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Speciation Analysis of Arsenic by Selective Hydride Generation-Cryotrapping-Atomic Fluorescence Spectrometry with Flame-in-Gas-Shield Atomizer: Achieving Extremely Low Detection Limits with Inexpensive Instrumentation

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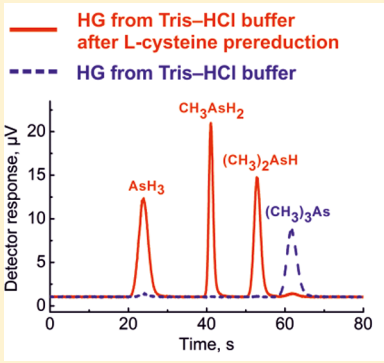
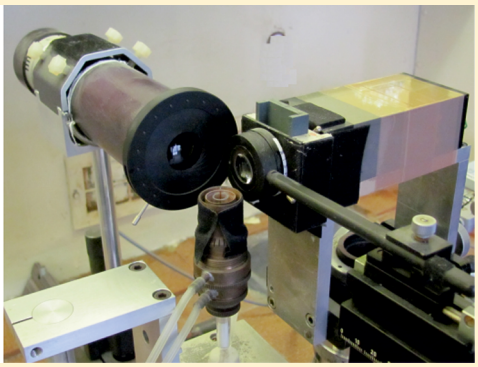
Supporting Information





ABSTRACT: This work describes the method of a selective hydride generation-cryotrapping (HG-CT) coupled to an extremely sensitive but simple in-house assembled and designed atomic fluorescence spectrometry (AFS) instrument for determination of toxicologically important As species. Here, an advanced flame-in-gas-shield atomizer (FIGS) was interfaced to HG-CT and its performance was compared to a standard miniature diffusion flame (MDF) atomizer. A significant improvement both in sensitivity and baseline noise was found that was reflected in improved (4 times) limits of detection (LODs). The yielded LODs with the FIGS atomizer were 0.44, 0.74, 0.15, 0.17 and 0.67 ng L^{-1} for arsenite, total inorganic, mono-, dimethylated As and trimethylarsine oxide, respectively. Moreover, the sensitivities with FIGS and MDF were equal for all As species, allowing for the possibility of single species standardization with arsenate standard for accurate quantification of all other As species. The accuracy of HG-CT-AFS with FIGS was verified by speciation analysis in two samples of bottled drinking water and certified reference materials, NRC CASS-5 (nearshore seawater) and SLRS-5 (river water) that contain traces of methylated As species. As speciation was in agreement with results previously reported and sums of all quantified species corresponded with the certified total As. The feasibility of HG-CT-AFS with FIGS was also demonstrated by the speciation analysis in microsamples of exfoliated bladder epithelial cells isolated from human urine. The results for the sums of trivalent and pentavalent As species corresponded well with the reference results obtained by HG-CT-ICPMS (inductively coupled plasma mass spectrometry).

Trivalent and pentavalent inorganic, mono-, di- and trimethylated arsenicals, the products of animal and human metabolism of arsenic, are analytes of toxicological significance: arsenite (iAs(III)), arsenate (iAs(V)), methylarsonite (MAs(III)), methylarsonate (MAs(V)), dimethylarsinite (DMAs(III)), dimethylarsinate (DMAs(V)) and trimethylarsine oxide (TMAs(V)O). These species, free or bound to proteins in biological systems,[1] have various toxicities including mutagenic, teratogenic and carcinogenic effects.[2−4] Therefore, it is necessary to identify and quantify them separately in biological systems to evaluate potential toxicological risk. The most common approaches to As speciation analysis rely on high performance liquid chromatography (HPLC) in combination with a sensitive specific element detector such as inductively coupled plasma mass spectrometry (ICPMS)[5] or postcolumn hydride generation (HG) with atomic fluorescence spectrometry (AFS).[6,7] However, both methods provide insufficient sensitivity for speciation analysis of all toxicologically relevant species at low or only slightly elevated As exposures. In addition, these methods are not suitable for direct analysis of

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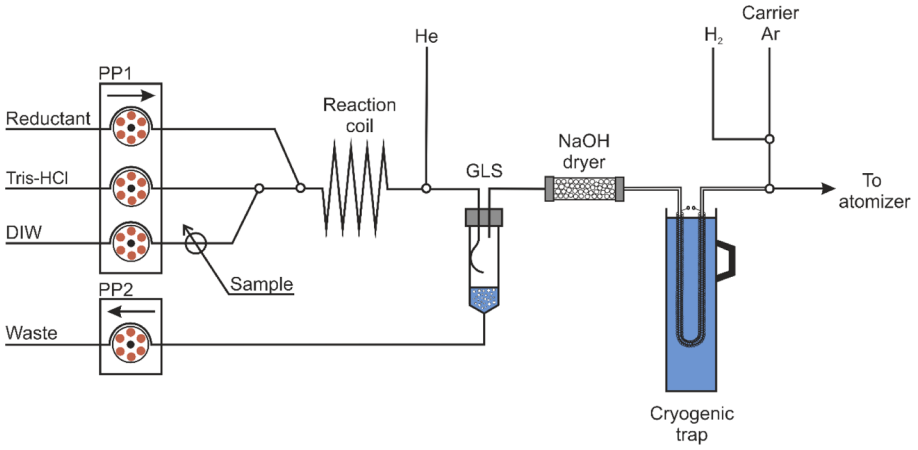

