EU Water Framework Directive 2008/105/EC [26] for surface waters. To the best of our knowledge, none of the earlier LOV platforms described in the literature for Hg(II) assays exploiting AFS and UV-spectrophotometric detection with LOQs > 180 ng L^{-1} Hg(II) [42–45] coped with the demands of the aforementioned European Commission Directive. Though the LOQ of the LOV method herein presented is of the same order of magnitude of flow systems coupled to AFS for Hg(II) assays with commercially available gas–liquid separators and conventional sample uptake [55,56], analysis of troublesome environmental waters (e.g. seawater) without sample clean-up is seldom reported.

Certified reference materials (CRMs) are deemed most appropriate for assessment of the method trueness (bias) in the course of analytical validation. A thorough survey of suppliers of CRMs (e.g., NIST in USA and JRC-IRMM in Europe) revealed that there is a scant number of materials with certified concentration of mercury in environmental waters which is hardly surprising given the instability of inorganic mercury in stored natural

Table 3
Analysis of drinking, ground and coastal seawater samples doped with ultratrace concentrations of Hg(II) for evaluation of method trueness.

Sample	Hg^{2+} (μg L^{-1})		Recovery[c] (%)
	Doped	Found[a]	
Drinking tap water from a city water reservoir	0.00	0.019 $\pm$ 0.007[b]	–
	0.05	0.063 $\pm$ 0.002	91%
	0.10	0.113 $\pm$ 0.007	95%
Ground water (Valldemossa, Mallorca)	0.00	0.024 $\pm$ 0.004[b]	–
	0.05	0.079 $\pm$ 0.008	107%
	0.10	0.107 $\pm$ 0.003	86%
Coastal seawater in highly populated (hotel resort) area (Puerto Pollença, NW Mallorca)	0.00	0.03 $\pm$ 0.01[b]	–
	0.05	0.08 $\pm$ 0.01	96%
	0.10	0.14 $\pm$ 0.02	101%
Coastal seawater in highly populated (hotel resort) area (Santa Ponça, SW Mallorca)	0.00	0.027 $\pm$ 0.004[b]	–
	0.05	0.08 $\pm$ 0.01	103%
	0.10	0.12 $\pm$ 0.01	95%
Spring water (NW, Mallorca)	0.00	< LOD	–
	0.05	0.046 $\pm$ 0.006	92%
	0.10	0.102 $\pm$ 0.007	102%

[a] Results are expressed as the mean of three replicates $\pm$ standard deviation.
[b] Estimated concentrations via external calibration.
[c] Relative recoveries are calculated as the ratio of found to expected concentrations. The latter are obtained as the sum of the doped level plus the original concentration in the sample.

water-samples [57]. In fact, there is merely a single CRM commercially available at present, the so-called BCR-579 (coastal seawater), which is unfortunately not applicable in our work because of the manufacturer's recommendation of using a minimum CRM volume of 30 mL for assays. Hence, an alternate procedure for ascertainment of the reliability and trueness of the proposed LOV method involves real-life sample spiking at trace level concentrations of Hg(II). Relative recoveries of drinking waters, coastal seawaters and hard groundwater doped at the 50 ng L^{-1} and 100 ng L^{-1} levels using external calibration ranged from 86% to 107% regardless of the sample matrix complexity (see Table 3), thereby demonstrating that the automatic LOV preconcentration procedure affords reliable and unbiased data for environmental assays of waterborne inorganic mercury.

4. Conclusion

We have herein proposed a pressure-driven mesofluidic platform for automatic on-chip μSPE in a disposable format for determination of waterborne inorganic mercury. The LOV unit integrates preparative sample clean-up and mercury preconcentration along with post-μSPE chemical processing of the eluate to trigger the release of volatile species. A dedicated flow-through interface has been harnessed to couple LOV-μSPE with a peripheral AFS. The sorptive capability of unmodified poly(divinylbenzene-N-vinylpyrrolidone) for selective and quantitative uptake of inorganic mercury at pH 2.3 in HCl regardless of the saline content of the samples was investigated in detail.

To the best of our knowledge this is the first micro/mesofluidic device featuring a LOQ (*viz.* 42 ng L^{-1} Hg(II)) below the maximum allowed contaminant level specified by current environmental (Water Framework Directive) and drinking water (WHO, EPA, EU Council) regulations within the range of 0.07–6.0 μg L^{-1} mercury.

Further research work is underway to explore the versatility of LOV to incorporate in-line UV-assisted photolysis or ultrasonic-probe degradation of alkylated organomercury species prior to μSPE for non-chromatographic speciation of mercury in environmental matrices. Chemical oxidation of alkylmercury species with nascent bromine evolved from potassium bromide and potassium bromate has been however proven unsuitable because of the fast degradation of the miniaturized sorptive column within the LOV conduits.

