Cold vapor ionic liquid-assisted headspace single-drop microextraction: A novel preconcentration technique for mercury species determination in complex matrix samples

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A novel technique named cold vapor ionic liquid-assisted headspace single drop microextraction (CV-ILAHS-SDME) was developed for Hg species determination at trace levels. Inorganic (InHg) and organomercury (OrgHg) species separation, preconcentration and determination were performed by *in situ* cold vapor generation (CV) followed by headspace extraction with a suspended microdrop of a low cost RTIL, *i.e.* tetradecyl(trihexyl)phosphonium chloride (CYPHOS® IL 101), and direct injection in electrothermal atomic absorption spectrometry (ETAAS). Stannous chloride ($SnCl_2$) was used to reduce Hg^{2+} to volatile Hg^0, while oxidation of OrHg species permitted the determination of total Hg. OrHg species concentration was evaluated based on the difference between total Hg and InHg concentration. Different variables of CV-ILAHS-SDME technique, such as cold vapor generation conditions, temperature, sample and solvent volume, extraction time, and stirring rate were carefully studied. The analytical sensitivity was enhanced by a factor of 75. A low detection limit (10 ng L^{-1}) and good precision (relative standard deviation of 4.6% at 0.25 μg L^{-1} Hg and $n = 10$) were achieved. Experimental results demonstrated that CV-ILAHS-SDME using CYPHOS® IL 101 is a rapid, cost-effective and green microextraction technique for Hg determination in samples with a complex matrix, such as sea water, fish tissues, hair and wine.

Introduction

Recently, the development of miniaturized methods has received increasing attention for counting on less contaminant and low-cost sample preparation procedures in trace analysis.[1–3] Solid phase microextraction (SPME) and single-drop microextraction (SDME) techniques have been widely used in the last years as powerful tools for the preconcentration and matrix separation of a variety of organic and inorganic compounds.[4,5] In SPME, a thin fused silica fiber coated with a stationary phase is exposed to the sample or its headspace, and partitioning of the target analytes between the sample matrix and the fiber coating takes place. However, the main drawback of SPME fibers is their limited lifetime, and additionally, precision may be affected by prolonged usage.[6] In liquid–liquid extraction based on SDME, a drop of solvent is suspended from the tip of a syringe needle and is exposed to headspace or immersed in a stirred aqueous solution containing the analyte to be extracted. This micro-extraction technique is a simple, inexpensive, effective and virtually solvent-free sample pretreatment. However, the use of traditional organic solvent and aqueous solution for SDME have the adverse consequences of prolonged extraction time and fast stirring rate of sample solutions might result in drop dissolution or evaporation.[3,6] Consequently, and to overwhelm these drawbacks, novel solvents are currently under study.[6]

Room temperature ionic liquids (RTILs) have been recently investigated as alternative solvents for SDME technique.[7] They are generally considered to be environmentally friendly solvents with unique characteristics (*e.g.* no effective vapor pressure, adjustable viscosity and immiscibility in water and other organic solvents) that can be tuned by changing the combination of different anions and cations.[8] RTILs are considered attractive extractant phases that may enhance analyte selectivity in direct immersion (DI)-SDME for metal[9,10] and organic compound[11] and headspace (HS) SDME for volatile organic compounds.[6,12,13] In addition, RTILs have been acknowledged in several works as a novel option for capturing gases and are considered to be prospective for separation of different volatile compounds.[14,15] Thus, Ji *et al.* synthesized and tested different RTILs, as coating absorbents on mesoporous silica gel for mercury vapor (Hg^0) capture.[16,17] However, these works were only focused on studying absorption properties of some RTILs for Hg^0 removal from flue gas, and no analytical chemistry application of these properties has been reported so far. On the other hand, the use of HS-SDME requires volatile or semivolatile analytes; whereas the possibility of avoiding extraction of potentially interferent non-volatile compounds, turns it into a very suitable technique for pre-concentration of analytes from complex matrix samples. Therefore, the combination of HS-SDME with RTILs as extractant phases could results into a very attractive approach for extending micro-extraction techniques towards trace element analysis.

Since Hg has become a proved cause of concern, due to its high neurotoxicity and widespread occurrence, its monitoring has attracted special attention.[18,19] Particularly, methylmercury (MeHg), the most toxic Hg species, can cause severe neurological

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damage to humans.[19,20] Potential health risks from low levels of Hg are a subject of intense debates and the accurate determination and speciation analysis at trace levels is a current analytical challenge. SDME has been used in recent years as a powerful tool for the preconcentration and matrix separation of different Hg species.[21] This technique has also been used in combination with a chromatographic separation step for Hg species.[10] Alternatively, direct coupling of SDME technique with a sensitive atomic spectrometry instrument which allows direct sample injection at microvolume scale, such as ETAAS, can lead to lower detection limits and faster Hg determinations and species separation with a substantially simplified method.[21]

In this work, a detailed analytical study on Hg atomic vapor capture by a RTIL has been developed. Phosphonium-containing RTIL, tetradecyl(trihexyl)phosphonium chloride CYPHOS® IL 101, is proposed for the first time in combination with HS-SDME technique for the separation, preconcentration, and determination of Hg species. The metal vapor was captured into a CYPHOS® IL 101 microdrop followed by direct injection into ETAAS for elemental detection. The combination of CYPHOS® IL 101 with common oxidant agent, such as $KMnO_4$, was assayed to obtain a high sensitivity enhancement factor. Our technique shows substantial improvements on various aspects as compared to earlier works, by making feasible analyte separation from complex matrix samples (*i.e.* sea water, fish tissues, hair, and wine) and subsequent preconcentration with a minimal amount of solvent required for analysis. Therefore, a green, simple, sensitive, and cost-effective determination of total Hg and its species are among its main advantages.