Figure 1. HG-CT system. GLS, gas–liquid separator; PP1 and PP2, peristaltic pumps.

complex biological samples without previous preparation steps, such as extraction, filtration or centrifugation, which can result in losses of species or speciation change.

Selective generation of arsine and methylsubstituted arsines followed by preconcentration and separation in a cryogenic trap (CT) is a convenient approach for ultrasensitive speciation analysis of these arsenicals because of its two inherent features: (i) effective preconcentration and (ii) separation capability. Although this approach is limited because only four arsines can be formed (i.e., AsH_3, CH_3AsH_2, $(CH_3)_2AsH$ and $(CH_3)_3As$), the selectivity can be enhanced by the selectivity of HG itself. At pH 6, only trivalent species and TMAs(V)O are quantitatively converted to their corresponding arsines while iAs(V) and MAs(V) are not (<1%) and DMAs(V) only to a small extent (4–6%). After prereduction with L-cysteine (L-cys), arsines are quantitatively generated from both tri- and pentavalent species with the exception of TMAs(V)O. This selective HG-CT based approach was successfully coupled to atomic absorption spectrometry (AAS)[8] with various applications in our laboratories.[9−13] However, for toxicological studies generating small amounts of biological material (e.g., limited numbers of cells collected in population or laboratory studies) only the most sensitive ICPMS detector yields satisfactory sensitivity.[14] For example, determination of As species in small samples of human cells and mouse pancreatic islets by HG-CT-ICPMS has been successfully used in studies examining risk and mechanism of diabetes associated with chronic exposure to iAs.[15,16] However, the high investment and running costs associated with ICPMS use may not be the only solution.

AFS instruments (coupled to HG) can provide analytical performance comparable to that of ICPMS at substantially lower costs[6] and can be assembled in the laboratory from commercially available parts.[17] Commercially available AFS instruments consist of a boosted-output hollow cathode lamp as the radiation source and a miniature diffusion flame (MDF) as the atomizer.[6,17−19] For in-house assembled instruments, the use of commercially available electrodeless discharge lamps (EDLs) was determined to be a much better solution[20] owing to higher radiation intensity and the resulting lower limits of detections (LODs).[21] When MDF is replaced by a flame-in-gas-shield atomizer (FIGS), a highly fuel rich hydrogen oxygen micro flame burning in an argon shield, the sensitivity and baseline noise can be further improved.[17,22−24]

The HG-CT-AFS combination has been scarcely mentioned in the As speciation analysis literature.[25−27] This work aims to demonstrate the potential of the selective generation of arsine and methylsubstituted arsines combined with cryotrapping and AFS detection and to assess advantages of the FIGS over the MDF atomizer. The feasibility of the selective generation of arsines combined with cryotrapping and AFS detection using a FIGS atomizer is illustrated by speciation analysis in certified water reference materials, an example of samples with extremely low As concentrations, and in human bladder exfoliated cells (BECs), an example of limited-size tissue samples typically collected in population or clinical studies.

■ EXPERIMENTAL SECTION

If not explicitly stated otherwise, the experiments described were performed at the Institute of Analytical Chemistry (IAC) in Prague.

Atomic Fluorescence Spectrometry. The in-house assembled nondispersive atomic fluorescence spectrometer of similar concept as described by D'Ulivo was used.[20] It is equipped with a commercially available As EDL (System II) as the radiation source, similar to that described in ref 28, an interference filter to isolate fluorescence radiation from the atomizer described below and a solar blind photomultiplier as the detector. The feeding power for the EDL was square-wave modulated at 40 Hz. This modulation enabled signal measurement in two modes alternately during the EDL cycle: (i) the lamp turned on (fluorescence signal and emission of the flame were recorded) and (ii) the lamp turned off (only emission of the flame was recorded). By subtracting the values obtained in these two modes, the fluorescence signals were corrected to the emission of the flame. See the Supporting Information for a detailed description of the spectrometer.

