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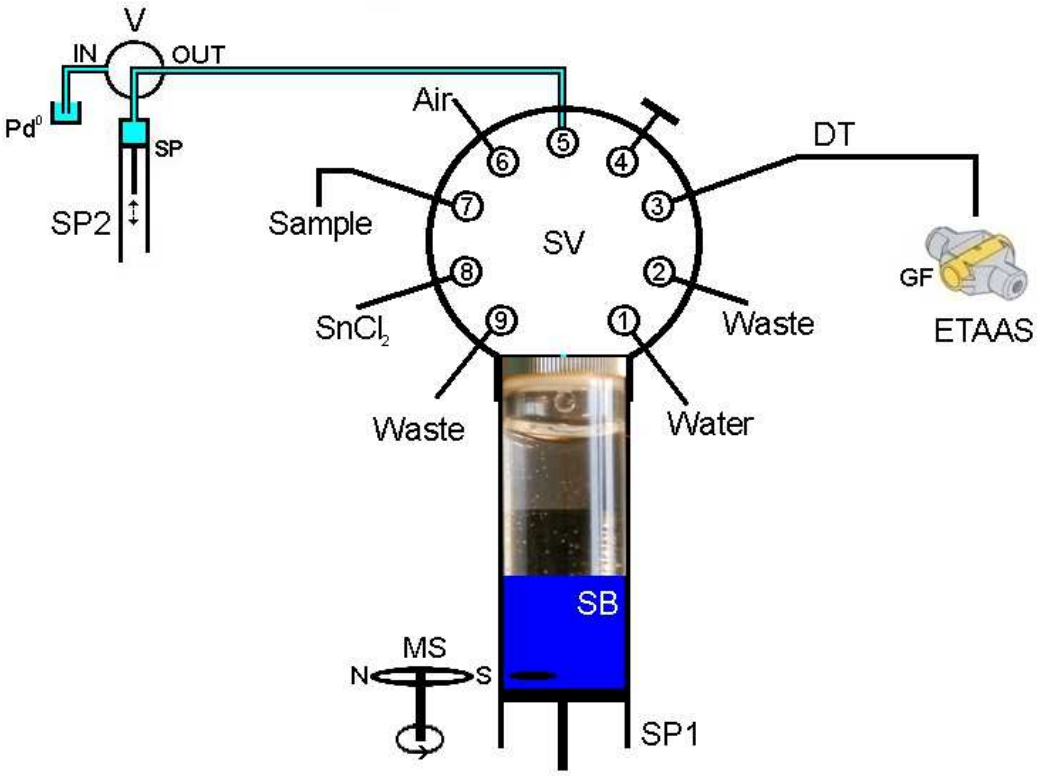

Automated lab-in-syringe headspace single drop microextraction (LIS-HS-SDME) hyphenated to ETAAS for mercury determination

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Automated headspace single-drop microextraction via a lab-in-syringe platform for mercury electrothermal atomic absorption spectrometric determination after *in situ* vapor generation

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Abstract

A novel fully automated headspace single drop microextraction system based on a programmable lab-in-syringe platform hyphenated to electrothermal atomic absorption spectrometry (ETAAS) for in-situ vapor generation assays, was developed. Mixing of precise metered volumes of sample and stannous chloride solutions along with generation of mercury vapor were accomplished inside the microsyringe under reduced pressure environment in a closed manner excluding the possibility of analyte losses. The released of Hg^0 vapor was trapped on the surface of a 25 μL aqueous microdrop consisted of a finely dispersed Pd^0 via amalgamation process. Reduced pressure conditions during the preconcentration step increased the extraction rates, resulting in a shorter cycle of analysis and higher sampling frequency. The proposed preconcentration system was fully characterized through optimization of the relevant parameters affecting the generation and sequestration of the vapor of mercury. For 3.5 mL sample, an enhancement factor of 75 was obtained. The detection limit and the precision expressed as relative standard deviation (RSD) were 0.48 μg L^{-1} and 4.2 % (at 5.0 μg L^{-1} Hg(II) concentration level) respectively. The proposed LIS-SH-SDME-

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ETAAS method was evaluated by analyzing the IAEA-350 and BCR 278-R certified reference materials as well as environmental water samples.

Keywords: headspace single drop microextraction; lab-in-syringe; on-line preconcentration; atomic absorption spectrometry; mercury determination.

Introduction

In the last decades, the concern for the environmental pollution and human health has led to the development of green analytical methods eliminating or reducing the consumption of organic solvents as well as using simple, rapid and economical sample preparation / preconcentration methodologies.[1] In this context, Jeannot and Cantwell[2] introduced a solvent-minimized sample pretreatment approach with the term of "single drop microextraction (SDME)□. In principal, SDME is based on the exposure of a hanging micro drop of an immiscible extraction solvent to an aqueous sample solution. Thus, high enrichment factors can be obtained as a result of the great reduction of the extractant phase-to-sample volume ratio. The advantages of SDME that make it particularly attractive include: simplicity without the need of any dedicated equipment, low cost, reduced solvent consumption and waste generation as well as lack of sample carryover.[3, 4]