Experimental

Instrumentation

Elemental detection was performed using a PerkinElmer 5100ZL atomic absorption spectrometer (PerkinElmer, Norwalk, CT, USA) equipped with a pyrolytic graphite tube (PerkinElmer) and a transversely heated graphite atomizer Zeeman-effect background correction system. A Hg electrodeless discharge lamp (EDL) (PerkinElmer) operated at a current of 170 mA (modulated operation) and a wavelength of 253.7 nm with a spectral band pass of 0.7 nm was used. All measurements were made based on absorbance signals with an integration time of 5 s. The temperature *vs.* time program for the atomizer is fully depicted in Table 1.

Table 1 Instrumental and experimental conditions for Hg determination

Instrumental conditions				
Wavelength/nm				253.7
Spectral band width/nm				0.7
EDL lamp current/mA				170
Modifier volume/μL				20
Modifier mass/μg				10 μg Pd [$Pd(NO_3)_2$]
Graphite furnace temperature program				
Step	*T*/°C	Ramp Time/s	Hold Time/s	Argon flow rate/mL min^{-1}
Drying	110	1	10	250
	130	10	40	250
Pyrolysis	400	10	20	250
Atomization	1300	1	5	0
Cleaning	2400	1	2	250
Extraction conditions				
Sample volume				10 mL
RTIL microdrop volume				6 μL
Hg^{2+} standard solution concentration				0.25 μg L^{-1}
$SnCl_2$ concentration				7% (w/v)
Cold vapor generation time				3 min
Extraction time				10 min
Stirring rate				1100 rpm
MeOH volume (washing solution)				20 μL

Reagents

All the reagents were of analytical grade and the presence of Hg was not detected within the working range.

CYPHOS® IL 101 was kindly donated by Prof. Ullastiina Hakala (University of Helsinki, Finland) and supplied by CYTEC (Canada); C.A.S. number: 258864-54-9. A 1000 μg mL^{-1} Hg^{2+} stock solution was prepared from mercury(II) chloride (Merck, Darmstadt, Germany) in 0.1 mol L^{-1} nitric acid (Ultrex® II Mallinckrodt Baker, Phillipsburg, NJ, USA). Lower concentrations were prepared by diluting the stock solution with 0.1 mol L^{-1} nitric acid. Stock MeHg and phenylmercury (PhHg) solutions (1000 mg L^{-1}) were prepared from methylmercury chloride and phenylmercury chloride (Merck) in ethanol (Merck) and methanol (Merck), respectively. Working standard solutions were prepared daily. The OrgHg solutions were stored away from light at 4 °C to prevent decomposition.

A 7% (w/v) $SnCl_2$ (Fluka, Milwaukee, WI, USA) solution in 20% (v/v) HCl (Ultrex® II Mallinckrodt Baker) was used as reducing agent. Sodium tetrahydroborate reagent was freshly prepared daily by dissolving appropriate amount of $NaBH_4$ (Merck) in 0.05% (w/v) sodium hydroxide solution (Aldrich). After dissolution of the reagents, the solution was filtered through a Whatman No. 42 filter paper to remove undissolved solids. Potassium permanganate (Merck, p.a., max. 0.000005% Hg, ACS), potassium dichromate (Merck) and potassium persulfate (Merck) individual solutions were daily prepared by dissolving 25 g of analytical-reagent grade oxidizing agents in 500 mL of ultrapure water and heating gently on a hot-plate until complete dissolution.

A 1000 mg L^{-1} palladium solution used as chemical modifier was prepared from $Pd(NO_3)_2 \cdot 2H_2O$ (Fluka, Buchs, Switzerland) in 0.1% (v/v) HNO_3. A 150 mg L^{-1} $Mg(NO_3)_2$ (Merck) and 2500 mg L^{-1} $NH_4H_2PO_4$ (Merck) stock solutions were tested as chemical modifier. These solutions were prepared in 0.1% (v/v) HNO_3.

A 10^{-2} mol L^{-1} 2-(5-bromo-2-pyridylazo)-5-diethylaminophenol (5-Br-PADAP) (Aldrich) solution was prepared in ethanol.

A $NaNO_3$ (Merck) solution 2 mol L^{-1} was used in order to adjust ionic strength.

Ultrapure water (18 MΩ cm) was obtained from a Millipore Continental Water System (Bedford, MA, USA).