Standards and Reagents. Deionized water (DIW; <0.2 μS cm^{-1}, Ultrapur, Watrex, USA) was used for the preparation of all solutions. A 1000 mg L^{-1} As standard solution (Merck, Germany) was used as the iAs(V) stock standard solution. Stock solutions of 1000 mg L^{-1} As were prepared for MAs(V), DMAs(V) and TMAs(V)O species in DIW using the following compounds: $Na_2CH_3AsO_3{\cdot}6H_2O$ (Chem Service, USA), $(CH_3)_2As(O)OH$ (Strem Chemicals, Inc., USA); $(CH_3)_3AsO$ was obtained courtesy of Dr. William Cullen (University of British Columbia, Canada). The total As content of methylated As species standards was confirmed by liquid sampling graphite furnace-AAS (GF-AAS) in 100 μg L^{-1} solutions of individual species using a PerkinElmer Analyst 800 instrument. End-capped transversely heated tubes modified permanently by 4 μg

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of Ir were used at the program and conditions recommended by the manufacturer. Assuming that the sensitivity for individual As forms were identical in the GF-AAS, the values obtained were taken as the true content.[8]

Working standards were prepared for individual As species by serial dilution of stock solutions in DIW. Mixed standards were used only after the last dilution, i.e., at the sub μg L^{-1} level. When the sums of trivalent and pentavalent forms were analyzed (with the exception of TMAs(V)O), prereduction by L-cysteine hydrochloride monohydrate (L-cys; Merck, Germany) added into the standard solution to final concentration of 2% (m/v) was carried out at least 1 h prior to the analysis. A reducing solution containing 1% (m/v) $NaBH_4$ (Fluka, Germany) in 0.1% (m/v) KOH (Lach-Ner, s.r.o., Czech Republic) was prepared fresh daily. A 0.75 M Tris−HCl buffer was prepared from the reagent grade Trizma hydrochloride (Tris(hydroxymethyl)aminomethane hydrochloride; Sigma, Germany) and adjusted to pH 6 by the addition of approximately 1.5 mL of 10% (m/v) KOH per 250 mL of the buffer solution.

Hydride Generator with a Cryotrap. The hydride generator described previously[14] was used with minor modifications (Figure 1). Briefly, reductant, Tris−HCl buffer and DIW were pumped by a peristaltic pump (PP1, Reglo Digital, Ismatec, Switzerland) at flow rates of 1 mL min^{-1}. Samples were injected into the flow of DIW by a manual six-port injection valve (Rheodyne, USA) with a 0.6 mL sample loop volume unless stated otherwise. The dryer, incorporated between the gas−liquid separator (GLS) and the CT, was filled with solid sodium hydroxide pellets (p.a., Lachner, Czech Republic, pearls of 3 mm o.d.). The CT device consisting of a 300 mm glass U-tube (2.4 mm i.d.) packed with Chromosorb WAW-DMCS 45/60, 15% OV-3 (Supelco, USA) was previously described.[14] The U-tube was wrapped with a resistance wire with a current for heating of 1.6 A (~23.5 V). The flow rate of carrier He was 90 mL min^{-1}.

Procedure. Approximately 3/4 of the U-tube was manually immersed in liquid N_2 before the beginning of the cycle. The cycle started with switching on PP1, and after 5 s, the sample was injected into a flow of DIW. The PP1 was switched off after 90 s. Another 90 s was allowed to complete the reaction and for transport of arsines from the GLS to the CT. Subsequently, the volatilization step started by removing the flask with liquid N_2 and switching on heating of the U-tube. To improve the resolution between $(CH_3)_2AsH$ and $(CH_3)_3As$ peaks, the heating was manually switched off at the time corresponding to CH_3AsH_2 signal maximum and switched on again after 20 s to remove incidental byproducts from the CT. The signal was recorded during the entire volatilization step (70−80 s read time). Subsequently, PP2 was switched on to remove the waste liquid from the GLS and the heating was switched off. The total cycle time was less than 6 min.

Atomizers. Unless stated otherwise, the FIGS atomizer displayed in Figure 2 was used. This atomizer consisted of a vertical tube (support tube) made of quartz (6 mm i.d., 8.5 mm o.d.) that introduced gases from the HG-CT system, i.e., He, Ar and H_2 carrying arsines. The support tube was surrounded by a two-channel brass shielding unit that formed two outer concentric shielding Ar flows around the atomizer. The dimensions of the shielding unit were as follows: inner shielding unit, 14.9 mm i.d. and 16.0 mm o.d.; outer shielding unit, 20.8 mm i.d. and 22.4 mm o.d.[29] An inner quartz capillary (0.53 mm i.d.) centered in the support tube with its tip 5 mm