Typically, two main approaches of SDME are used for the determination of various analytes: the direct immersion (DI-SDME) and the headspace (HS-SDME).[5] In HS-SDME, a microdrop of organic solvent or aqueous solution suspended at the tip of a micro-syringe is exposed in the headspace of a sample solution in order to extract and preconcentrate volatile or semi-volatile analytes. In this case the analyte is distributed between the three phases (aqueous, headspace and microdrop), while rapid stirring of the sample solution can be employed without the undesirable impact on the stability of the droplet. HS-SDME can be

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easily coupled with atomic spectrometric techniques that require sample volumes in microliters such as electrothermal atomic absorption spectrometry (ETAAS) and electrothermal vaporization-inductively coupled plasma optical emission spectrometry/mass spectrometry (ETV-ICP-OES/MS).[6] Bendicho et al.[7, 8] reported a novel solvent-free mode of HS-SDME for the preconcentration of hydride forming or vapor generating elements onto a noble metal containing an aqueous drop prior detection by ETAAS.

Although several efforts have been made towards the improvement of sample preparation techniques, they are still tedious and laborious with multistep protocols involving repeated manual manipulation of the extracts. Sample pretreatment is estimated as the two-thirds of the total analysis time and is considered to be the primary source of errors. Hence, proper selection and optimization of the sample preparation procedure are essential points that can significant affect the accuracy and precision of the final results.[9] A challenging task in analytical procedures is the controllable and reproducible handling of the solutions and samples prior the final determination. Automated flow approaches have attracted the researchers' attention due to the fact that all chemical and physical manipulations can be made on-line in an enclosed environment resulting in minimum risk of sample contamination and increased safety of the operator as well.[10]

Recently, Cerda et al.[11] introduced an interesting automated system, the so-called lab-in-syringe, which proved to be an excellent tool for downscaling fluidic manipulations with the concomitant in-syringe accommodation of wet chemical or heterogeneous reactions at will. Similar methodology was later reported for spectrophotometric assays of copper and aluminum after in syringe dispersive liquid−liquid microextraction[12, 13] and for automatic cold vapor atomic absorption spectrometric determination of mercury using a novel integrated lab-in-syringe/gas−liquid separation (LIS/GLS) programmable batch-flow system.[14] An inherent

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advantage of LIS systems is the fact that the syringe pump is an entirely closed reaction vessel with great potential for accommodation of vapor generation approaches.

Ouyang et al.[15] developed a semi-automatic HS-SDME method for liquid samples in discrete vials using a robotic auto-sampler apparatus, CTC Combipal autosampler (Zwingen Switzerland).[16] In this work, all the operational parameters involved in the headspace microextraction process could be precisely and conveniently controlled by the autosampler and the associated cycle composer software. The above system has been successfully applied for organic volatile compounds,[17] however, there is no other application in the literature for inorganic substances like hydride or vapor forming elements.

In the present work, a novel fully automated headspace single drop microextraction system based on a lab-in-syringe platform is presented for inorganic mercury determination via in-situ cold vapor generation and ETAAS. To the best of our knowledge, this is the first paper reporting the potential use of LIS for HS-SDME. On the basis of versatile and precise programmable flow, the LIS setup is amenable to expedient mixing of the sample and reducing solutions in a batch-flow format followed by the release and sequestration of the volatile vapor of Hg^0 into the microdrop consisted of a finely dispersed Pd^0 into the aqueous solution. Moreover, reduced pressure conditions were adopted for the first time in a LIS system for automatic sample derivatization. The proposed preconcentration system was fully characterized through optimization of the relevant parameters affecting the generation and sequestration of the vapor of mercury.

Experimental

Reagents and samples

All chemicals were of analytical grade. Ultra-pure quality water was obtained from a Milli-Q system (Millipore, Bedford. USA, www.millipore.com). Mercury working standard

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solutions were prepared by appropriate stepwise dilution of 1000 mg L^{-1} Hg(II) stock standard solution in 0.5 mol L^{-1} HNO_3 (Merck Titrisol). The standard and sample solutions were acidified to 0.01 mol L^{-1} HCl (pH ~ 2.0) by dilute HCl. The reductant solution, 4.0% (*m/v)* $SnCl_2$ in 0.5 mol L^{-1} HCl was freshly prepared from $SnCl_2 \cdot 2H_20$ (Merck, <0.000001% mm Hg). The $SnCl_2$ solution was purified from possible traces of elemental mercury by 30 min degassing with argon prior use. The trapping agent solution was prepared by dilution of 1000 mg L^{-1} Pd stock standard solution $Pd(NO_3)_2$ in 0.5 mol L^{-1} HNO_3 (Merck Titrisol). L(+) ascorbic acid (Merck) was used to obtain a dispersion of Pd^0 in aqueous solution. All glassware were cleansed for at least 24 h in 10% (v/v) nitric acid solution and rinsed with ultra-pure water prior use.

In order to evaluate the proposed method, the following standard reference materials were used: BCR 278-R (Community Bureau of Reference Brussels, Belgium) trace elements in mussel tissue and IAEA-355 tuna fish tissue homogenized. An amount of ca. 0.4 g of tissue was precisely weighed into sealed Teflon crucibles and wetted by a mixture of HNO_3-$HClO_4$-H_2O_2 in a volume ratio of 3:2:0.5. The digestion procedure was carried out in a stainless-steel pressurized bomb at 120 ± 5 °C for 2 h, according to the recommendations of the manufacture. After cooling the system, the digests were properly diluted in ultra-pure water and used for the total determination of mercury.