Sample collection and conditioning

The manipulation and analysis of the samples were developed in a clean laboratory environment. All the material used were previously washed with a 10% (v/v) HNO_3 solution and then with ultrapure water before drying in a clean air hood. Sea water samples were taken at different points of the coast of Valparaiso city (Chile). A clean sample collection procedure was followed in order to reduce contamination of sea water samples. The water samples were collected in 1000 mL borosilicate glass bottles and filtered through 0.45 μm pore size membrane filters (Millipore, Bedford, MA, USA). Immediately after sampling, the 1000 mL-aliquots were acidified with 5 mL of 12 M HCl and stored at 4 °C. Hair samples were collected from men and women volunteers, aged between 25 to 35 years, living in Mendoza city (Argentina). Hair samples were obtained using the following standardized cutting and washing procedure:[22] hair samples were collected from the occipital area, by cutting strands of hair close to the scalp. The hair length ranged from 5 cm to 10 cm. The hair was first cut into approximately 0.3 cm pieces and mixed to allow a representative sub-sampling of the hair specimen. After cutting, each sample was washed four times with a 1 : 200 (v/v) dilution of Triton X-100. The samples were then rinsed with acetone and allowed to drain. This was followed by three rinses with ultrapure water and two rinses with acetone. The samples were then dried in an oven at 40 ± 5 °C. Tuna fish samples were obtained from the local market. Tuna fish samples were mashed, homogenized and dried in an oven at 40 ± 5 °C.

An ultrasound-assisted acid leaching procedure was adopted for human hair and fish samples.[23,24] About 0.1 g human hair or 0.2 g fish sample were weighed into a 50 mL plastic centrifuge tube. Then 3 mL of 5 mol L^{-1} HCl was added, extraction was performed in an ultrasound bath for 30 min at room temperature. After centrifugation, the clear supernatant was collected into another 50 mL plastic centrifuge tube. The residue was extracted again as described above, and the supernatant was combined with the first one. The residue was then extracted with 5 mL pure water for 30 min at room temperature under sonication, and the aqueous supernatant was again mixed with the combined acidic supernatant. After vortex mixing, the extractant solution was filtered through a membrane of 0.45 μm pore size into a 50 mL volumetric flask, and diluted to the mark with water. A 10 mL aliquot of the prepared solution was taken and then subjected to CV-ILAHS-SDME technique.

Bottled wine samples were bought from the local market. A wet digestion procedure was followed for wine sample conditioning; 25 mL of sample, 3 mL nitric acid and 2 mL hydrogen peroxide were added in a glass beaker. The mixture was subsequently heated under reflux in a water-bath maintained at 100 °C for 3 h. The solution was allowed to evaporate up to 20 mL. After cooling, the solution was transferred to a 25 mL volumetric flask and diluted to the mark with water. An aliquot of 10 mL of the resulting solution was used for the determination.

Extraction and preconcentration with CV-ILAHS-SDME technique

The extraction/preconcentration procedure was performed as follows: Initially, an amount of 100 μg of CYPHOS® IL 101 and 0.1 mL of 0.18% (w/v) $KMnO_4$ solution were placed in a centrifuge tube, and the resulting system was shaken for 2 min with a vortex stirrer. In order to separate the phases, the turbid solution was centrifuged during 7 min at 2000 rpm (1058 × g) and the aqueous phase was removed with a transfer pipette. The resulting CYPHOS® IL 101- $KMnO_4$ solution was used as acceptor phase.

CV-ILAHS-SDME was performed using a 20 mL vial sealed with a silicone rubber septum placed on top as shown in Fig. 1. Two syringe needles were passed through the septum. One syringe was used to inject the reductant into the vial and the other to suspend the RTIL-microdrop. A volume of 10 mL of Hg-containing sample solution was placed in the glass vial containing a magnetic stirring bar. The vial was capped with the septum and placed on a magnetic stirrer at 55 °C. Then, 1 mL of the reductant solution ($SnCl_2$) was injected into the vial by one syringe, while simultaneously stirring the mixture (1100 rpm). After 3 min, the needle of a 50 μL microsyringe (PerkinElmer) was passed through the septum and a 6 μL drop of CYPHOS® IL 101-$KMnO_4$ solution was suspended at the needle tip and exposed to the headspace. Inside the vial, Hg^{2+} was reduced by $SnCl_2$ to yield the corresponding Hg^0 cold vapor, which was subsequently extracted into the RTIL microdrop. Headspace sampling was performed for 10 min. Thereafter, the drop was retracted back into the microsyringe and subsequently injected into the graphite furnace of ETAAS for Hg determination. Further washing of the microsyringe with 20 μL of MeOH solution served to remove any remaining of analyte, and this solution was also injected. ETAAS determination was performed under the conditions showed in Table 1. Calibration was performed against aqueous standards submitted to the same preconcentration procedure. Likewise, blank solutions were analyzed in the same manner as standard and sample solutions.

Mercury speciation analysis

Since $SnCl_2$ reductant can selectively react with Hg^{2+} species, forming Hg cold vapor, only InHg is released into the headspace of vial and hence separated from OrgHg species. In order to evaluate total Hg concentration, the pretreated samples were irradiated for 3 h with a 15 W UV lamp in order to photooxidize OrgHg species.[25] The difference between total Hg and InHg determined the OrgHg content in the sample. In the case of wine samples, only total Hg was determined just to evaluate the applicability of CV-ILAHS-SDME technique in presence of another complex matrix submitted to a digestion procedure.