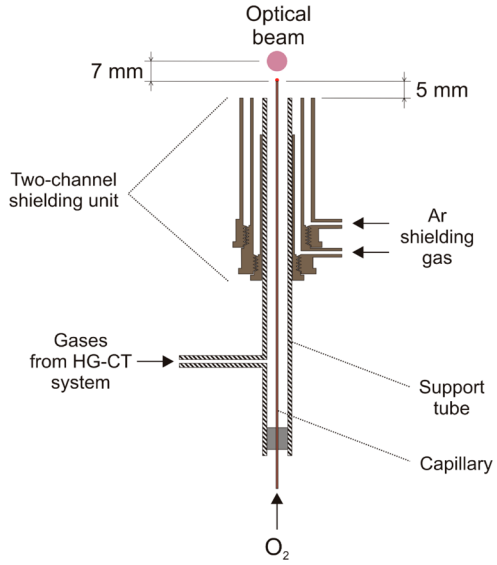

Figure 2. FIGS atomizer.

above the support tube top introduced O_2 forming a tiny, nearly invisible, flame at the capillary tip. The optical axis of the spectrometer, i.e., the axis of the radiation beam, intersected the (vertical) axis of the support tube at a height of 7 mm above the capillary tip. For comparative purposes, the atomizer also served as a miniature diffusion flame (MDF) when O_2 and Ar shielding flows were stopped, the capillary tip was aligned with the support tube top and the radiation beam axis was at a height of 7 mm above the capillary tip/support tube top which was optimal for both atomizers. All gas flow rates, controlled by mass flow controllers (FMA 2400 Series, Omega Engineering, Inc., USA) or by rotameters with needle valves, are summarized in Table 1.

Table 1. Gas Flow Rates (L min^{-1}) to the FIGS and MDF Atomizers

	FIGS	MDF
carrier Ar	0.5	0.5
H_2	0.3	0.3
O_2	0.005	
shielding Ar (channel I)	1.5	
(channel II)	1.5	

Sample Preparation. *Water Samples and CRMs.* The developed method was verified by analyzing two certified reference materials and two samples of bottled drinking water purchased from a local store in Prague. The reference materials SLRS-5 (river water) and CASS-5 (nearshore seawater) were obtained from the Institute for National Measurement Standards (National Research Council, Canada). The samples were measured directly for iAs(III) and TMAs(V)O determination, whereas for iAs(III+V), MAs(V) and DMAs(V) determination, 2% (m/v) solid L-cys was added at least 1 h prior to analysis. Quantification of As species was performed against external calibration of aqueous mixed standards treated with 2% L-cys.

Bladder Exfoliated Cells (BECs). Thirty midstream urine samples (~100 mL) were collected for this study from residents of Chihuahua, Mexico who are exposed to iAs in drinking water. The collection and preparation of the BEC

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samples is described in detail elsewhere[14,15] and is summarized in the Supporting Information. The lysed BEC samples were first analyzed at the University of North Carolina at Chapel Hill (UNC) for trivalent species and subsequently for the sum of tri- and pentavalent species by HG-CT-ICPMS. The remaining aliquots were shipped on dry ice to IAC in Prague where the sums of tri- and pentavalent species were measured by HG-CT-AFS. Six BEC lysates were also quantified for trivalent species to examine the stability of MAs(III) and DMAs(III). Diluted sample aliquots were introduced to the HG-CT system using a 1 mL plastic pipette tip connected directly to the peristaltic pump tubing of the hydride generator, followed by a 350 μL DIW rinse (the injection valve was disconnected in this case). Quantification of As species in both laboratories was performed against external calibration of aqueous mixed standards treated with 2% L-cys. Reported amounts of As species are normalized per 10 000 cells.

Reference Methods. A HG-CT-AAS system with a multiatomizer[30,31] was used at IAC as a reference method. The manual HG-CT system was identical to that coupled with AFS. An AAnalyst 800 AAS spectrometer (PerkinElmer, USA) equipped with FIAS 400 flow injection accessory was employed. The multiatomizer was heated to 900 °C. The details of this method are described in ref 8. Standard addition technique liquid sampling ICPMS (Agilent 7700x) was used for determination of total As concentration in the samples of bottled drinking water. The conditions are given in the Supporting Information, Table S-1. A reference analysis of BEC samples was carried out by HG-CT-ICPMS using an Agilent 7500cx system as described elsewhere.[14]

