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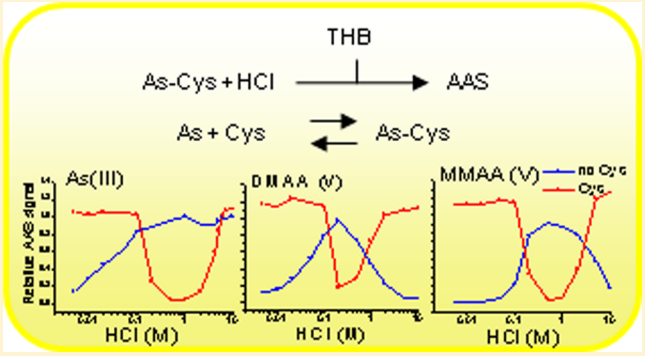
Chemical Generation of Arsane and Methylarsanes with Amine Boranes. Potentialities for Nonchromatographic Speciation of Arsenic

Emanuela Pitzalis,*[,†] Massimo Onor,[†] Marco Carlo Mascherpa,[†] Giacomo Pacchi,[†] Zoltan Mester,[‡] and Alessandro D'Ulivo[†]

[†]C.N.R., Consiglio Nazionale delle Ricerche, Institute of Chemistry of OrganoMetallic Compounds, Via G. Moruzzi 1, 56124 Pisa, Italy

[‡]National Research Council of Canada, 1200 Montreal Road, Ottawa, ON K1A 0R6, Canada

ABSTRACT: The efficiency of chemical generation of arsanes from inorganic arsenic, monomethylarsonic acid (MMAA) and dimethylarsinic acid (DMAA), to arsane, AsH_3, monomethylarsane, CH_3AsH_2 (MMA), and dimethylarsane, $(CH_3)_2AsH$ (DMA), has been investigated in different reaction media with the aim to better elucidate the mechanisms controlling their generation process and to find the experimental conditions to implement a nonchromatographic arsenic speciation analytical method, which is based on the selective determination of some arsenic species. Studies were performed by continuous flow hydride generation coupled with atomic spectrometry (CF-HG-AS), using different reductants such as borane−ammonia (AB), borane-*tert*-butylamine (TBAB), and sodium tetrahydridoborate (THB) in HCl and $HClO_4$ media, in the presence or absence of L-cysteine (Cys). The efficiency of HG processes for MMA and DMA is mainly controlled by the reactivity of the substrates with the borane, which could be strongly influenced by the formation of ion couples. The protonation of arsane did not play a significant role in the employed reaction system. By taking advantage of the different reactivity pattern of As species in selected generation conditions, DMAA and MMAA could be selectively determined in 0.5 and 10 M $HClO_4$ solutions, respectively, in the presence of Cys, with AB as the reducing agent. The presence of Cys as a masking agent and the peculiar reducing properties of AB ensured a good control of interferences, as far as it has been observed for Co(II), Ni(II), Cu(II), Fe(II), Fe(III). The overall time needed to complete the prereduction step has been verified for MMAA and DMAA at different acidities in order to achieve the best selectivity. The selective determination of DMAA with AB/Cys in $HClO_4$ has been optimized and applied to certified reference materials (CRMs) of natural waters CASS-4, SLRS-4, and NASS-4 (NRCC). The estimation of DMAA concentration allows us to correct the concentration of As(III) for the interference of DMAA in the selective determination of As(III) according to a selective HG method recently reported.



Arsenic speciation analysis is of fundamental importance in the evaluation of the toxicological impact of this element in a wide range of samples, as for example, natural waters,[1−3] soils/sediments,[4] and food,[5] besides giving information about transport processes in the environment;[6,7] moreover, it is extremely useful to understand arsenic biotransformation in humans and animals that have been exposed to environmental contamination.[8,9]

Methods employed for the speciation of the different arsenic forms, like hydride generation, voltammetry, and chromatography (liquid chromatography, including high-performance liquid chromatography and gas chromatography), which are usually coupled with numerous sensitive detectors, have been extensively published and reviewed.[4,10−12]

Chemical generation of volatile hydrides (CHG) coupled with atomic and mass spectrometric techniques represents one of the most important analytical tools for the determination and speciation of hydride-forming elements at trace and ultratrace levels.[13,14] With respect to the determination of As(III), As(V), monomethylarsonic acid (MMAA), and dimethylarsinic acid (DMAA), CHG can be considered one of the most important analytical approaches when it is employed in combination with either chromatographic[15−17] or nonchromatographic methods.[30,18−22] The second approach is included in those several speciation strategies, reviewed by De la Guardia et al.[23] and by Campos et al.,[24] that take advantage of different chemical properties of the species or employ different separation techniques; they can often be easily accessible and fast, yet providing adequate information on the chemical forms of the

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analyte. In these cases,[18−22] the methods are based on the complex reactivity of different arsenic species, As(III), As(V), MMAA, and DMAA with aqueous tetrahydroborate(III) (THB), that allows the determination of each species in the presence of the others, when not responding.

Besides THB, other borane compounds have been used as reducing agents in hydride generation techniques.[25] Amine borane complexes such as borane−ammonia, AB, borane-*tert*-butylamine, TBAB, sodium cyanoborohydride, CBH, borane-dimethylamine, DMAB, were employed as useful reagents in hydride generation coupled with atomic absorption spectrometry, HG-AAS, in aqueous solutions for the determination of traces of most hydride forming elements. Interesting features were observed, like good selectivity and control of interferences, with sensitivities in many cases comparable with those obtained with THB; in addition, their aqueous solutions showed better stability, in some cases even in the absence of NaOH. In particular, the efficiency of AB in hydride generation for many analytes was proven to be generally high and very similar to the one of THB, even though with differences in the pattern of dependence from acidity, in HCl.[26]

CHG reaction efficiency of As species is severely affected by the presence of L-cysteine,[27,28] Cys, or other thiolic compounds,[20,29] with different yield patterns depending on pH and on the kind of acid medium employed. The effect of Cys on arsanes generation from inorganic arsenic with THB and its modification with acidity has been thoroughly studied with HCl, $HClO_4$, H_2SO_4 and acetic acid, indicating a strong interaction both with analyte and reductant, and its use as masking agent allowed good control of metal interfering agents.[30]

Owing to the good generation efficiency generally described for AB and TBAB, these two reagents were employed with the different As species on varying HCl and $HClO_4$ concentration, and the effect of Cys presence in these systems was tested. The different curves we registered for arsanes generation from inorganic and organic As derivatives suggested us to search for the experimental conditions to selectively determine each of the species in the presence of the others, without separation steps.