Results and discussion

Evaluation of thermal and matrix effects of CYPHOS® IL 101

An initial study focusing on CYPHOS® IL 101 thermal and spectral behaviors during Hg measurements by ETAAS was performed. Thus, a 75 μg L^{-1} Hg^{2+} solution, with an equal volume of RTIL that was used for CV-ILAHS-SDME technique (6 μl), was injected into the graphite furnace. On the other hand, a more accurate study applying CV-ILAHS-SDME technique was performed by analyzing a 2.5 μg L^{-1} Hg^{2+} solution. Since thermal behavior of RTILs shows that the onset weight loss for CYPHOS® IL 101 occurs at 350 and 290 °C under nitrogen and

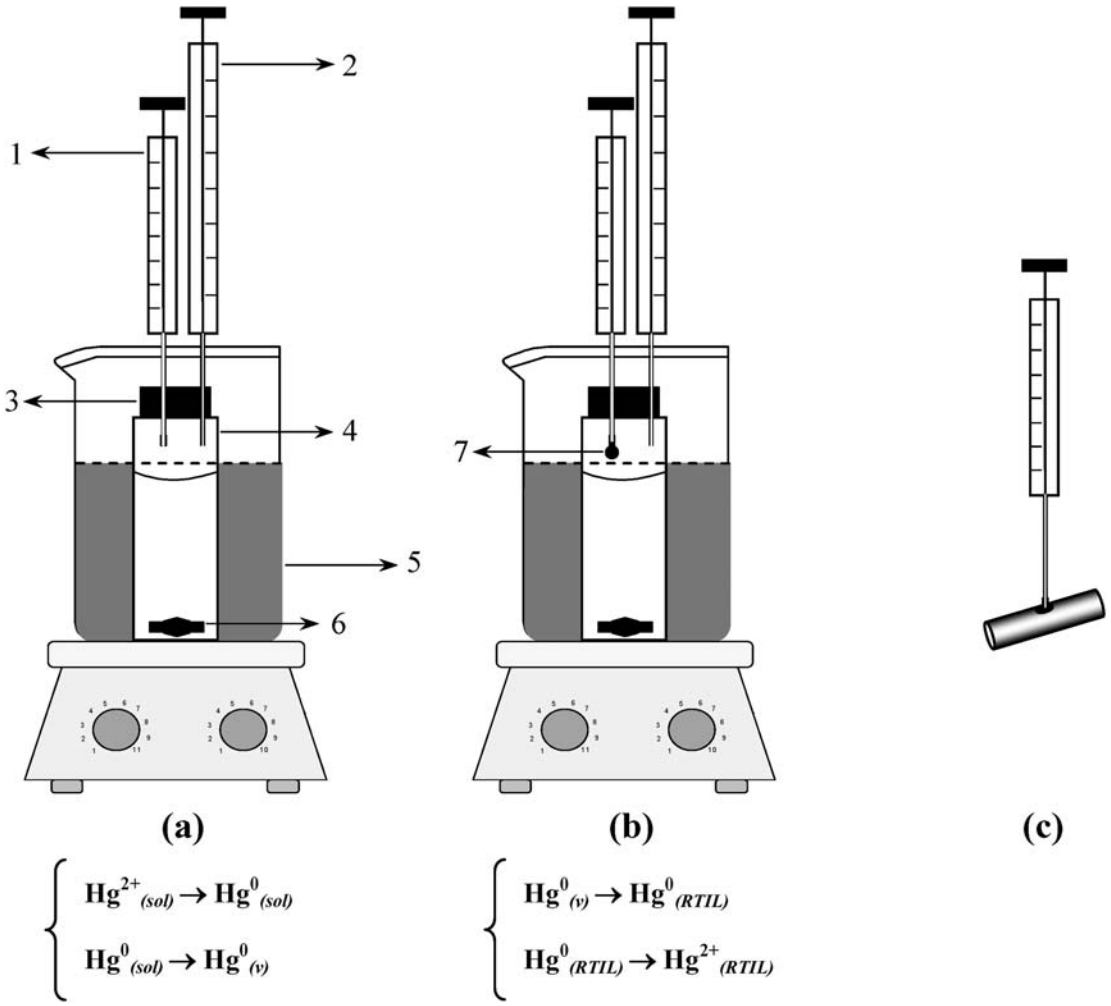

Fig. 1 Schematic diagram of CV-ILAHS-SDME experimental set-up. (a) Cold vapor generation step. (b) Extraction step. (c) Injection of IL microdrop into the graphite furnace and ETAAS detection. (1) Microsyringe; (2) Syringe (reductant); (3) Septum; (4) Vial containing sample solution; (5) Thermostatic bath; (6) Stirring bar; and (7) IL microdrop.

air, respectively;[26] the application of higher pyrolysis temperatures would be desirable to discompose the RTIL organic matrix and reduce background signal observed during atomization step. Consequently, it was necessary to reduce Hg losses in the atomizer over 350 and 290 °C. This problem was minimized with the use of chemical modifiers, which stabilize Hg at higher temperatures. Different amounts of $NH_4H_2PO_4$, $Mg(NO_3)_2$, $Pd(NO_3)_2$ and mixtures of them were tested as chemical modifiers. The stabilization of Hg in the atomizer by Pd (10 μg) was the most effective. It is generally assumed that noble metals form stable amalgams with Hg,[27] in this case, Hg was retained up to 400 °C without significant losses and a reduced background absorption during atomization. Therefore, 400 °C was chosen as the pyrolysis temperature with Pd injected after the sample as chemical modifier. Once selected pyrolysis temperature, the effect of atomization temperature on Hg absorption signal was studied within the range of 800–1400 °C. The maximum signal was obtained at 1300 °C under stop flow conditions. Final conditions for ETAAS detection are shown in Table 1.