The analyzed environmental water samples were collected from the Northern Greece region. The seawater sample was from Thessaloniki bay, while the waste-water sample was from a central ditch of the industrial area of Thessaloniki. All samples were filtered through 0.45 μm cellulose acetate membrane filters (Millipore), acidified to 0.01 mol L^{-1} HCl and stored in acid-cleaned polyethylene bottles prior analysis.

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Apparatus and Software

A Perkin-Elmer Model 5100 PC atomic absorption spectrometer (Perkin-Elmer, Norwalk, CT, USA, http://las.perkinelmer.com) with Zeeman-effect background correction and transversely heated graphite tube atomizer (THGA) with circulating cooling unit was employed as detection system. The furnace graphite system was connected with the AS-71 autosampler for automatic injection of samples into THGA. Argon 99.996 % was used as purge and protective gas. Pyrolytically coated THGA (Perkin-Elmer) with integrated L'vov platform were used throughout the measurements. The graphite furnace temperature/time program for mercury determination is summarized in Table 1. A Perkin Elmer mercury electrodeless discharge lamp (EDL) was used as a light source operated at 5 W. The wavelength was set at 253.7 nm resonance line and the monochromator spectral bandpass (slit) was 0.7 nm. Integrated absorbance (peak area) was used exclusively for signal evaluation.

A diagram of the entire LIS-HS-SDME system is presented schematically in Fig. 1. A high precision bi-directional micro-syringe pump, SP1 (MicroCSP-3000, FIAlab Instruments, Bellevue, WA) consisting of a glass syringe barrel (SB) with a capacity of 5000 μL and a nine-position Teflon/Kel-F selection valve, SV at the head of it, was used as the main sample processing unit. An additional micro-syringe pump, SP2 (Cavro, Sunnyvale, CA) with a capacity of 1000 μL and a two-position (IN/OUT) valve, V at the head of it, which was part of a FIAlab®-3000 (Alitea FIAlab, USA) sequential injection (SI) system, was used for precise and accurate formation of the headspace microdrop. The two-position valve, V facilitates the communication of SP2 with either an external reservoir (drop solution) or with port 5 of the SV. The operational procedure of the lab-in-syringe head-space single drop microextraction (LIS-HS-SDME) system was computer controlled by the FIAlab application

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software for windows v. 5.9.245 (http://www.flowinjection.com) and synchronized with the commands for the activation of the ETAAS program through an electric interface.[18, 19]

The length of PTFE tubing used for all connections as well as that for microdrop delivery into the graphite tube was kept as short as possible in order to minimize the dead volume in the proposed system.

A Metrohm (http://www.metrohm-autolab.com/) E649 magnetic stirrer was used for strong mixing of the aqueous phase to produce turbulence and release of mercury vapor.

Fig. 1

Table 1

The LIS-Headspace device

The glass barrel of the micro-syringe SP1, termed as LIS-Headspace (LIS-HS) devise, operated as a closed reaction chamber eliminating potential interfering effects from external sources as well as possible loss of volatile compounds. The sample solution and the reducing agent were aspirated subsequently into the SB by means of the SV in a similar way as in lab-on-valve systems.[14] A prominent advantage of the LIS-HS devise is the fact that reduced pressure (< 0.2 atm) into the SB can be easily produced by downwards movement of the piston while SV valve is in a plugged port (Fig. 1, port 4). The vapor release from the aqueous phase in a reduced pressure environment significantly increases the liberation rate resulting in a decreased time of analysis.

An effective stable and reproducible aqueous microdrop, with a large interfacial area hanging at the top of the SB-headspace was achieved after appropriate modification of the conduit connector (CC) (15 mm length, 2 mm i.d.) between the SB and SV. In this context, a capillary tube (18 mm length, 0.3 mm i.d.) made of polytetrafluoroethylene (PTFE) was

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placed into this CC in such a way to protrude from the top of the SB into the headspace for about 3 mm length as shown in Fig. 2b,c. This modification enables the minimization of the dead volume of the CC resulting in a larger active hanging drops formation. The volume of stable microdrops that could be formed with the above set-up was ranged up to 25 μL, which is a considerable advantage of the proposed method over the majority of HS-SDME ones, which use microdrops with volume smaller than 5 μL. It should be mentioned that in case of a hydrophilic capillary tube (made of glass), the formation of relatively larger aqueous (hydrophilic) drops would be facilitated. However, taking into account the technical difficulties of placing the glass capillary into the CC of the SV as well as the fact that a drop of 25 μL was adequate for the developed ETAAS method, a PTFE capillary tube was adopted instead of a glass one.

Fig. 2

Table 2

Operating procedure

The complete operational sequence for automatic on-line determination of Hg(II) using the LIS-HS-SDME scheme is listed in Table 2, and summarized as follows:

a) Loading (sample - reductant). Initially, SP1 aspirated consecutively 100 μL of reductant solution and 3500 μL of sample solution into SB. Next, a volume of 200 μL of air was aspirated into the SB in order to provide appropriate headspace for the microdrop formation. In the meantime, the magnetic stirrer (MS) was activated and the mercury vapor was generated.