Acknowledgments

Lindomar A. Portugal is indebted to the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior—CAPES (Brazil) for allocation of a travel grant to conduct research at the University of the Balearic Islands and to Programa de Pós-graduação em Química from the University Federal of Bahia. Manuel Miró acknowledges financial support from the Spanish Ministry of Economy and Competitiveness (MINECO) through Project CTM2010-17214. Luis M. Laglera is supported by a Ramon y Cajal (MICINN) fellowship. The authors thank Dr. V. Cerda for the loan of analytical instrumentation.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2013.02.013.

References

[1] M.L. Kovarik, P.C. Gach, D.M. Ornoff, Y.-L. Wang, J. Balowski, L. Farrag, N.L. Allbritton, Anal. Chem. 84 (2012) 516–540.
[2] Y. Xu, K. Jang, T. Yamashita, Y. Tanaka, K. Mawatari, T. Kitamori, Anal. Bioanal. Chem. 402 (2012) 99–107.
[3] D. Pappas, K. Wang, Anal. Chim. Acta 6001 (2007) 26–35.
[4] J. Chen, J. Li, Y. Sun, Lab Chip 12 (2012) 1753–1767.
[5] J. Nilsson, M. Evander, B. Hammarström, T. Laurell, Anal. Chim. Acta 649 (2009) 141–157.
[6] M. Landreh, J. Astorga-Wells, J. Johansson, T. Bergman, FEBS J. 278 (2011) 3815–3821.
[7] Y.-H. Zhang, P. Ozdemir, Anal. Chim. Acta 638 (2009) 115–125.
[8] F. Ahmad, S.A. Hashsham, Anal. Chim. Acta 733 (2012) 1–15.
[9] J. Hu, C.J. Easley, Analyst 136 (2011) 3461–3468.
[10] Y. Xu, X. Yang, E. Wang, Anal. Chim. Acta 683 (2010) 12–20.
[11] P.T. Anastas, Crit. Rev. Anal. Chem. 29 (1999) 167–175.
[12] R.E. Oosterbroek, A. van den Berg, Lab-on-Chip: Miniaturized Systems for Bio(Chemical) Analysis and Synthesis, Elsevier, Amsterdam, The Netherlands, 2003.
[13] K.E. Herold, A. Rasooly (Eds.), Lab on a Chip Technology: Biomolecular Separation and Analysis, vol. 2, Caister Academic Press, Norfolk, UK, 2009.
[14] B.C. Giordano, D.S. Burgi, S.J. Hart, A. Terray, Anal. Chim. Acta 718 (2012) 11–24.
[15] C.-C. Lin, J.-L. Hsu, G.-B. Lee, Microfluid. Nanofluid. 10 (2011) 481–511.
[16] A. Ríos, M. Zougagh, M. Avila, Anal. Chim. Acta 740 (2012) 1–11.
[17] Nurinam, B. Kuswandi, W. Verboom, Sens. Actuators B 157 (2011) 438–443.
[18] W.S. Abdul-Majeed, J.H. Lozano-Parada, W.B. Zimmerman, Anal. Bioanal. Chem. 401 (2011) 2713–2722.
[19] C.-G. Chen, J.-C. Zhang, Y. Du, X.-R. Yang, E.-K. Wang, Analyst 135 (2010) 1010–1014.
[20] C.-G. Chen, Y. Du, J. Li, X.-R. Yang, E.-K. Wang, Anal. Chim. Acta 738 (2012) 45–50.
[21] L. Marle, G.M. Greenway, Trends Anal. Chem. 24 (2005) 795–802.
[22] H.-F. Li, J.-M. Lin, Anal. Bioanal. Chem. 393 (2009) 555–567.
[23] J.C. Jokerst, J.M. Emory, C.S. Henry, Analyst 137 (2012) 24–34.
[24] M. Baeza, N. Ibanez-Garcia, J. Baucells, J. Bartrolí, J. Alonso, Analyst 131 (2006) 1109–1115.
[25] B. Kuswandi, Nuriman, J. Huskens, W. Verboom, Anal. Chim. Acta 601 (2007) 141–155.
[26] European Commission, Directive 2008/105/EC of the European Parliament and of the Council on Environmental Quality Standards in the Field of Water Policy and Amending Directive 2000/60/EC, Official Journal of European Communities: Legislation L348, 84–97, 2008.