A critical experimental observation was made regarding the injection of the RTIL phase into the graphite furnace of ETAAS. The high viscosity of the resulting RTIL phase can avoid an efficient and reproducible injection of the analyte into the graphite furnace. Therefore, the application of an additional injection step using a suitable solvent was considered to overcome this drawback. After injection of the microdrop into ETAAS, further washing with methanol served to remove any sample still present in the microsyringe. Methanol was assayed in volumes ranging from 5 to 50 μL. A volume of 20 μL was suitable for total elution of the RTIL phase from the syringe. Smaller volumes did not remove completely the RTIL and caused signal reduction. The resulting alcohol-RTIL phase did not show differences in thermal behavior with respect to RTIL and hence optimal pyrolysis and atomization temperatures were the same as mentioned above in this section. Thus, the resulting phase was successfully analyzed by ETAAS under the conditions showed in Table 1.

Mercury cold vapor generation and capture in IL microdrop

The cold vapor generation from the samples was carefully studied in order to reach the best conditions for Hg^0 generation

and liberation into the headspace of the reaction vial. Both $NaBH_4$ and $SnCl_2$ were assayed for the reduction and vapor generation of Hg^0. Sodium tetrahydroborate was not a suitable reagent as it produced high pressures in the microextraction vessel leading to vapor leakage and microdrop instability. Moreover, the use of $NaBH_4$ might cause poisoning of the trapping medium due to co-evolution of hydride-forming elements.[28] This problem was avoided by using $SnCl_2$ as reducing agent since it does not generate hydrogen as a sub-product of the reaction with Hg^{2+} ion. The $SnCl_2$ concentration was an important parameter to be optimized as it allowed cold vapor generation even in the eluent medium. Only 1 mL of $SnCl_2$ solution at different concentrations was added to the sample in the closed vial. The best analytical sensitivity for Hg was obtained with a concentration of 7.0% (w/v) $SnCl_2$. Higher concentrations of the reducing agent did not produce a significant change on Hg signals. Hence, the above-mentioned $SnCl_2$ concentration was adopted as the working concentration. The sample solution for the final analysis was made to contain 0.6 mol L^{-1} HCl.

Optimization of CV-ILAHS-SDME experimental conditions

Important considerations were made during sample volume selection to carry on the analysis. The suitable sample volume was chosen considering geometry and dimensions of the reaction vial, as well as the resultant headspace volume. In HS-SDME technique, an increase of sample volume causes a reduction of the headspace volume of the vial, which could enhance extraction kinetics of target analytes improving the sensitivity of the method. Furthermore, concentration of target analytes transferred into the headspace is larger and as such, a net increase of the total analyte mass to be extracted is also expected. In order to evaluate the effect of sample volume upon extraction, experiments were performed using 20 mL-vials containing sample volumes ranging from 5 to 15 mL.[5] The results showed a net increase of the analytical signal upon an increase of the aqueous sample volume (Fig. 2). A volume of 10 mL was appropriate to achieve a satisfactory headspace-to-sample volume ratio and enhancement factor.

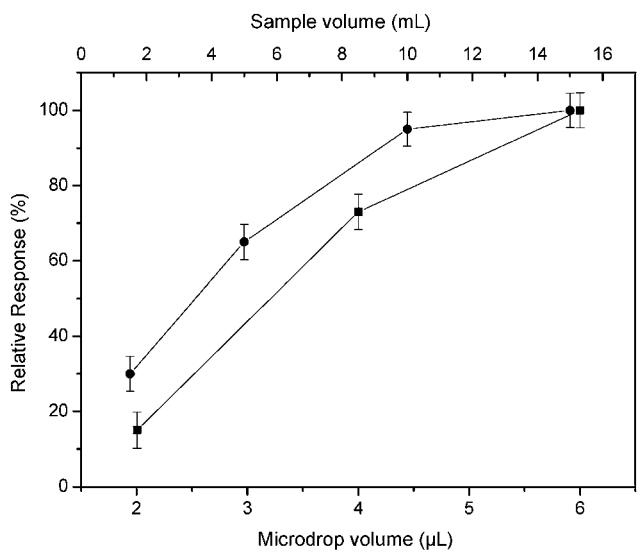

Fig. 2 Influence of (■) CYPHOS® IL 101 and (●) sample volumes on the extraction efficiency of the system. Experimental conditions are listed in Table 1.

The volume of the microdrop is one of the variables determining the extraction efficiency in CV-ILAHS-SDME technique, as it affects both superficial area of the drop and interfacial layer between RTIL and gas phases. Therefore, a more efficient mass transfer from aqueous solution to the organic drop can be expected when the superficial area increases. The effect of CYPHOS® IL 101 drop volume on Hg absorbance signal was investigated in the range of 2 to 8 μL. The results illustrated in Fig. 2 show a slight increase on integrated absorbance when the drop volume was changed from 3 μL to 6 μL. For RTIL volumes larger than 6 μL, the microdrop became unstable and it was easily released from the tip of the syringe needle. Thus, a 6 μL-droplet was selected in this study. Additionally, mixing the RTIL with other solvents like methanol or toluene was studied in order to evaluate their effect on Hg capture by CYPHOS® IL 101. This strategy was assayed based on the fact that surface tension of the RTIL could be diminished in the presence of another solvent, improving the diffusion of Hg into the RTIL.[29] However, this approach was not successful as undesirable dilution of the RTIL phase was also provoked.