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b) Headspace microdrop formation. A minute volume (25 μL) of the trapping solution was aspirated by SP2 and subsequently dispensed via the CC in order to the microdrop into the headspace.

c) Preconcentration. Consequently, the releasing mercury vapor into the headspace was being trapped on the microdrop surface resulting in the preconcentration of Hg^0. For enhanced mercury vapor release, a reduced headspace pressure was applied by downward movement of the SP1-piston, while SV was in the plugged port 4. Next, the reduced pressure was equilibrated prior the microdrop transportation to ETAAS by activating SP1.

d) Transportation and measurement. In the following steps (9-13) transportation of the microdrop from the headspace to graphite furnace of ETAAS via the delivery tube (DT) took place. The ETAAS program was automatically activated and the tip of autosampler arm moved into the dosing hole of the graphite tube for the injection of the microdrop and mercury atomization/quantification.

e) Cleaning. The cleaning operation of the manifold and its preparation for the next analytical cycle was synchronized with the ETAAS program, to be accomplished during the measurement step.

The whole procedure is given in a representative video in the supplementary material (S1).

Results and Discussion

Trapping agent

A preconcentration technique involving trapping of mercury by amalgamation is generally regarded as the most sensitive for its determination. For this purpose, noble metals such as gold, platinum and palladium have been successfully used for the coating of the

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graphite tubes followed by atomic absorption spectrometric detection.[20-22] Recently, Bendicho et al. reported a novel organic-free mode of HS-SDME for vapor forming elements preconcentration onto a noble metal-containing aqueous microdrop prior to detection by ETAAS.[6-8] In these works the HS-SDME method was based on the use of palladium nitrate solution as sorbent in the drop, which also acts as matrix modifier for the electrothermal atomization of mercury according the manufacturer recommendations. The sequestration mechanism was based either on the catalytic decomposition of the hydrides or on the amalgamation of Hg^0 with the finely dispersed Pd^0 formed on the drop surface.[7] Pd^0 arises as a result of the reducing action caused by the nascent hydrogen gas that evolves in the headspace after the sodium tetrahydroborate decomposition.

Due to the fact that in the proposed LIS-HS-SDME method, $SnCl_2$ was adopted as more convenient reducing agent for mercury vapor generation instead of $NaBH_4$, Pd^0 could not be formed on the microdrop surface during the extraction procedure and thus, mercury vapor could not be trapped. This problem was addressed by off-line preparation of the trapping solution by reducing Pd(II) to Pd^0 adding an appropriate amount of ascorbic acid, which acts as a reduction agent. In this way, a finely dispersion of Pd^0 was produced throughout the entire mass of the trapping solution. In the proposed LIS-HS-SDME system this trapping solution was used for the hanging drop formation. The obtained drop was more repeatable and homogeneous with higher amount of dispersed Pd^0 comparing with that of previous reported batch methods. Considering the trapping solution (TS), it was freshly prepared prior analysis using ascorbic acid in ten times the stoichiometric amount (ca. 0.03% m/v ascorbic acid in TS). The produced Pd^0 was remained dispersed into the aqueous phase without any precipitation for at least 24-hours. It should be mention that reduction of Pd(II) to Pd^0 using a much higher amount of ascorbic acid could lead to aggregation and fast precipitation of the dispersed Pd^0.

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Fig. 3

The effect of palladium concentration in the trapping solution was studied in the range 0 – 50 mg L^{-1}. The volume of the microdrop was 25 μL. As it is shown in the diagram of Fig. 3, no sequestration of Hg^0 was observed in the absent of palladium, while for concentrations higher than 20 mg L^{-1} the recorded absorbance was maximum and remained almost constant. Thus, a concentration of 20 mg L^{-1} Pd(II) was used for further studies.

Effect of the microdrop volume

The volume of the drop defines the interfacial layer between the liquid phase of the drop and the gaseous phase into the headspace. As expected more efficient mass transfer was observed for higher drop volume in the range 5 – 25 μL. Taking into consideration the extraction efficiency of the proposed manifold and the fact that stable microdrops with volume up to 25 μL could be prepared in a repetitively way, a microdrop of 25 μL was adopted for further experiments. This volume was also suitable for injection into the graphite furnace.

Reduced pressure and stirring

The application of reduced pressure conditions during headspace solid phase microextraction (HS-SPME) sampling had been considered in the past but overlooked.[23] Recently, Psillakis et al. proposed the vacuum-assisted HS-SPME (Vac-HS-SPME) extraction procedure in which the mass transfer from the aqueous phase to the headspace is the rate-determining step.[24, 25] As demonstrated within short sampling times, under non equilibrium conditions, Vac-HS-SPME results in higher extraction efficiency compared to regular HS-SPME due to enhancement of the evaporation rates.

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An inherent advantage of the proposed LIS-HS-SDME system is the fact that reduced pressure conditions can be easily applied into the syringe barrel without the need of any external vacuum pump. In the proposed manifold, the increase in headspace from 200 μL to 1400 μL with vertical movement of the syringe piston downwards (Table 2; steps 3, 7) leads to reduced pressure which ranged up to 0.14 atm. In this manner, the liberation rate of mercury vapor into the HS was increased significantly.