RESULTS AND DISCUSSION

Interfacing the HG-CT System to AFS Atomizers. The basic parameters of selective HG-CT were optimized in our previous studies when connected to AAS[8,32] or ICPMS.[14] The crucial component of the HG-CT-AFS system is a dryer situated between the GLS and the CT that removes water vapor and aerosol from the gaseous phase. There are at least two reasons to employ the dryer: (i) the CT could be blocked by frozen water vapor and (ii) water vapor released from the CT in the volatilization step can be responsible for fluorescence signal fluctuations because water molecules absorb radiation at a wavelength of 193.7 nm.[33] Commercial AFS spectrometers employ a tube dryer with a Nafion membrane for water removal.[18] However, recent evidence indicates that these dryers are unsuitable for use with the HG-CT due to pronounced losses of methylated arsines.[34] Instead of the Nafion tube, a dryer filled with NaOH pellets had sufficient drying ability and was safe for all arsines at the 2 μg L^{-1} level.[34] To exclude the presence of unfavorable sorption of arsines in the NaOH dryer at 20 times lower concentration level, i.e., 100 ng L^{-1}, the measured peak areas corresponding to individual arsines were compared with those measured without the NaOH dryer. Thus, the GLS outlet was connected directly to the U-tube. Absolutely no losses of arsines in the dryer were observed because the relative responses (with dryer/without dryer) for iAs(V), MAs(V), DMAs(V) and TMAs(V)O were 101.5 ± 2.9%, 100.4 ± 3.0%, 97.9 ± 3.5% and 103.5 ± 3.6%, respectively. The use of the NaOH dryer in the HG-CT-AFS is critical because it results in improved performance without the danger of accidental U-tube blocking by ice and in better baseline stability. The NaOH dryer also significantly improved the shape of AsH_3 peak that was deformed at its trailing edge without the dryer.

The baseline separation of all arsines that are quickly released from the CT, typically within 50 s, is also crucial. The early problem with unsatisfactory original resolution between $(CH_3)_2AsH$ and $(CH_3)_3As$ peaks was solved when the U-tube heating was manually switched off at the time corresponding to CH_3AsH_2 peak maximum (approximately in 42 s), delaying the release of $(CH_3)_3As$ from the CT. The typical signals obtained from iAs(V), MAs(V) and DMAs(V) measured with L-cys prereduction and of TMAs(V)O measured without prereduction for optimized HG-CT conditions are shown in Figure 3. The full width at half-maximum (fwhm) and

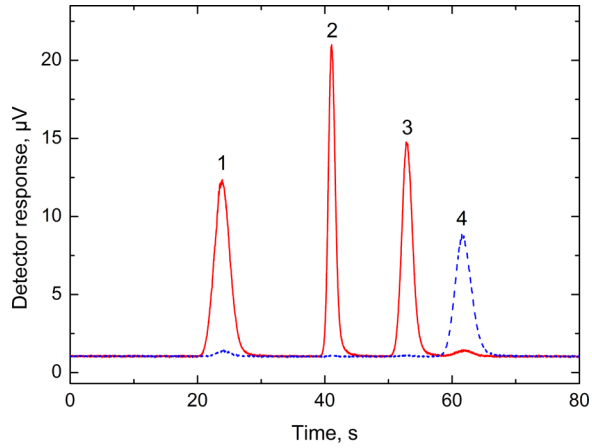

Figure 3. Typical chromatograms measured at optimized conditions for 100 ng L^{-1} of iAs(V), MAs(V) and DMAs(V) with L-cys prereduction (red solid line) and for 80 ng L^{-1} of TMAs(V)O without prereduction (blue dashed line). 1, AsH_3; 2, CH_3AsH_2; 3, $(CH_3)_2AsH$; 4, $(CH_3)_3As$. PMT voltage 1300 V.

peak asymmetry factor (A_s; A_s is equal to b/a where a is the width of the front half of the peak and b is the width of the back half of the peak measured at 10% of the peak height from the leading or trailing edge of the peak to a line dropped perpendicularly from the peak maximum) were compared with those obtained by HG-CT-AAS with the multiatomizer. The same HG-CT system, measurement procedure and total gas flow rate through the U-tube (He + H_2) were employed. The comparison was done only for iAs(V) and MAs(V) because their release from the U-tube was not influenced by the pause in heating. The fwhm for AAS detector was 3.53 ± 0.11 s and 1.5 ± 0.02 s, respectively, whereas for the AFS detector, it was slightly improved to 3.10 ± 0.15 s and 1.21 ± 0.05 s, respectively. However, the improvement in peak asymmetry when replacing AAS with the AFS detector was substantial: from A_s values of 1.79 ± 0.11 and 2.14 ± 0.14 to 1.16 ± 0.05 and 1.24 ± 0.05 for iAs(V) and MAs(V), respectively. This is because analyte free atoms remain for a brief period of time in the observation volume of the FIGS atomizer. The free atoms are quickly flushed by high carrier gas flow as opposed to the multiatomizer where the "physical" removal of free atoms from the inner volume of the atomizer is apparently responsible for the observed peak tailing. It should be highlighted that for the AFS detector, peak shapes of all signals observed with the MDF atomizer did not significantly differ from those with the FIGS atomizer.