It was supposed that stirring rate could influence Hg^0 generation and releasing from the solution. As can be observed in Fig. 3(a) a plateau region was not reached with the stirring rates assayed. This is reasonable, as for high stirring rates of the sample, a faster mass transfer towards the headspace occurs due to the high diffusion coefficient in the gas phase as well as the convection in the solution. However, values for this variable higher than 1100 rpm caused a significant spreading of the liquid towards the walls of vessel or even the microdrop. Therefore, the stirring rate was limited to 1100 rpm.

The effect of the extraction temperature on the uptake and generation of Hg vapor was also studied. The temperature was regulated by immersing the extraction vial in a thermostated water bath placed on a magnetic stirring plate. The water level in the bath was the same as the sample solution in the extraction vial. The effect of temperature was examined within a range of 20 to 75 °C. As the temperature was increased, it was observed an enhancement of the analytical response, possibly due to a higher Hg^0 partial pressure and hence major releasing of the vapor from the solution into the headspace. An optimum extraction temperature of 50 °C was selected. Higher temperatures produced sample evaporation and condensation on the surface of the drop and the inner walls of the reaction vial, affecting the reproducibility of the methodology.

Time-dependant processes involved in CV-ILAHS-SDME technique were studied. Both, time required for total cold vapor generation and extraction of Hg^0 were evaluated. Since mass transfer of Hg^0 into the RTIL-drop occurs mainly by a diffusion phenomenon, a maximum concentration of Hg^0 in the headspace has to be initially reached before the RTIL microdrop is exposed. Thus, cold vapor generation time was studied in the range of 1 to 10 min. Only 3 min were necessary to obtain the highest enhancement on analytical signal (Fig. 3(b)). The extraction time was another important variable to be studied, to achieve an efficient sequestration of the vapor. This time was defined as that elapsed during microdrop exposure to headspace. The extraction

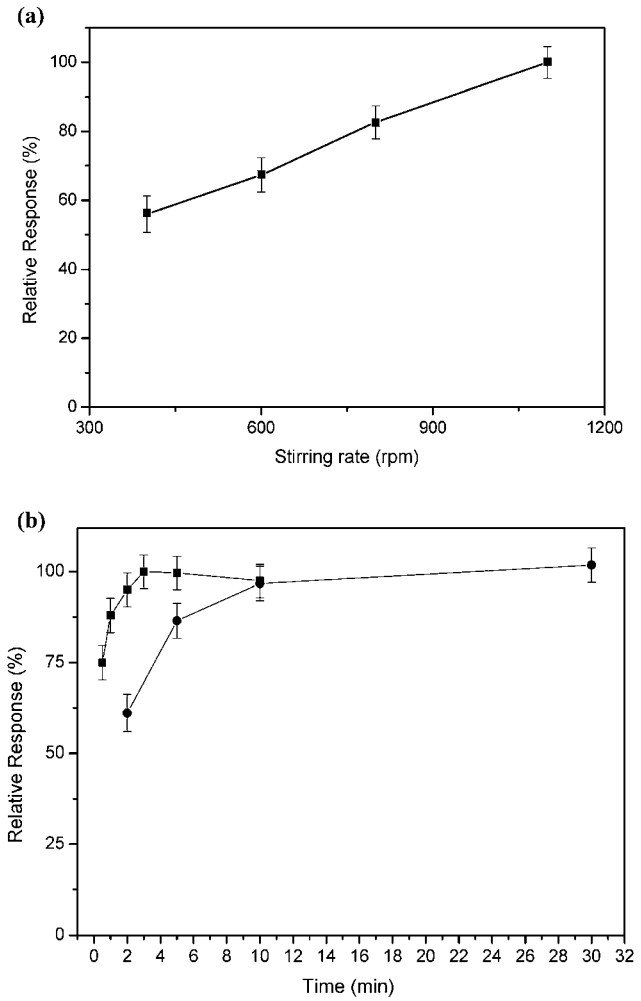

Fig. 3 (a) Effect of stirring rate on the cold vapor generation and extraction capacity of the system. (b) Effect of cold vapor generation time (■) Effect of the extraction time on the extraction efficiency of the system (●). Experimental conditions are listed in Table 1.

time was studied in the range of 3–30 min. A dramatic increase of extraction efficiency was observed up to 10 min and after that, the increase was slower (Fig. 3(b)). As a compromise between sensitivity and sampling frequency, a maximum extraction time of 10 min was adopted throughout the experiments.

The ionic strength effect was assayed by adding different amounts of $NaNO_3$ in the sample solution prior to develop the CV-ILAHS-SDME procedure. No further improvements in analytical sensitivity were achieved by addition of salt. On the other hand, only a negative effect on the sensitivity was observed at high salt concentrations (>2 mol L^{-1}). Consequently, salt addition was not adopted in this work.

Different alternative strategies to enhance Hg^0 capture in the RTIL microdrop were attempted in this work. Thus, 0.1 g of CYPHOS® IL 101 was mixed with 100 μL of a 10^{-2} mol L^{-1} ethanol-containing solution of the complexing agent 5-Br-PADAP. The pH value was adjusted to 9 with ammonia buffer solution. This strategy did not provide efficient trapping of Hg cold vapor. These kind of compounds act as chelating agents for Hg^{2+} ions in solution, but were useless to improve Hg^0 sequestration from the headspace.