In general, stirring of the solution is expected to increase the mass transfer of mercury vapor in headspace and consequently enhance the amount of analyte extracted regardless of the pressure conditions inside the sampling vessel.[26] Taking into account the high density of mercury vapor, strong mixing of the aqueous phase is necessary in order to produce turbulence, allowing the vapor to reach the interface area faster and thus frequent exchanges between the aqueous phase and the microdrop surface be performed. The effect of stirring rate was studied in the range 200-1900 rpm. The integrated absorbance was increasing by increasing the stirring rate without any impact on the drop stability. Thus, the higher level of stirring (1900 rpm) was used for further experiments.

Extraction time

HS-SDME technique is an equilibrium-based process regarding the mass transfer from the liquid phase to headspace and from headspace to liquid phase of the hanging drop. The time needed to attain partition equilibrium is depended from parameters such as reduced pressure and stirring. However, due to the fact that in automatic systems all the operation sequences and conditions are highly reproducible, it is not necessary to measure in thermodynamic equilibrium conditions. In this context, the effect of the extraction time on the absorbance of mercury was investigated in the range 0–900 s under either normal or reduced pressure conditions. The obtained results showed an increase in the integrated absorbance

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with the increase of the extraction time up to 300 s for reduced pressure and up to 600 s for normal pressure. After the above extraction times, the absorbance remained practically unchanged. As a compromise between sensitivity and sampling frequency, an extraction time of 300 s was adopted.

Sample volume

In general, in preconcentration methods sample volume significant affects the sensitivity, although high volume of liquid phase can lead to inhibition of the release of the volatile substances.[27] The effect of sample volume on the integrated absorbance was studied within the range 1.0 to 3.5 mL. A positive correlation of the absorbance with the sample volume in the studied area was observed. On the other hand, keeping constant the mass of mercury amount into the reaction chamber (syringe barrel), the signal slightly decreased by increasing the sample volume, confirming the result of liquid phase on vapor release. For high sensitivity, a sample volume of 3.5 mL was selected.

Reductant type, volume, concentration

Regarding the reducing agent for cold vapor generation, two reductants like $NaBH_4$ and $SnCl_2$ were assayed. As it was proved, $NaBH_4$ was incompatible for in-syringe headspace SDME due to the elevated pressure arising from nascent hydrogen generation into the syringe barrel, which resulted in microdrop instability and inhibition of vapor liberation.[28] In addition, $NaBH_4$ reduces both either inorganic or organic mercury, limiting the selectivity of the method. In contrast, by using $SnCl_2$ all the above problems were overcome. Moreover, it facilitated the drop formation closer to the sample upper surface without the risk of drop dislodging because of aqueous phase splashing, as happened in previous systems.[6] For higher

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sample volume, 100 µL of $SnCl_2$ solution was adopted. A concentration of 4.0% (*m/v)* $SnCl_2$ in 0.5 mol L^{-1} HCl was selected as the working reduction solution.

Interfering studies

Determination of mercury using the cold vapor technique offers an inherent advantage of limited interferences which is focused on the elements which are responsible for inhibition of vapor releasing. The interference effect on the determination of inorganic mercury was investigated by analyzing a standard solution of 10.0 µg L^{-1} Hg(II) containing a given metal species, using the proposed LIS-HS-SDME system under the optimum conditions described above. A recovery deviation more than ± 5 % was considered as significant interference. Experimental results revealed that the method could tolerate concentrations of Al(III), Cr(IV), Co(II), Fe(III), Cu(II), Pb(II), Mn(II) and Zn(II) at least up to 4 mg L^{-1}. In addition, commonly encountered matrix components in natural water samples like Ca(II), Ba(II) and Mg(II) were tolerated at least up to 1000 mg L^{-1} and NaCl up to 35 g L^{-1}.

Analytical features

The analytical performance characteristics of the proposed LIS-HS-SDME method for inorganic mercury determination, under the optimum conditions are summarized in Table 3. For 300 s extraction time and 3.5 mL of sample, the calibration curve was linear in the range 1.6 – 40.0 µg L^{-1} with a good correlation coefficient (r) of 0.9988. The limit of detection (c_L), defined as 3σ criterion (3 times the standard deviation of the blank solution measurements divided by the slope of the corresponding calibration curve), was 0.48 µg L^{-1} and the limit of quantitation (q_L, 10σ criterion) was 1.6 µg L^{-1}. The precision, in terms of repeatability, expressed as relative standard deviation (RSD), was 4.2 at 5.0 µg L^{-1} Hg(II) concentration level. The sampling frequency was 8 h^{-1}. The enhancement factor, defined as the ratio of the

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slopes (S) of the calibration curves obtained with (S = 0.0075) and without (S = 0.0001) preconcentration by direct injection of 25 μL of aqueous standard solutions, was calculated as 75. The recovery was investigated by analyzing spiked coastal seawater and ditch water samples with standard amount of Hg(II) before any pretreatment and ranged from 96 to 98 %.

Table 3

Validation of the method

The accuracy of the proposed LIS-HS-SDME method was estimated by analyzing two standard reference materials: BCR 278-R (Community Bureau of Reference Brussels, Belgium) trace elements in mussel tissue and IAEA-355 tuna fish tissue homogenized.