Previously we implemented a nonchromatographic method for the speciation of inorganic trivalent arsenic,[30] based on a selective HG procedure. That method could be employed in samples with low content of organic arsenic, as it showed a good As(III)/MMAA selectivity (140) but a poor As(III)/DMAA one (8). By taking into consideration that accurate quantification of As(III) is of great importance, as this species is the most toxic among As species in natural samples, we aimed to extend the method to As organic derivatives. Therefore in the present work for the first time AB has been employed as reductant in the selective generation of monomethylarsane, MMA, and dimethylarsane, DMA, from MMAA and DMAA, respectively. The data obtained by this procedure were used to correct the value of As(III) found in natural water CRM samples, CASS-4, SLRS-4 and NASS-4 (NRCC) that at those conditions was altered by the DMAA contribution, and validated by comparison with literature results obtained with different methods.

EXPERIMENTAL SECTION

Chemicals. Aqueous solutions of 0.2 M AB, TBAB, and THB were prepared daily by using the following solid reagents: borane−ammonia complex (Sigma Aldrich, assay 97%), borane−*tert*-butylamine complex (Sigma Aldrich, pellets, assay 97%), sodium borohydride (Sigma Aldrich, powder, reagent grade >98.5%). All solutions were clear and colorless and did not require filtration. In all experiments 0.2 M AB, TBAB, and THB solutions contained 0.005 M NaOH, commonly employed to stabilize THB; a 0.2 M THB solution stabilized with 0.1 M NaOH was used with As(III) solution in 1.0 M HCl, as reference measure, S_0. Stock solutions of As(III) and As(V) were prepared by dilution of commercial Fluka 1000 mg L^{-1} AAS standard solutions. Monomethylarsonic acid (MMAA) and dimethylarsinic acid (DMAA) stock solutions (1000 mg L^{-1} As) were prepared from $CH_3AsO(ONa)_2{\cdot}6H_2O$ (Carlo Erba, Codex, assay >99.4%) and $(CH_3)_2AsO(ONa){\cdot}3H_2O$ (Carlo Erba, Codex, assay >96%), respectively. Purity check on MMAA and DMAA stock solutions were performed by HPLC-HG-AFS[17] and indicated that inorganic As(V) was the main impurity, while As(III) was not detectable (<0.5%). L-Cysteine (powder, assay >99.0%) was purchased from Fluka. $CoCl_2{\cdot}6H_2O$ (Carlo Erba, powder, assay >99.0%), $NiCl_2{\cdot}6H_2O$ (Carlo Erba, powder, assay >98.0%), $FeSO_4{\cdot}7H_2O$ (Merck, powder, assay >99.5%), $FeNH_4(SO_4)_2{\cdot}12H_2O$ (Carlo Erba, powder, assay >99.0%), $Fe(ClO_4)_3{\cdot}9H_2O$(Carlo Erba, powder, assay >98%), $CuCl_2{\cdot}2H_2O$ (Carlo Erba, powder, RPE), $CuSO_4{\cdot}5H_2O$ (Merck, powder, assay >99.0%), and Na_2SO_4 (Carlo Erba, powder, assay >99.5%, RPE) were employed in the range of metal and SO_4^{2-} ion concentration of 1−1000 mg L^{-1}. All of the other chemicals were of analytical grade or higher. CASS-4, SLRS-4, NASS-4 certified reference materials were purchased from NRC, Canada. Ultra pure water purified by the Purelab Pro (USF) system was used in all the operations.

Apparatus. The schematic representation of the chemifold setup A and B employed in the present work is reported in Figure 1. A continuous flow HG coupled with an atomic absorption spectrometric detection system (Perkin-Elmer 503 atomic absorption spectrophotometer) (CF-HG-AAS) equipped with an Ar−H_2 miniflame atomizer[31] was employed for optimization and diagnostic purposes (limit of detection, 3σ, 2 ng mL^{-1}). Atomic absorption experiments were performed with an As electrodeless discharge lamp (Perkin-Elmer EDL System II) that was employed at the current recommended by the manufacturer. Absorbance measurements were performed at 193.7 nm with a spectral bandwidth of 0.7 nm.

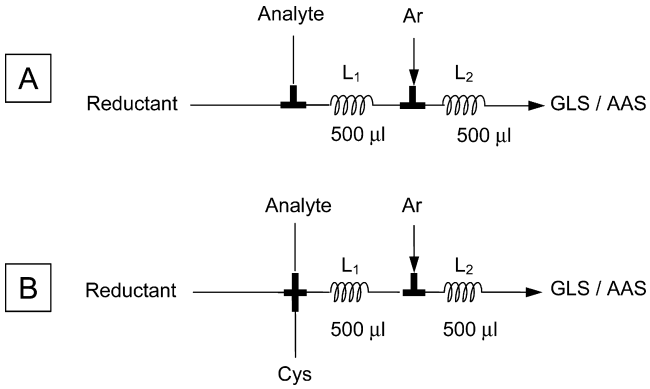

Figure 1. Schematic representation of the chemifold setup employed in the present work. Details are reported in the Experimental Section.