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Table 2. Slopes of Calibration, Relative Sensitivities and LODs Obtained with FIGS Atomizer and Comparison of LODs Obtained with HG-CT Coupled to Other Detectors

				LODs (ng L^{-1})/(pg)		
As species	slope[a] (μV s L ng^{-1})	relative sensitivity[b] (%)	R^2	AFS (this work)	AAS[13]	ICPMS[14]
iAs(III)[c]	N.D.[d]			0.44/0.26[e]	30/15	1.2/0.6
iAs(III+V)[f]	0.283 ± 0.003	100.0 ± 1.4	0.9992	0.74/0.45	63/32	3.4/1.7
MAs(III+V)[f]	0.276 ± 0.002	97.6 ± 1.1	0.9997	0.15/0.09	30/15	0.055/0.027
DMAs(III+V)[f]	0.279 ± 0.004	98.8 ± 1.8	0.9982	0.17/0.10	30/15	0.14/0.071
TMAs(V)O[c]	0.298 ± 0.001	105.4 ± 1.0	0.9999	0.67/0.40	30/15	0.1/0.049

[a]Uncertainty expressed as SD. [b]Relative to iAs(III+V) sensitivity; uncertainty expressed as combined SD. [c]Without prereduction. [d]Not determined. [e]Only blanks measured, for LOD assessment the sensitivity of iAs(III+V) taken. [f]With L-cys prereduction.

In summary, the AFS peaks, regardless of the atomizer, were narrower and more symmetric (without significant tailing) than with multiatomizer/AAS detection. Consequently, the separation of all arsines was more easily achieved.

Analytical Performance. To demonstrate the exceptional analytical performance of HG-CT-AFS using FIGS and its advantages over MDF atomizers, the following critical parameters were investigated: (i) sensitivity, (ii) repeatability and (iii) limit of detection (LOD).

(i). Sensitivity. The sensitivities (slopes of calibration curve evaluated from peak areas) obtained with the FIGS atomizer are listed in Table 2. Calibration curves were linear in the tested concentration range up to 1500 ng L^{-1}. 100% hydride generation efficiency and the same sensitivities for both trivalent and pentavalent hydride forming As species were achieved in our recent works employing the same experimental parameters for HG-CT when detection was performed by AAS with the multiatomizer[8,11,32] or by ICPMS.[14] The slopes obtained by HG-CT-AFS with FIGS also exhibited good uniformity for iAs(V), MAs(V) and DMAs(V) after L-cys prereduction and for TMAs(V)O measured without prereduction because all the relative sensitivities were between 97.6 and 105.4% (Table 2).

With the MDF atomizer, iAs(V) sensitivity was 3.16 ± 0.06 times lower. This can be attributed to (i) much higher temperature and thus thermal expansion in the observation volume of the MDF atomizer and (ii) to chemical reactions of analyte free atoms with ambient O_2 that penetrates into the flame of the MDF atomizer more easily than to the observation volume of the FIGS atomizer.[23] Nevertheless, analogously as in the case of the FIGS atomizer comparable relative sensitivities (relative to iAs(V) = 100%) were also achieved: 96.2 ± 1.6% and 105.6 ± 1.6% for MAs(V) and DMAs(V), respectively.

(ii). Repeatability. The repeatability of the method was determined as RSD for nine measurements of individual As species at 100 ng L^{-1}. The repeatability of peak areas with the FIGS atomizer was 2.1, 2.0, 2.7 and 2.0% for iAs(V), MAs(V), DMAs(V) and TMAs(V)O, respectively. The repeatability measured with the MDF atomizer was similar, 1.6, 1.8 and 2.6% for iAs(V), MAs(V) and DMAs(V), respectively.

(iii). LOD. For LOD evaluation, the baseline noise was evaluated as SD of peak areas. The instrument baseline level and baseline noise were observed when no gases were introduced to an atomizer. When the FIGS atomizer was switched on, no significant contribution either to baseline level (no FIGS emission) or to noise was observed. Consequently, the signal correction to the flame emission (see atomic fluorescence spectrometer description) was not used. In contrast, the MDF atomizer was characterized by significant short time fluctuations of its flame emission, increasing approximately 6 times the baseline noise. Therefore, using the correction of the flame emission was essential for measurements with the MDF atomizer. However, even with correction, the MDF baseline noise was 2 times worse compared with FIGS. Therefore, only the FIGS atomizer was used for further detailed LOD characterization.