Student t-test was used to examine the statistically significant differences between the certified values and the obtained results. The measured concentrations for total mercury after wet digestion and $t_{exp,}$ values for mercury determination are given in Table 4. Since all $t_{exp,}$ values were lower than the $t_{crit,\ 95\%} = 4.30$, no statistically significant differences were found at the 95% probability level. Due to the environmental significance of mercury, representative samples of costal seawater and ditch water were analyzed. The corresponding results are presented in Table 5. The good values of recoveries (96 and 98 %), demonstrated the applicability of the proposed method for trace analysis of similar environmental samples.

Table 4

Table 5

Conclusions

An automated on-line lab-in-syringe system for headspace single drop microextraction hyphenated to ETAAS has been presented for mercury determination. This platform

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constitutes an effective alternative approach to conventional mode of HS-SDME which is characterized by simplicity, accuracy and elimination of analyte losses due to automatic solutions' handling in a closed manner. Another inherent advantage of the proposed system is the ability of the syringe pump to produce reduced pressure conditions at will, facilitating the vapor release and resulting in shorter cycle of analysis. The proof of concept of the creation of reduced pressure condition by means of a syringe pump was herein presented for the first time for mercury HS-SDME determination. The present method is considered as a Green Analytical Method because of complete eliminating the use of organic solvents using aqueous microdrop consisted of a finely dispersed of palladium. The LIS-HS-SDME-ETAAS system was successfully applied to Hg determination in complex matrix samples like tuna fish tissues, mussel tissue as well as sea and waste water with good accuracy and precision.

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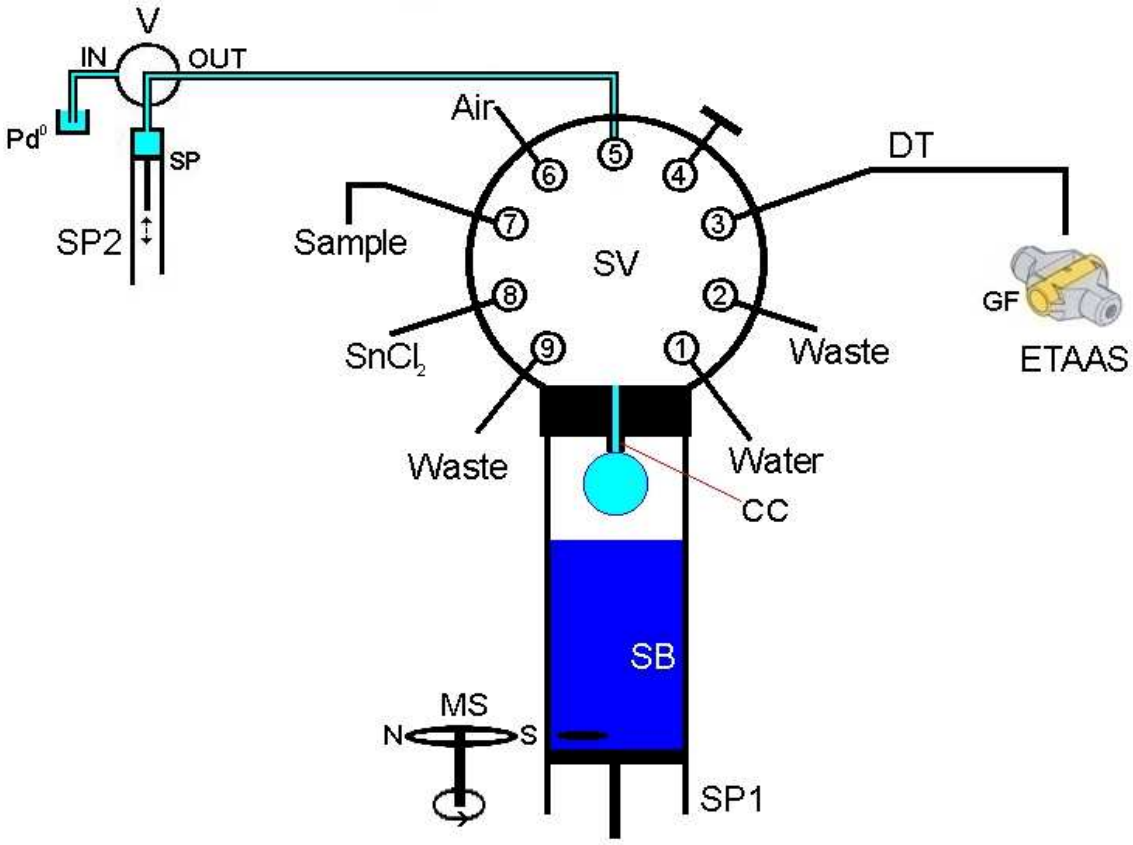

Fig. 1. Schematic diagram of the LIS-HS-SDME system coupled with ETAAS for mercury determination. SP1, SP2, syringe pumps; V, 2-port selection valve; SV, 9-port selection valve; DT, delivery tube; SB, syringe barrel; CC, conduit connector; MS, magnetic stirrer; GF, graphite furnace of ETAAS.