Analytical determinations were performed by a similar apparatus in which the AAS detector was replaced by a more sensitive atomic fluorescence detector (CF-HG-AFS, limit of detection, 3σ, ≤ 0.01 ng mL^{-1}) and using the same chemical

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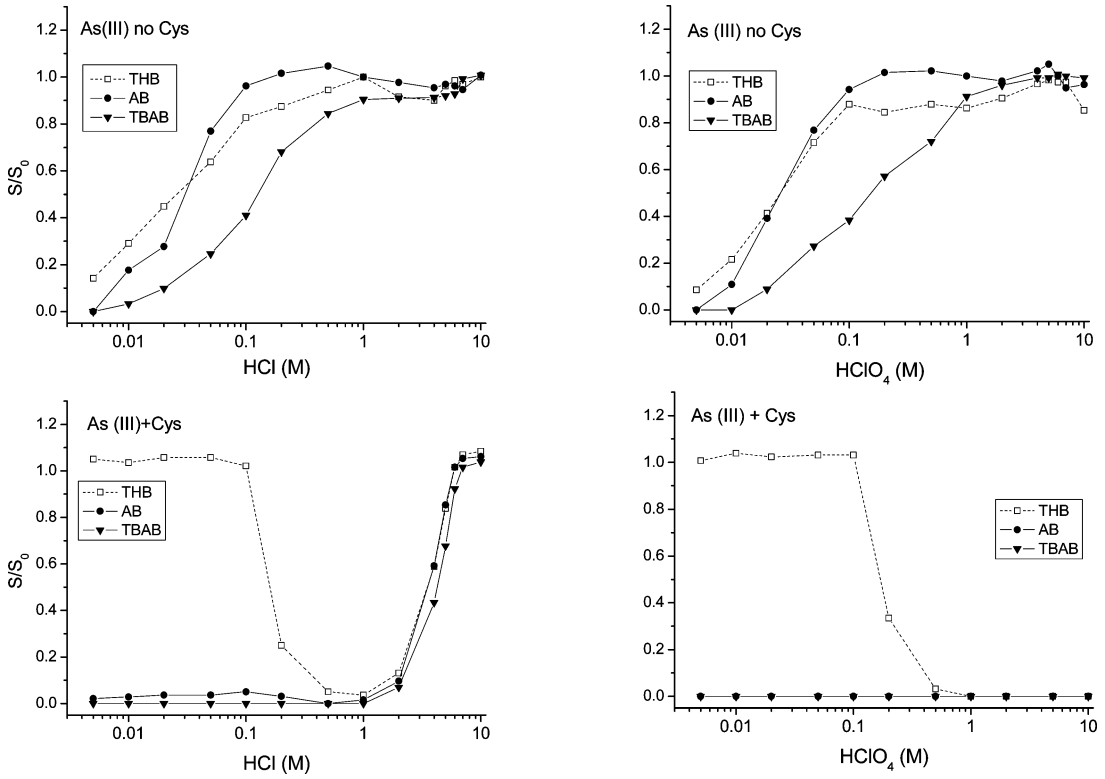

Figure 2. Effect of HCl and $HClO_4$ concentration on arsane generation from 0.2 mg L^{-1} As(III) in the absence and in the presence of 0.2 M Cys, added to the analyte in batch for 24 h: 0.2 M THB, AB, and TBAB, in 0.005 M NaOH. Recorded with analytical setup A.

reaction conditions optimized by CF-HG-AAS. The AFS detector was a laboratory-built nondispersive atomic fluorescence spectrometer, described previously[32] except for the following modifications. A commercially available radiation source and power supply (As electrodeless discharge lamp, EDL System 2, Perkin-Elmer) was used. The feeding power for EDL was square-wave modulated at 500 Hz with a 50% duty cycle, and the operating current was set at 400 mA. A 190 nm optical filter model 190-B (Acton Research Corporation, MA) with 28.4% peak transmittance and 37.8 nm fwhm was inserted just in front of the photocathode of a solar-blind photomultiplier (R166, Hamamatsu). Signal output from the lock-in amplifier was continuously monitored at a frequency of 20 s^{-1} and stored on a PC.

The atomizer, for both CF-HG-AAS e CF-HG-AFS, was a miniature argon−hydrogen diffusion flame[31] supported on a quartz tube of 6.5 mm inside diameter. The miniature diffusion flame was supported with a total argon and hydrogen flow rate of 70 and 240 mL min^{-1}, respectively. Argon was introduced after reaction coil L_1, while hydrogen was introduced between the gas−liquid separator and the atomizer. The gas liquid separator was in borosilicate glass (60 mm long, 10 mm i.d., inlet and outlet tubing 6 mm o.d. and 2 mm i.d.). The waste liquid solutions were pumped off by a peristaltic pump; the flow rate of the waste channel was experimentally adjusted in order to find a compromise, which allowed us to achieve the minimum level of waste solution inside the gas−liquid separator.

The CF-HG system was assembled with a peristaltic pump (Ismatec pump head MS/CA4−12 fitted on a Masterflex L drive H-7519−25). Ismatec Tygon microtubings of appropriate diameters were used for propelling reductant and waste solutions. Ismatec Viton microtubings were used for propelling sample and Cys acid solutions. Analyte and reductant solution flow rates were 4 and 2 mL/min, respectively; Cys solution flow (setup B) was 4 mL/min. All the mixing T-junctions and X-junctions were from Ismatec (Kel-F, 0.8 mm i.d.). The reaction coils were in Teflon PFA (0.5−0.8 mm i.d.).

Procedure. Two different chemifold arrangements were employed in the present work (Figure 1). The system A represents the analytical setup commonly employed in hydride generation techniques, where the reductant and the analyte solutions (with or without the addition of additives) are allowed to react for times that relied on the volume of the coil L_1; after argon introduction and stripping of volatile compound in the coil L_2, phases are separated in a gas−liquid separator. The vapor phase is carried to the spectrometer together with the hydrogen flow, and the liquid phase is discarded. The setup B illustrates the setup employed to allow the contemporary interaction among three reagent solutions online, before the steps already described; in this work, these three reagents are analyte, reductant, and Cys solutions.