Retention mechanism of Hg in the RTIL microdrop

RTILs have been widely used in many chemical fields. There are successful demonstrations in the literature reporting the application of RTILs in the area of gas capture[14,15] and gas solubility studies in these media.[30] Based on these previous studies, we propose that the possible mechanism involved in Hg^0 capture by CYPHOS® IL 101 can be vapor solubilization into the microdrop. Likewise, Marek has shown that Hg^0 dissolved much faster in highly oxidizing solutions.[31] In fact, some works have shown the possibility of using RTILs as oxidizing media. For example, H. Kumar *et al.* and S. P. Panchgalle *et al.* have studied the oxidation of benzylic alcohols to carbonyl compounds[32] and the oxidation of alkyl and aryl pyridines,[33] respectively. Thus, the addition of a strong oxidant could enhance Hg^0 extraction into the RTIL due to oxidation of Hg^0 solubilized in the microdrop to Hg^{2+}. The overall result of oxidizing conditions could be a faster Hg^0 removal from headspace and higher extraction capacity of the RTIL microdrop. In order to evaluate the above mentioned effects, different oxidant reagents ($KMnO_4$, $K_2S_2O_8$ and $K_2Cr_2O_7$) at 10^{-3} mol L^{-1} concentration were individually mixed with CYPHOS® IL 101 followed by the extraction step described early. Potassium permanganate yielded the best Hg signal, *i.e.* a 300% improvement of analytical sensitivity with respect to the same situation but with no addition of $KMnO_4$ to the RTIL phase. Therefore, $KMnO_4$ was chosen as oxidant agent. The concentration of $KMnO_4$ in the solution that was put in contact with the RTIL before the preconcentration step was also optimized. A $KMnO_4$ concentration of 0.18% (w/v) was found to be optimum for the preconcentration system, while higher concentrations diminished analytical sensitivity and increased background signal (Fig. 4). It has to be pointed out, that the combination of $KMnO_4$ as an oxidant with CYPHOS® IL 101 as an extractant phase was feasible thanks to the wide electrochemical potential window of this RTIL, which allows to perform oxidation or reduction reactions with no degradation of the solvent.[26,34,35]

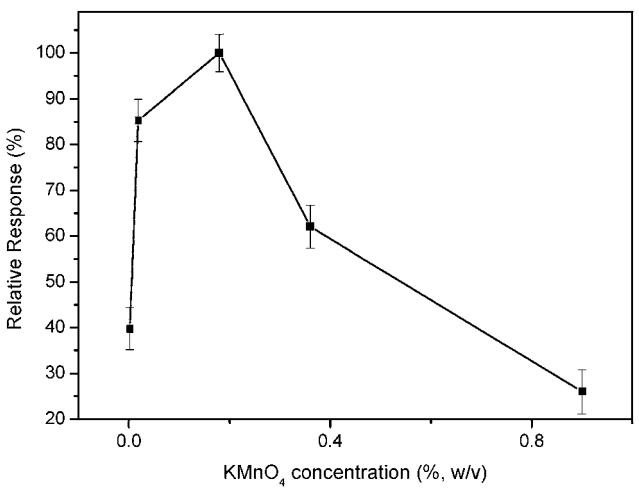

Fig. 4 Effect of $KMnO_4$ concentration on the extraction efficiency of the system. Other conditions were as indicated in Table 1.

Potential interfering species

Cold vapor generation offers the advantage of leaving behind in solution many substances which might potentially interfere with the determination of Hg.[36] Furthermore, when using $SnCl_2$ as a reductant instead of $NaBH_4$ hydride-forming elements are not released from solution.[37] Therefore, interferences of CV-ILAHS-SDME technique are only possible during the cold vapor generation process. In particular, this refers to concomitant elements which are responsible for non-quantitative releasing of Hg from the solution into the headspace.[37] The chemical vapor generation is essentially a chemical separation of elemental species from the sample solution, which is performed *in situ* and instantaneously. Thus, the high capacity of cold vapor generation for Hg isolation was profited in this work to apply the proposed methodology for Hg species separation and determination in complex matrix samples.

Table 2 Analyte recovery study in real samples (95% confidence interval; $n = 6$)

	Spiked Hg as/ μg L^{-1}		InHg		OrgHg	
Sample	InHg	OrgHg (MeHg, PhHg)	Found/ μg L^{-1}	Recovery (%)[a]	Found/ μg L^{-1}	Recovery (%)[a]
Sea water	—	—	0.09 ± 0.01	—	< LOD	—
	0.5	—	0.57 ± 0.03	96	< LOD	—
	—	0.5	0.09 ± 0.01	—	0.50 ± 0.05	100
	0.5	0.5	0.59 ± 0.03	100	0.48 ± 0.05	97
Hair	—	—	0.15 ± 0.01	—	< LOD	—
	0.5	—	0.67 ± 0.04	105	< LOD	—
	—	0.5	0.15 ± 0.01	—	0.51 ± 0.05	101
	0.5	0.5	0.63 ± 0.04	96	0.48 ± 0.05	97
Fish tissues	—	—	0.10 ± 0.01	—	0.17 ± 0.02	—
	0.5	—	0.59 ± 0.03	98	0.17 ± 0.03	—
	—	0.5	0.10 ± 0.01	—	0.66 ± 0.06	98
	0.5	0.5	0.60 ± 0.04	100	0.65 ± 0.06	95
Wine	—	—	0.12 ± 0.01	—	–	—
	0.5	—	0.60 ± 0.04	96	—	—
	—	0.5	0.12 ± 0.01	—	—	—
	0.5	0.5	0.64 ± 0.04	104	—	—

[a] 100 × [(Found-base) / added].