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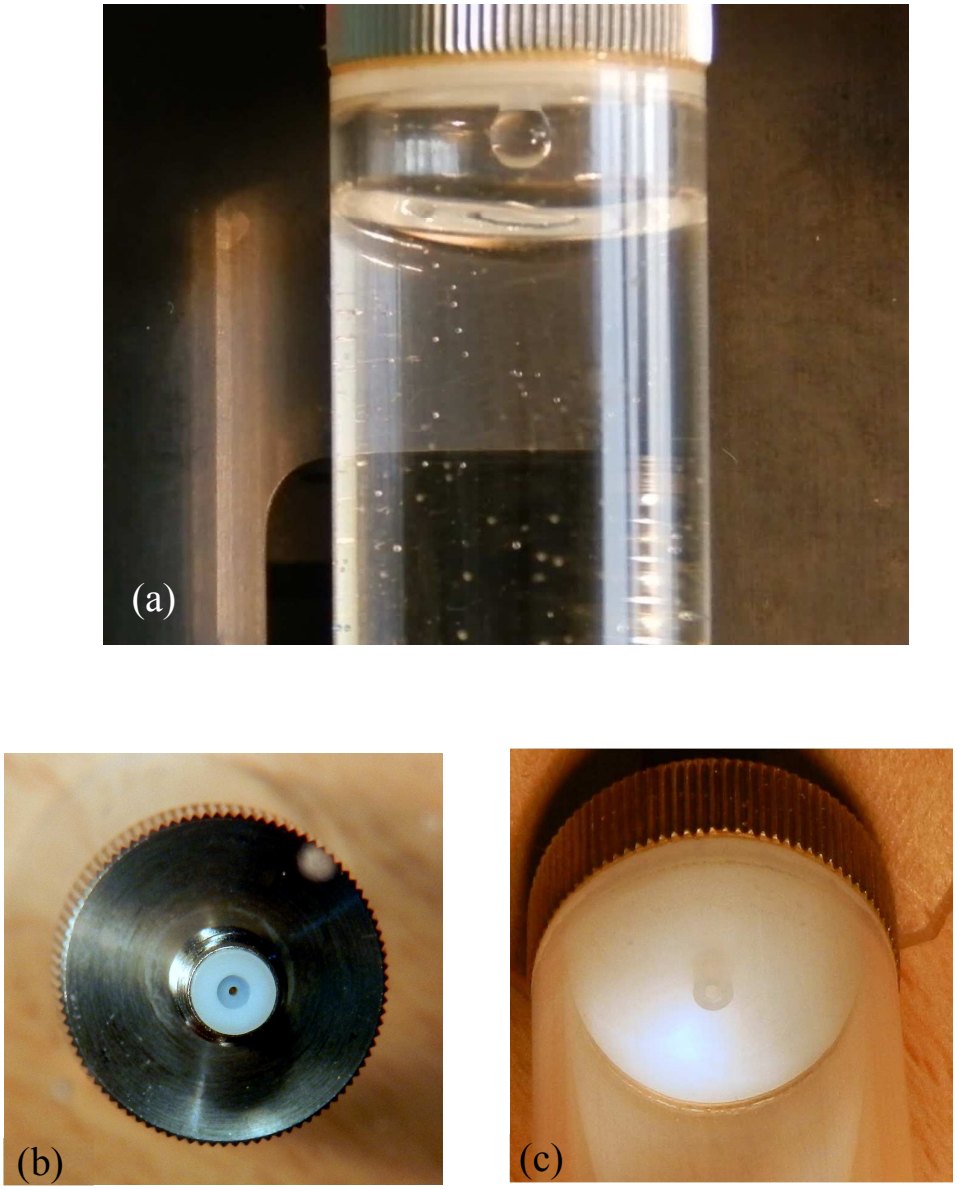

Fig. 2. a) Photo of the syringe barrel (SB) and the hanging microdrop; b) and c) Photos of the conduit connector with the capillary tube into it.

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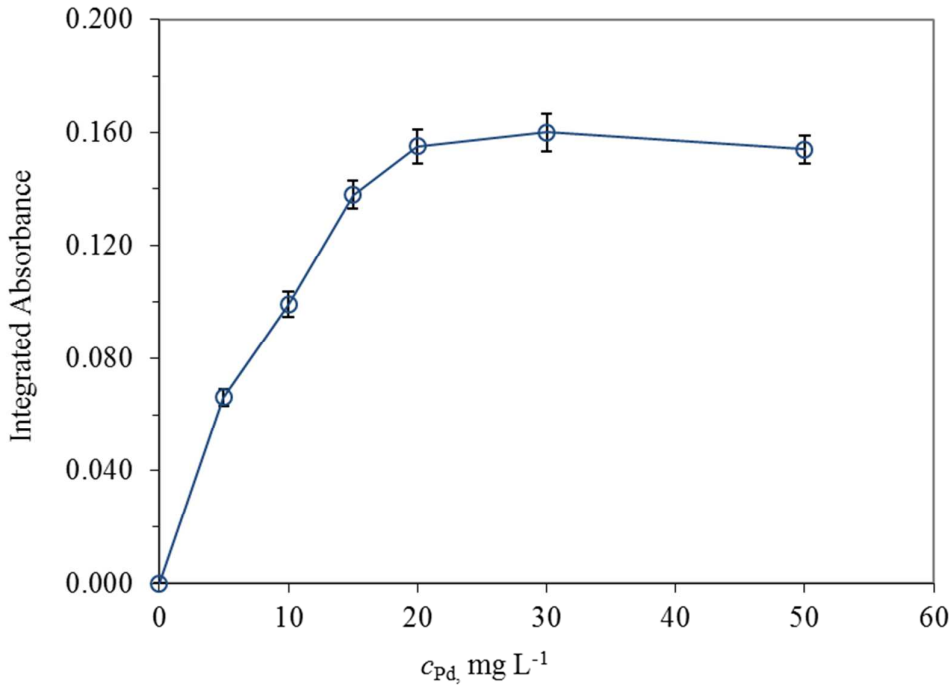