According to the mathematical model developed for CF-HG-AAS,[13] the absorbance signal, S, depends on the number of analyte atoms delivered to the atomizer in the unit time, which is linearly related to hydride generation efficiency, β_g, sample flow rate, f_s, and analyte concentration C_0. Assuming that under optimized conditions for As(III) (1.0 M HCl, 0.2 M THB, setup A) the measured absorbance signal for the generated AsH_3, S_0, corresponds to a hydride generation efficiency $\beta_g = 1$, then the ratio $S/S_0 = \beta_g$ will be reported as the parameter giving the relative generation efficiency of methylarsanes with respect to arsane, under different reaction conditions.

The plots giving S/S_0 versus acid concentration (Figures 2, 3, 4, and 5) or S/S_{DMAA} versus interference concentration (Figure 6) are typically reproducible within 6%.

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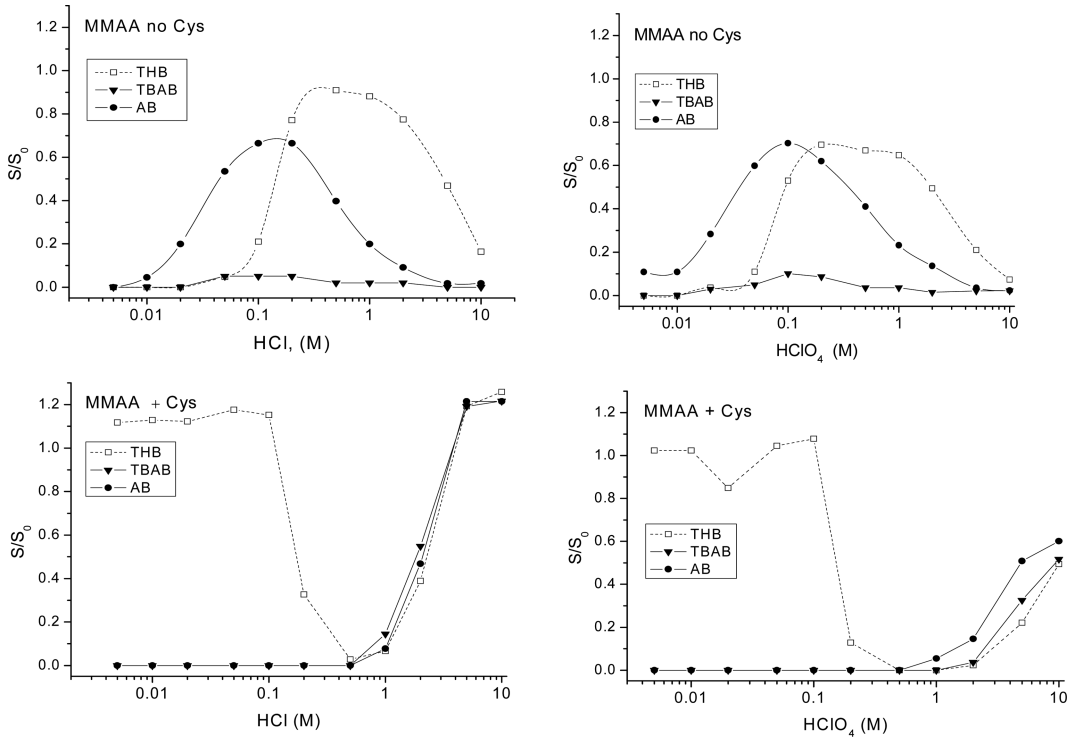

Figure 3. Effect of HCl and $HClO_4$ concentration on MMA generation from MMAA (0.2 mg L^{-1} As) in the absence and in the presence of 0.2 M Cys, added to the analyte in batch for 24 h: 0.2 M THB, AB, and TBAB in 0.005 M NaOH. Recorded with analytical setup A.

RESULTS AND DISCUSSION

Reactivity. Acidity curves of As(III), MMAA and DMAA, 200 ng mL^{-1} As, in 0.005−10 M both HCl and $HClO_4$ were recorded with the experimental setup A reported in Figure 1: 0.2 M THB, AB, and TBAB, in 0.005 M NaOH aqueous solution were employed as reagents; the same measurements were recorded after analyte prereduction with 0.2 M Cys.[30] In fact, inorganic As, MMAA and DMAA, are known to react with Cys according to the following reactions:[33]

$$H_3AsO_3 + 3RSH \rightarrow As(SR)_3 + 3H_2O \quad (1)$$

$$H_3AsO_4 + 5RSH \rightarrow As(SR)_3 + RSSR + 4H_2O \quad (2)$$

$$MeAs(O)(OH)_2 + 4RSH \rightarrow MeAs(SR)_2 + RSSR + 3H_2O \quad (3)$$

$$Me_2As(O)OH + 3RSH \rightarrow Me_2As - SR + RSSR + 2H_2O \quad (4)$$

Total As species are known to respond with the same efficiency by CF-HG-AS after 30 min prereduction with Cys in 0.1 M HCl[30]. In order to verify the reaction times between analytes and Cys at different acidities and in $HClO_4$ solution, the change of absorbance signals were monitored over time, up to the achievement of a stable signal. Measurements were performed at 0.05, 1, and 10 M acid concentration for all the species. For As(V), the absorbance signals had stabilized within 30 min in both HCl and $HClO_4$ media. For MMAA, stable signal was achieved within 30 min in HCl; however, in $HClO_4$, signal changes still occurred beyond 30 min, and signal stability was achieved after 24 h. For DMAA, stable signals were achieved after 30 min in all of these conditions, except for 5 M $HClO_4$. Owing to these findings, all acidity curves were recorded after prereduction with Cys for 24h, in order to obtain comparable curves.