Due to the extraordinary sensitivity the LODs (3σ, n = 13) obtained for the whole measurement procedure with HG-CT with the FIGS atomizer for iAs(III), iAs(III+V) and TMAs(V)O were controlled by blank contamination. The blank signals for iAs(III+V) and TMAs(V)O typically corresponded to concentrations of 7.1 and 2.3 ng L^{-1}, respectively. The amount of iAs(III) was approximately one half of the sum for iAs(III+V), 3.6 ng L^{-1} (see Figure 4 for a comparison of the blank signals with and without L-cys prereduction). Contamination from reagents was the main source of elevated blank signals because attempts to clean the whole generator with concentrated HNO_3 or HCl to eliminate memory effects did not reduce the blank values. Blank signals for MAs(III+V) and DMAs(III+V) were low (see Figure 4), around the LOD levels. The improvement factors of the LOD in comparison to the MDF atomizer were thus calculated only for these species because these LODs were not controlled by contamination but rather by baseline noise. The improvement factors for MAs(III+V) and DMAs(III+V) were 4.1 and 4.3, respectively, which is not far from the theoretical value of six predicted from the improvement in sensitivities (3 times) and from the reduction of baseline noise (2 times). As presented in Table 2, the LODs

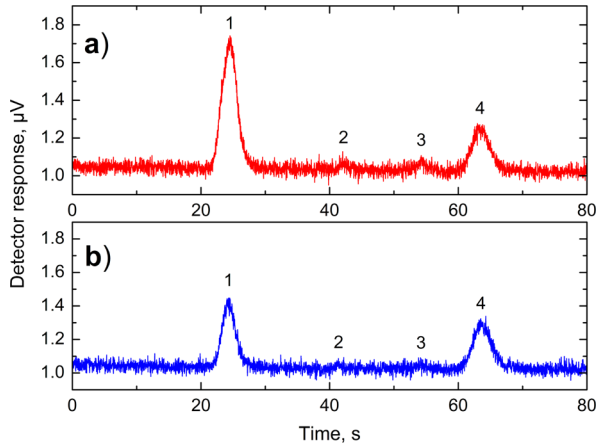

Figure 4. Chromatograms of typical blank signals (a) with L-cys prereduction (red line) and (b) without L-cys prereduction (blue line). 1, AsH_3; 2, CH_3AsH_2; 3, $(CH_3)_2AsH$; 4, $(CH_3)_3As$. PMT voltage 1500 V.

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Table 3. Determination of Individual As Species Content (ng L^{-1}) in Certified Reference Materials and in Two Samples of Bottled Drinking Water[a]

sample	iAs(III+V)	MAs(V)	DMAs(V)	TMAs(V)O	total As	reference value for total As
SLRS-5	295.5 ± 6.1	39.2 ± 2.7	45.6 ± 2.5	19.6 ± 0.7	400 ± 7	413 ± 39[b]
CASS-5	1103 ± 44	10.2 ± 2.4	116.0 ± 6.8	16.2 ± 1.6	1245 ± 45	1240 ± 90[b]
Water 1	409 ± 11	N.D.[c]	0.2−0.6[d]	N.D.[c]	409 ± 11	398 ± 34[e]
Water 2	260 ± 11	1.5 ± 0.5	7.3 ± 1.3	0.7−2.2[d]	269 ± 11	289 ± 35[e]

[a]Uncertainties presented as 95% confidence limits. [b]Certified value. [c]Value lower than LOD. [d]Value between LOD and LOQ. [e]Determined by standard addition technique liquid sampling Agilent 7700x ICPMS (conditions in Table S-1, Supporting Information).

obtained with the FIGS atomizer are extremely low and were improved by 2 orders of magnitude in comparison with our previous HG-CT system with AAS detection.[13] These LODs can even compete with those obtained with the HG-CT-ICPMS system in our laboratory recently.[14] Moreover, the absolute LODs (in pg) are at least 1 order of magnitude better than those achieved with other HG-CT-AFS based systems.[25−27] The LODs for trivalent methylated species, MAs(III) and DMAs(III), were also assessed. The determination is based on the observation that the generation efficiency of CH_3AsH_2 and $(CH_3)_2AsH$ from Tris−HCl medium (without L-cys prereduction) is 100%, and therefore, the sensitivities of MAs(III+V) and DMAs(III+V) for their determination can be safely utilized.[8] The obtained LODs were at the same level as for the sums of pentavalent species.

Analysis of Water Samples and CRMs. The accuracy of the developed HG-CT-AFS method was verified by speciation analysis of two certified reference materials, SLRS-5 (river water) and CASS-5 (nearshore seawater), with total certified As concentrations of 0.413 ± 0.039 and 1.24 ± 0.090 μg L^{-1}, respectively. The results are summarized in Table 3. Although these CRMs are certified only for total As content, the speciation agreed well with the previously reported values measured by several methods.[14] As presented in Table 3, iAs(V) was the prevalent species in both CRMs, but the pentavalent methylated species can also be conveniently quantified by our method. Trivalent methylated species were not detected/quantified at all and iAs(III) only to a small extent, reaching to approximately 1% of the iAs(III+V) concentration. Two samples of bottled drinking water were also analyzed (Table 3). The sums of As species were in agreement with the values obtained by liquid sampling ICPMS. Concentrations of As species were also measured with the reference HG-CT-AAS method; however, because of the limited detection capability, only the sums of iAs(III+V) were quantified. The determined values of 387 ± 28 and 254 ± 32 ng L^{-1} for water 1 and 2, respectively, corresponded well to the HG-CT-AFS results in Table 3.