Fig. 3. Effect of palladium concentration on the absorbance of 20.0 μg L^{-1} Hg(II). Error bars calculated based on standard deviation values (n=5). All other experimental parameters are shown in Table 2.

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Table 1. Graphite furnace temperature program for determination of mercury by ETAAS following the headspace microextraction

Step	Temperature (^{o}C)	Ramp time (s)	Hold time (s)	Argon flow rate (mL min^{-1})
Drying	110	10	20	250
Ashing	200	10	10	250
Atomization	1300	0	5	0
Cleaning	2000	1	2	250

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Table 2. Operation sequences of the LIS-HS-SDME system for inorganic mercury determination by ETAAS

Step	Position		Operation		OT (s)	Volume (μL)			Flow-rate (μL s^{-1})		Commentary
	V	SV	SP1	SP2		MS	SP1	SP2	SP1	SP2	
a) Loading (Sample-Reductant)											
1	IN	8	Aspirate		1.3	OFF	100	-	80	-	Reductant into SB
2	IN	7	Aspirate		17.5	ON	3500	-	200	-	Sample solution into SB
3	IN	6	Aspirate		1.0	ON	200	-	200	-	Air into SB
b) Headspace microdrop formation											
4	IN	5		Aspirate	2.5	ON	-	25	-	10	Drop solution into S1
5	OUT	5		Dispense	5.0	ON	-	25	-	5	Drop formation into headspace of SB
c) Preconcentration											
6	OUT	4	Aspirate		12	ON	1200	-	100	-	Headspace pressure reduction
7	OUT	4			300	ON	-	-	-	-	Hg vapor sequestration on the drop surface / preconcentration (300 s)
8	OUT	4	Dispense		12	OFF	1200	-	100	-	Headspace pressure equilibration
d) Transportation and measurement											
9											Activation of ETAAS program: tip of autosampler arm moves into the graphite tube
10	OUT	3	Dispense		5	OFF	100	-	20	-	Removal of the microdrop from the headspace
11	OUT	6	Aspirate		5	OFF	500	-	100	-	Air into SB
12	OUT	3	Dispense		25	OFF	500	-	20	-	Transportation of drop solution into the atomizer
13											Autosampler arm moves back to the original position; ETAAS runs the temperature program
e) Cleaning											
14	OUT	2	Dispense		12.5	OFF	3700	-	300	-	
15	OUT	1	Aspirate		10	OFF	3000	-	300	-	
16	OUT	2	Dispense		10	OFF	3000	-	300	-	
17	OUT	1	Aspirate		10	OFF	3000	-	300	-	Cleaning of Syringe Barrel and DT
18	OUT	3	Dispense		3.3	OFF	1000	-	300	-	
19	OUT	2	Dispense		6.6	OFF	2000	-	300	-	
20	OUT	6	Aspirate		3.3	OFF	1000	-	300	-	
21	OUT	3	Dispense		3.3	OFF	1000	-	300	-	

OT: Operation Time, MS: Magnetic Stirrer, SB: Syringe Barrel, DT: Delivery Tube

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Table 3. Analytical performance characteristics of the LIS-HS-SDME method for mercury determination

Sample volume (mL)	3.5
Drop volume (μL)	25
Sampling frequency (h^{-1})	8
Enhancement factor	75
Linear range (μg L^{-1})	1.6 – 40.0
Detection limit, c_L (μg L^{-1})	0.48
Precision, RSD, % (n = 9)	4.2 (at 5.0 μg L^{-1})
Regression equation	A = (0.0075 ± 0.0004) [Hg(II)] + (0.0031 ± 0.0079)
(8 standards; n = 5; [Hg] in μg L^{-1})	
Correlation coefficient (r)	0.9985

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Table 4. Analytical results for total mercury determination in CRMs after wet digestion

CRM	Certified value mg kg^{-1}	Found*	Recovery (%)	t_{exp}
IAEA-350 (Tuna homogenized)	4.10 (3.31 – 4.42)	3.9 ± 0.2	95.1	1.732
BCR 278-R (Mussel tissue)	0.196 ± 0.009	1.84 ± 0.01	93.9	2.078

*, mean value ± standard deviation based on three replicates; $t_{crit.}$ = 4.30 at 95 % probability level.

Table 5. Analytical results for Hg(II) determination in spiked environmental water samples

Sample	Added*	Found*	*R* (%)
Ditch wastewater	-	2.2 ± 0.1	-
	5.0	7.1 ± 0.2	98.0
Coastal seawater	-	< c_L	-
	5.0	4.8 ± 0.2	96.0

*, concentration in μg L^{-1}, mean value ± standard deviation (*n*=5); *R*, recovery.

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