As(V) behavior with THB, in the absence of Cys, in HCl and $HClO_4$ media was reported previously.[30] AB and TBAB activity was poorer than THB in generating arsane from As(V) in the whole HCl concentration range. For example, relative signals S/S_0 were in the range of 0.11−0.26 at 10 M HCl for AB and TBAB, versus 0.7 for THB, and AB had a maximum of 0.17 at 0.2 M; therefore, data from As(V) were not reported here.

Acidity curves of As(III) are reported in Figure 2. Without Cys, in both acids the signal increased, with different intensities, together with acid concentration in both HCl and $HClO_4$, according to the similar strength of the two acids; all the reductants reached the maximum value in the arsane generation.

After the addition of Cys, the arsane generation pattern changed. As already reported,[30] THB was active at low concentration in both acids, giving the maximum signal up to 0.1 M. The signal then decreased to zero, and only in HCl did it increase again, to reach the maximum at HCl concentration higher than 5 M, while no signal was observed at high acidity in the absence of the Cl^- ion. With resepect to the present results, AB and TBAB were not active at all in the whole concentration range examined for $HClO_4$. In HCl medium, the pattern was different, and both borane complexes behaved similarly: they were not active at low acid concentration, then the signal started to increase at 2 M HCl, reaching the reference value, $S/S_0 = 1$, at 5−10 M concentration. This evidence could be explained by the formation of an As-containing species where both Cys and chloride ion play a role in activating the analyte substrate to the reaction with the forms of THB or borane complex intermediates that are active at this acidity. Perchloric acid at a concentration above 1 M and in the presence of Cys

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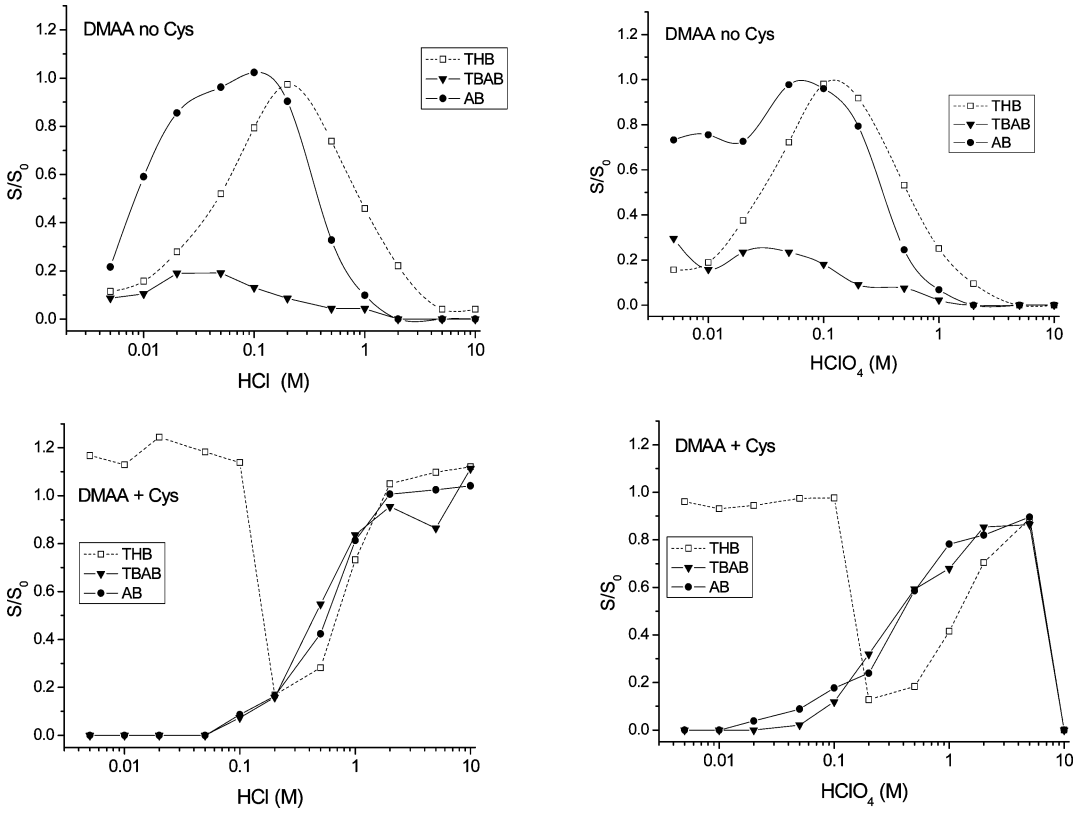

Figure 4. Effect of HCl and $HClO_4$ concentration on DMA generation from DMAA (0.2 mg L^{-1} As) in the absence and in the presence of 0.2 M Cys, added to the analyte in batch for 24 h: 0.2 M THB, AB, and TBAB in 0.005 M NaOH. Recorded with analytical setup A.

seemed here to behave as an interfering species on arsane generation from As(III).

Acidity curves of MMAA are reported in Figure 3. Without Cys, TBAB gave a very poor signal in both HCl and $HClO_4$. The MMAA acidity curves with THB and AB are very different from the ones of inorganic arsenic: they show poor or no activity at the extreme values of acidity examined in both media, while there is a maximum in the range of 0.05−0.2 M and 0.2−2 M on both HCl and $HClO_4$ for AB and THB, respectively. The relative signal values were the highest for THB in HCl. After the addition of Cys, the MMAA response in HCl was similar to that of inorganic arsenic, with a narrower lack of signal with THB; in $HClO_4$, the main difference was the presence of a generation signal for every reductant at high acid concentration, up to 0.6 units at 10 M, indicating that the presence of a methyl substituent bound to the arsenic atom seriously modifies the process. In fact, in the case of inorganic As/Cys, the signal at 10 M $HClO_4$ concentration was observed only after the addition of the chloride ion.[30]

Acidity curves of DMAA are reported in Figure 4. As in the case of MMAA, without Cys TBAB showed low activity in the whole range of acid concentration, and THB and AB had a maximum of generation at ∼0.1 and ∼0.2 M, respectively; poor or no activity was observed at the extreme values of acidity, except for AB, which at low $HClO_4$ concentration had generation efficiency close to 0.8. After the addition of Cys, the pattern in HCl is similar to the MMAA one, apart from a narrower and not complete lack of signal with THB at intermediate acidity. Similarly, in $HClO_4$, the signal increased from 0.1 M acidity; however, in this case, the signal dropped down at 10 M, where no generation was observed for any reagent. This evidence could be explained by the protonation of Me_2AsH just generated; otherwise, ion couple formation before generation between the protonated form of the substrate complex, $[Me_2As\text{-}Cys]H^+$, and the ClO_4^- anion could be considered.