Table 3 Concentration of Hg species in real samples (95% confidence interval; $n = 6$)

Sample	InHg	OrgHg
1[a]	0.09 ± 0.01	< LOD
2[a]	0.18 ± 0.02	0.56 ± 0.05
3[a]	< LOD	< LOD
4[b]	75 ± 5	< LOD
5[b]	230 ± 15	425 ± 35
6[b]	175 ± 10	260 ± 25
7[c]	25 ± 2.5	42 ± 5
8[c]	55 ± 5	95 ± 10
9[c]	< LOD	27 ± 5
10[d]	0.12 ± 0.01	—
11[d]	0.15 ± 0.01	—
12[d]	< LOD	—

[a] Sea water (μg L^{-1}). [b] Hair (μg kg^{-1}). [c] Fish tissues (μg kg^{-1}). [d] Wine (μg L^{-1}).

Analytical performance and determination of mercury in real samples

The relative standard deviation (RSD) resulting from the analysis of 10 replicates of 10 mL solution containing 0.25 μg L^{-1} of Hg^{2+} was 4.6%. Analytical sensitivity was enhanced by a factor of 75. The enhancement factor was obtained from the ratio of the calibration curve slopes for Hg with and without application of the extraction/preconcentration step. Calibration curve without preconcentration was obtained by directly injecting 20 μL of Hg standard solutions at different concentrations into ETAAS. The calibration graph obtained with the proposed method was linear with a correlation coefficient of 0.9996 at levels near the detection limits and up to at least 10 μg L^{-1}. The limit of detection (LOD) was calculated based on the signal at intercept and three times the standard deviation about regression of the calibration curve.[38] A LOD of 10 ng L^{-1} Hg was obtained for the proposed methodology.

In order to demonstrate the wide applicability of the proposed method, different complex matrix samples including seawater, tuna fish, hair and wine were specially considered for analysis in this work. A recovery study was developed on spiked samples containing known additions of InHg and OrgHg (as equimolar concentrations of MeHg and PhHg). The results are shown in Table 2. Recoveries of Hg varied between 95 and 105%. Additionally, the applicability of the proposed methodology for InHg and OrgHg determination was assayed by analysis of several real samples (Table 3). Regarding total Hg determination, the accuracy of the proposed method was evaluated by analysis of a certified reference material (CRM), QC Metal LL3 Mercury in Water (VKI Certified Reference Materials), with a Hg content of 6.48 ± 0.51 μg L^{-1}. Concentration of Hg found in this CRM by the proposed method was 6.56 ± 0.12 μg L^{-1} (95% confidence interval; $n = 6$).

In comparison to other methods reported in the literature for Hg determination based on on-line CV, the proposed method requires lower volume of sample and reagents, reducing the residue production in the laboratory. In addition, our method shows a lower limit of detection, better precision and higher

Table 4 Characteristic performance data obtained by using the proposed method and others reported for Hg determination based on HS-SDME

Method	LOD/ng L^{-1}	RSD (%)	Enhancement factor	Sample consumption/mL	Speciation	Detection technique	Ref.
(Pd–Pt)[a][b]	5000 (Pd) 4000 (Pt)	7	40	5	No	ETAAS	40
Pd[a]	800	8.7	72	5	No	ETV-ICP-MS	39
IL[a]	10	4.6	75	10	Yes	ETAAS	This work

[a] Trapping agent. [b] Methylmercury was determined in that work.

enhancement factor with respect to other works using HS-SDME technique based on Hg amalgamation with noble metals for analyte preconcentration (Table 4).[39,40] Furthermore, the use of $SnCl_2$ as a reductant allowed Hg species separation by selective Hg vapor generation, which was not achieved by other works previously reported. Thus, the excellent analytical performance of the proposed method associated with ILs introduction in HS-SDME technique opens up an attractive alternative in the area of preconcentration methodologies for metal species determination.

Conclusion

An innovative method involving species separation and preconcentration based on headspace capture of Hg cold vapor into a RTIL microdrop followed by ETAAS detection was developed in this work. The identification of CYPHOS® IL 101 as a sequestration phase for an atomic vapor provides a novel and simple approach for extraction and preconcentration of metal species. Our method combines the advantages of SDME technique (*i.e.*, miniaturization and low solvent consumption) with the use of RTILs as extractant phases (*i.e.*, environmentally friendly and undetectable vapor pressure) for the preconcentration and speciation of Hg. Thus, for low-level Hg speciation, CV-ILAHS-SDME is a convenient alternative to other extraction techniques as it integrates extraction, preconcentration and sample introduction into a single step without the need of common organic solvents, some of which might be harmful and contaminate the environment due to high vapor pressure. Finally, CV-ILAHS-SDME technique was successfully applied to Hg determination in complex matrix samples such as sea water, wine, tuna fish tissues and hair with good accuracy and reproducibility. Moreover, it should be pointed out that CYPHOS® IL 101 is a cost-effective ionic liquid; and hence, the proposed method could be generally applicable for routine analytical laboratories.

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