[27] European Commission, Council Directive 98/83/EC of 3 November 1998 Relating to the Quality of Water Intended for Human Consumption, Official Journal of European Communities: Legislation L330, 32–54, 1998.
[28] Edition of the Drinking Water Standards and Health Advisories, EPA 820-R-11-002, Office of Water, United States Environmental Protection Agency (US-EPA), 2011.
[29] World Health Organization (WHO), Guidelines for Drinking Water Quality, vol. 1, third edition, Geneva, 2008. Available on-line at 〈http://www.who.int/water_sanitation_health/dwq/fulltext.pdf〉, (accessed 07.07.12).
[30] M. Miró, E.H. Hansen, Anal. Chim. Acta 600 (2007) 46–57.
[31] J.-H. Wang, E.H. Hansen, Trends Anal. Chem. 22 (2003) 225–231.
[32] M.D. Luque de Castro, J. Ruiz-Jiménez, J.A. Pérez-Serradilla, Trends Anal. Chem. 27 (2008) 118–126.
[33] M. Miró, E.H. Hansen, Anal. Chim. Acta 750 (2012) 3–15.
[34] E.H. Hansen, M. Miró, Appl. Spectrosc. Rev. 43 (2008) 335–357.
[35] J.M. Karlinsey, Anal. Chim. Acta 725 (2012) 1–13.
[36] J. Ruzicka, Analyst 125 (2000) 1053–1060.
[37] J.-H. Wang, E.H. Hansen, M. Miró, Anal. Chim. Acta 499 (2003) 139–147.
[38] X.-B. Long, M. Miró, R. Jensen, E.H. Hansen, Anal. Bioanal. Chem. 386 (2006) 739–748.
[39] X.-B. Long, M. Miró, E.H. Hansen, J.M. Estela, V. Cerdà, Anal. Chem. 78 (2006) 8290–8298.
[40] Y.-L. Yu, Y. Jiang, M.-L. Chen, J.-H. Wang, Trends Anal. Chem. 30 (2011) 1649–1658.
[41] M. Miró, S. Kradtap-Hartwell, J. Jakmunee, K. Grudpan, E.H. Hansen, Trends Anal. Chem 27 (2008) 749–761.
[42] H. Erxleben, J. Ruzicka, Anal. Chem. 77 (2005) 5124–5128.
[43] Y.-L. Yu, Z. Du, J.-H. Wang, J. Anal. At. Spectrom. 22 (2007) 650–656.
[44] Y.-L. Yu, Z. Du, M.-L. Chen, J.-H. Wang, J. Anal. At. Spectrom. 22 (2007) 800–806.
[45] A.N. Anthemidis, V. Cerda, M. Miró, J. Anal. At. Spectrom. 25 (2010) 1717–1723.
[46] G. Cutter, P. Andresson, L. Codispoti, P. Croot, R. Francois, M. Lohan, H. Obata, M.R. van der Loeff, Sampling and Sample-handling Protocols for GEOTRACES Cruises, GEOTRACES Standards and Intercalibration Committee, 2010, Virginia, USA, pp. 52–61. Available at 〈http://www.obs-vlfr.fr/GEOTRACES/libraries/documents/Intercalibration/Cookbook.pdf〉, (accessed 07.12.02).
[47] D.C. Montgomery, Design and Analysis of Experiments, 7th ed., John Wiley &Sons, 2009, pp. 168–177 (Chapter 5).
[48] T. Lundstedt, E. Seifert, L. Abramo, B. Thelin, Å. Nyström, J. Pettersen, R. Bergman, Chemom. Intell. Lab. Syst. 42 (1998) 3–40.
[49] B. Dajaegher, Y. Vander Heyden, LC–GC Eur. 21 (2008) 96–102.
[50] K. Leopold, M. Foukes, P. Worsfold, Anal. Chim. Acta 663 (2010) 127–138.
[51] B. Horstkotte, J.C. Alonso, M. Miró, V. Cerda, Talanta 80 (2010) 1341–1346.

[52] M. Miró, J.M. Estela, V. Cerdà, Talanta 63 (2004) 201–223.
[53] J.P. Gustafsson, Visual MINTEQ 3.0, KTH, Sweden. Available at 〈http://www2.lwr.kth.se/english/OurSoftware/Vminteq/download.html〉, (accessed 07.12.12).
[54] A. Sugii, N. Ogawa, H. Imamura, Talanta 26 (1979) 941–944.
[55] A.M. Serra, J.M. Estela, V. Cerda, Talanta 77 (2008) 556–560.
[56] B.F. Reis, E. Ródenas-Torralba, J. Sancenón-Buleo, A. Morales-Rubio, M. de la Guardia, Talanta 60 (2003) 809–819.
[57] C. Ibáñez-Palomino, J.F. López-Sánchez, A. Sahuquillo, Anal. Chim. Acta 720 (2012) 9–15.