In order to clarify the low generation of methylarsanes at high acidities, the protonation of the arsane just generated was taken into consideration, and tests on protonation after generation were performed: solutions of inorganic As, MMAA or DMAA, with or without Cys, were allowed to react with THB in the L_1 coil, in the best conditions to generate arsanes, before introducing online acid solutions at variable concentration. In all these cases, the generation efficiency did not change significantly, indicating that the protonation could not be taken into account to justify the decrease of the signal in the present experimental setup.

The hypothesis of ion couple formation seems to be more realistic because the effect is strictly related to the presence of perchlorate anion in strongly acidic media. Indeed, if the depressive effect of $HClO_4$ in the presence of Cys for all arsenic species is considered, it is evident that it increases in the order DMAA, MMA, As(III). Under these conditions, according to reactions 1−4, the arsenic substrates are the corresponding thiolate complexes: $Me_2As{-}S{-}CH_2{-}CH(NH_2)(COOH)$, $MeAs{-}[S{-}CH_2{-}CH(NH_2)(COOH)]_2$, and $As{-}[S{-}CH_2{-}CH(NH_2)(COOH)]_3$. Then, the depressive effect decreases with the number of cysteines bound to the arsenic. Protonation of these substrates could take place on an amino group, but it seems unlikely that the formation of ion couples far from the As central atom could hinder the attack of borane on As. Protonation of sulfur could be another hypothesis because the formation of $-As{-}SH^+-$ will lead to the formation of an ion couple which is close to the As central atom. However, the

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protonation of sulfur is much less favored than nitrogen and oxygen. The last possibility could be the protonation of the arsenic central atom to form an arsonium cation. The protonation of arsenic would be favored by the presence of sulfur donor groups, which means that protonation of As is more favored in $As(SR)_3$ with respect to $MeAs(SR)_2$ and $Me_2As(SR)$. Then, the formation of an ion couple on the As central atom would hinder the borane attack to form the final arsane.

Analytical Application. On the basis of our previously published method for the speciation of inorganic arsenic,[30] (i) total arsenic can be determined by sample treatment with 0.2 M Cys for 30 min (acidity 0.1 M HCl, 0.2 M THB, by CF-HG-AFS (setup A)); (ii) As(III) is selectively determined in 0.005 M CH_3COOH by adding Cys online in a cross chemifold that allows simultaneous mixing of sample, 0.2 M Cys and 0.1 M THB (Figure 1, setup B); (iii) As(V) does not respond under the conditions (ii) and can be determined by difference. With this method, the selectivity of As(III)/As(V) is 220; with respect to methylated species, As(III)/MMAA selectivity is 140, but the selectivity As(III)/DMAA is reported to be 8. Therefore, this method can be employed only in those samples where DMAA is a minor fraction. The different curves observed in arsane generation from inorganic and organic As derivatives allowed us to find the experimental conditions to selectively determine methylated As species in the presence of the others, without separation steps. Among the different patterns, we chose to operate at 0.2 M AB in 0.005 M NaOH as the reductant with analytes in $HClO_4$ in the presence of Cys (0.2 M): looking at the relative curves, assembled in Figure 5, 0.5 and 10 M $HClO_4$ concentrations could be suitable to selectively determine DMA and MMA, respectively.

The prereduction of the analytes with Cys in $HClO_4$ was prolonged for 24 h: at 0.5 M $HClO_4$, DMAA completely reacted with Cys in 3 h, but MMAA reaction was slower. Thus,

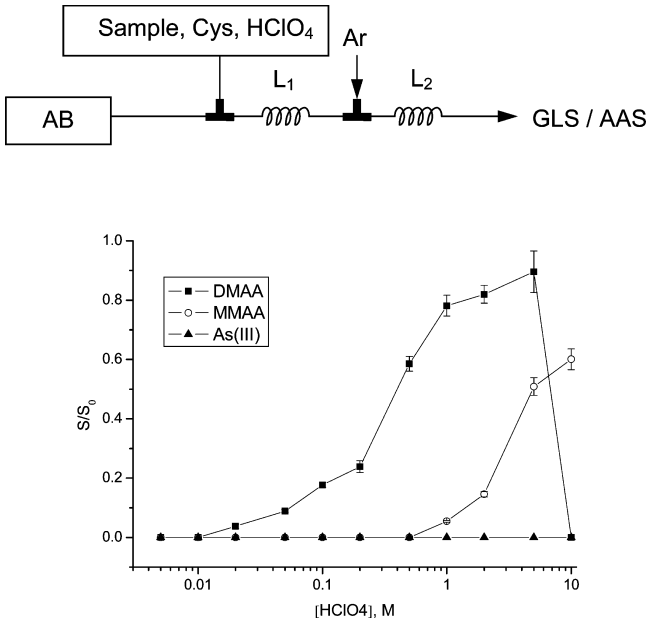

Figure 5. Comparison among generation efficiency trends (S/S_0 is reported) of arsane, MMA, and DMA from As(III), MMAA, and DMAA (0.2 mg L^{-1} As) $HClO_4$ solutions, vs acid concentration, in the presence of 0.2 M Cys, added to the analyte in batch for 24 h. Reductant is 0.2 M AB in 0.005 M NaOH.

the reaction time was chosen to allow the complete reaction of MMAA with Cys, a process that improved the selectivity of DMAA/MMAA. Under these conditions, As(III) did not respond as well as As(V), previously reduced to As(III) by Cys; the presence of Cys also allowed a good control of interferences, as described later. Under the same conditions, similar results were obtained with TBAB as the reductant (cf. Figures 2, 4, and 5), but TBAB solutions caused foaming in the gas–liquid separator, and therefore, AB was preferred. THB was not taken into consideration because its response in DMA generation was about one-third of both AB and TBAB (cf. Figure 4).