HG-CT-AFS Analysis of BECs Samples and Comparison with HG-CT-ICPMS. The excellent analytical performance of the HG-CT-AFS with the FIGS atomizer and the extremely low LODs for all As species allows for convenient analysis in microsamples of biological material. Here, BEC samples collected from Chihuahua residents with high chronic exposure to iAs from drinking water[15] were analyzed at IAC by the newly developed HG-CT-AFS method, and the results were compared with results of the speciation analysis by HG-CT-ICPMS at UNC. Only the sums of tri- and pentavalent species after L-cys prereduction are presented in Figure 5 and Table S-2 (Supporting Information). $TMAs^{V}O$ was not determined in those samples because its contents were very low. When the whole dataset of 30 measured BEC samples is considered, the

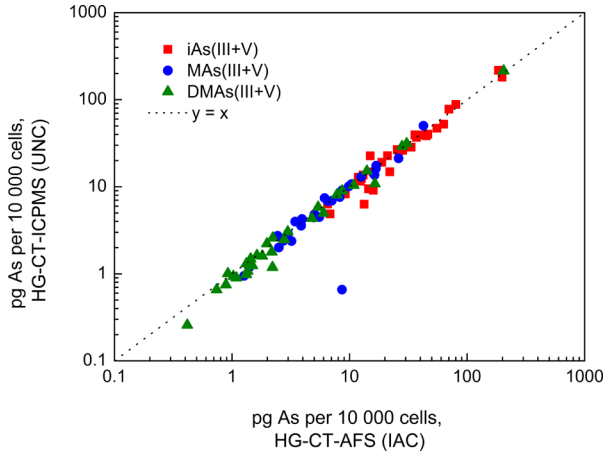

Figure 5. Comparison of results of analyses of BECs by HG-CT-AFS and HG-CT-ICPMS. ■, iAs(III+V); ●, MAs(III+V); ▲, DMAs(III+V).

concentrations of iAs(III+V), MAs(III+V) and DMAs(III+V) determined by HG-CT-AFS versus HG-CT-ICPMS (Figure 5) exhibited good linearity with the slopes close to 1 (1.016 ± 0.025 with R = 0.9861; 1.02 ± 0.04 with R = 0.9721; 1.044 ± 0.006 with R = 0.9995). Such an agreement between both laboratories indicates good stability of As species methylation even after long storage and transport. For six BEC samples, the oxidation state of trivalent species was also determined when they were analyzed without L-cys treatment. The DMAs(III) signals were corrected for the limited selectivity of HG at pH 6 by spiking experiments when DMAs(V) was found to generate with 5.0−6.8% efficiency without L-cys prereduction. Conversely, the contribution of MAs(V) to MAs(III) was negligible (<1%). The percentages of As species found at IAC in the trivalent form in comparison to the values found at UNC varied from 45−100% for iAs(III), 39%−100% for MAs(III), and 0%−52% for DMAs(III), with medians of 91%, 82% and 19%, respectively. Currier et al.[9] observed that MAs(III) and DMAs(III) species in cell lysates were stable for at least 3 weeks when these samples were stored at −80 °C. Our results indicate that these species in the lysed cells were not completely preserved after storing for several months and shipping overseas on dry ice. Especially DMAs(III), which was nearly completely oxidized. Oxidation during thawing of the BEC samples and during their preparation before analysis can also play a role.

CONCLUSION

The selective generation of arsines combined with cryotrapping and AFS detection using a FIGS atomizer is an extremely sensitive approach, as demonstrated by the determination of the toxicologically important As species in water samples and in

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human cells. Even though the required instrumentation is relatively simple and inexpensive, the yielded LODs outperform the much more frequently used HPLC-ICPMS by several orders of magnitude and are comparable with HG-CT-ICPMS. The advantage of the FIGS atomizer compared with a MDF atomizer lies in improvement of LODs (4 times). The equal sensitivities for all As species observed with both the FIGS or with the MDF atomizers suggest equal generation and atomization efficiencies for all arsines. This suggests that the HG setup and both atomizers perform well, which has never been reported with AFS instruments due to either different atomization efficiency of arsines[35] and/or different generation efficiency from HCl medium.[36−38] Our results confirm that single species standardization by using iAs(V) standards can result in accurate quantification of all iAs and methylated As species. This is important because the trivalent methylated As standards are very unstable and are not readily available from commercial sources.[39]

■ ASSOCIATED CONTENT

Supporting Information

Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org/.

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Notes

The authors declare no competing financial interest.

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