MMAA could be selectively determined in 10 M $HClO_4$; however, the MMAA determination method has a poor application in water analysis, owing to the high acid concentration that would critically dilute the analyte; so, the method was not pursued further, although it could be useful in the analysis of solid samples like food, soils, or sediments.

DMAA could be selectively determined at 0.5 M $HClO_4$. The sensitivity (slope of the calibration curve, m, au/μg,) and the selectivity ($m_{DMAA}/m_{As\ species}$) of the DMAA determination method are reported in Table 1 for all As species; these

Table 1. Sensitivity and Selectivity for Different As Species in HG-AFS

As species	As (III)	As (V)	MMAA	DMAA
sensitivity, m (au/μg)	0.125	0.124	0.649	28.9
selectivity $m_{(DMAA)}/m_{(As\ species)}$	230	233	45	1

parameters were obtained by CF-HG coupled to atomic fluorescence spectrometry, owing to the better sensitivity of the technique. The data are relevant to a 24 h pretreatment.

The sensitivity m for DMAA did not change after 3 h, indicating that the process was complete at that time, while the one for MMAA was 0.864 au/μg after 3 h, with a selectivity value 33 ($m_{(DMAA)}/m_{(MMAA)}$). It then continued to decrease, reaching the value of 0.649 au/μg, at 24 h, with the best selectivity ($m_{(DMAA)}/m_{(MMAA)}$ = 45) that could be obtained.

Interference Effects. Interference curves are reported in Figure 6. Relative signals normalized with respect to the reference signal, S_{DMAA}, recorded without interferent, were obtained by the CF-HG-AAS technique, by varying the interferent content in the analyte solution, DMAA (0.2 mg L^{-1} As) in 0.5 M $HClO_4$, in different conditions: (i) according to the method described in the present work, with 0.2 M AB in 0.005 M NaOH, 24 h prereduction with Cys 0.2 M; (ii) with 0.2 M AB in 0.005 M NaOH as reductant, without Cys; (iii) with 0.2 M THB in 0.005 M NaOH as reductant, without Cys; (iv) the same as (i), without analyte, to exclude the presence of As impurities in the interferent. Co(II), Ni(II), Cu(II), Fe(II), and Fe(III) were examined as interferents in the concentration range of 1–1000 mg L^{-1}; in the case of Cu(II), both Cl^- and SO_4^{2-} salts of the metal were employed to test the effect of the counterion; in addition, a salt of a noninterferent metal was used to check only the SO_4^{2-} effect. Ni and Co did not interfere using AB, even without Cys, while with THB, they showed a strong depressive effect, probably due to the higher strength of the reductant, similar to what happened with Cu(II). Fe(II) and Fe(III) interfered over 100 mg L^{-1}, and this could not be attributed to the SO_4^{2-} ion, if present, because it had no effect up to 1000 mg L^{-1}. In reality, in the case of Fe(II) and Cu(II), with the use of Cys and AB, a signal enhancement effect was

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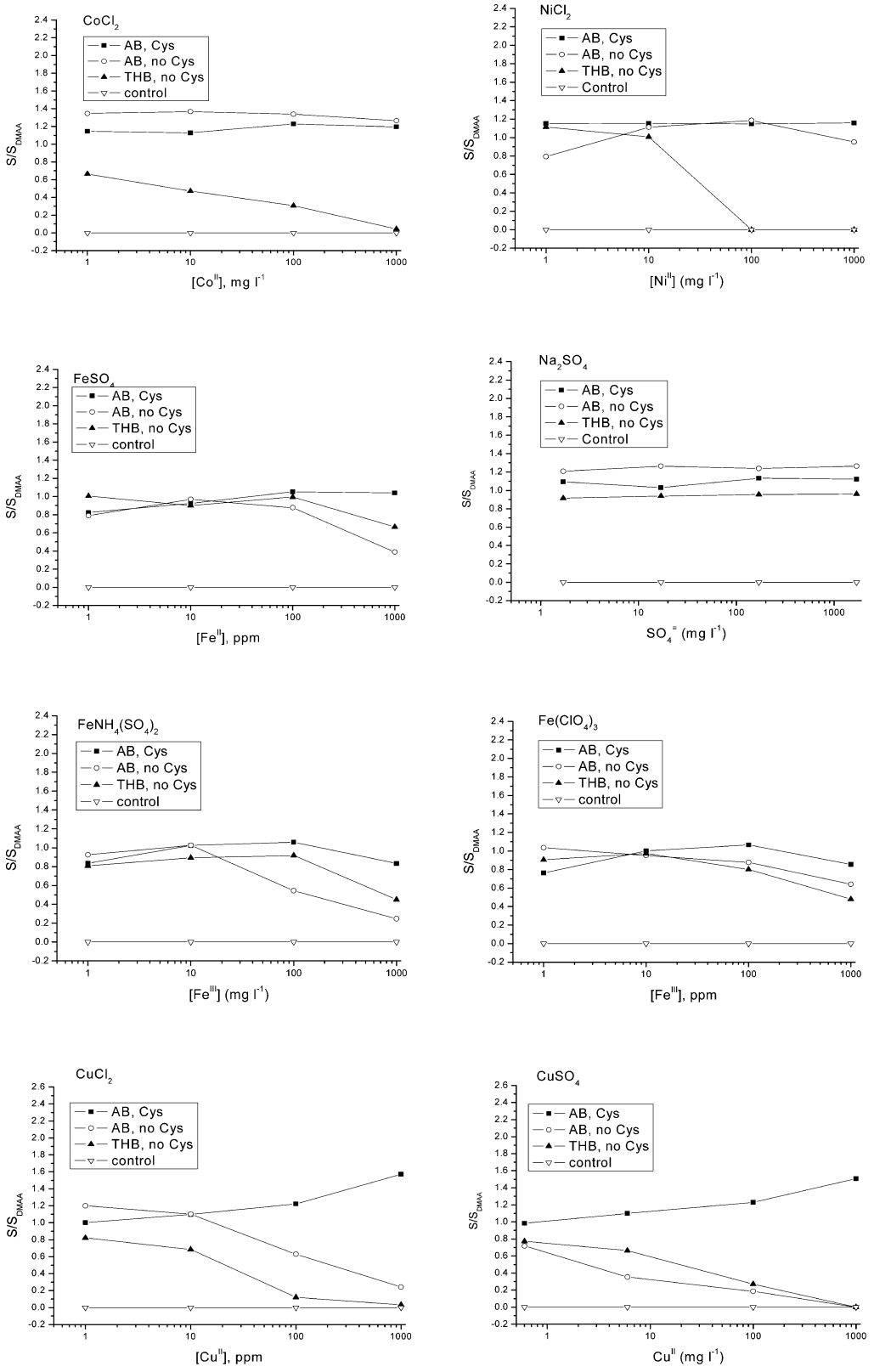

Figure 6. Interference effect of selected transition metal ions in the determination of 0.2 mg L^{-1} DMAA employing the analytical setup A, DMAA (0.2 mg L^{-1} As) in 0.5 M $HClO_4$, at different conditions: (i) according to the method described in the present work, with 0.2 M AB in 0.005 M NaOH, 24 h prereduction with Cys 0.2 M; (ii) 0.2 M AB in 0.005 M NaOH, without Cys; (iii) 0.2 M THB in 0.005 M NaOH, without Cys; (iv) at the same conditions as (i), without analyte, to exclude the presence of As impurities in the interferent compound.

registered, and the signal increased with the metal concentration; with 1000 mg L^{-1} Cu(II), both chloride and sulfate raised the DMAA signal up to more than 150%, allowing the signal to reach the As(III) reference signal intensity ($S/S_0 = 1$). Transition metals are reported to take part in the mechanism of hydrogen transfer from borane complex to arsenic;[34] there is

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Table 2. Selective Determination of DMAA in Certified Water Reference Materials

CRM	certified As_{tot} (μg As L^{-1})	found As_{tot}[a] (μg As L^{-1})	As(III)[a] (μg As L^{-1})	DMAA[a] (μg As L^{-1})	As(III) corrected[b] (μg As L^{-1})	relative slope (R[c])	DMAA/As_{tot} (%)	DMAA[d] (μg As L^{-1})
CASS-4 nearshore seawater	1.11 ± 0.16	1.01 ± 0.10	0.09 ± 0.01	0.16 ± 0.01	0.07 ± 0.01	1.269	14.4	0.094 ± 0.001
								0.080 ± 0.001
								0.095 ± 0.004
SLRS-4 river water	0.68 ± 0.06	0.69 ± 0.03	0.09 ± 0.01	0.11 ± 0.01	0.076 ± 0.01	0.927	16.1	0.090 ± 0.001
								0.072 ± 0.001
								0.086 ± 0.002
								0.077 ± 0.023
NASS-5 seawater	1.27 ± 0.12	1.29 ± 0.22	0.07 ± 0.01	0.10 ± 0.01	0.057 ± 0.01	1.280	7.8	0.047 ± 0.001
								0.040 ± 0.001
								0.046 ± 0.002

[a] $N = 3$ replicates. [b] As(III) corrected = As(III) − 1/8 DMAA. [c] $R = m_s/m_0$, where m_s is the slope of calibration graph obtained for the analyzed sample, and m_0 is the slope of calibration graph in pure aqueous solution (see text for explanation). [d] Data obtained by Matousek et al. by HG-CT-ICP-MS (ref 8).

evidence that the metal can interact with the borane−As reaction system. In our case, the metal could form metal−borane complexes with different reducing properties than AB, which could be able to modify the generation efficiency of DMA in the selected conditions. The enhancement did not lead to a better selectivity because in the presence of Cu(II), higher signals were also observed for AsH_3 and MMA generation with AB. Nevertheless, this method showed a good control of interferences, so it has been proved to be easily applicable to natural waters analysis, which are matrices with much lower metal content.

Analysis of Real Samples. The method developed in this work for DMAA determination was employed together with the speciation method described in the section "Reactivity" for inorganic As,[30] in order to verify the contribution of DMAA to the As(III) content found by that method, which would cause an overestimation of this species. In all cases, the analytes were detected by atomic fluorescence spectrometry, owing to the better sensitivity of this technique, by the analyte addition method.

Analyses were carried out on the water reference materials CASS-4, a nearshore seawater, SLRS-4, a river water, and NASS-4, a seawater (NRCC, Ottawa); although these materials are certified only for total arsenic content, a validation could be done by comparison with methylated species content obtained by Matoušek et al.[8] through different methods in the same reference materials.

In order to compensate for the variations of the analytical response of DMAA in different samples, calibration curves were obtained for each real sample by addition of a known concentration of DMAA (0.2, 0.5, and 1.0 ng mL^{-1}): the slope of calibration curves obtained by linear fitting of experimental data, m_s, were compared with that in pure aqueous solution, m_0. Total As, As(III), and DMAA values found in our laboratories according to the two different methods described before are reported in Table 2, along with the ratio $R = m_s/m_0$, and the value of certified total As content; As(III) values corrected for the amount found for DMAA, multiplied by its response factor in the As(III) method, 1/8, are reported in the fourth column.

The MMAA contribution to the DMAA signal could be neglected, as DMAA/MMAA selectivity in these conditions is 1/45. As recently reported by Matoušek et al.,[8] MMAA content in the reference water samples is such that its interference can be expected to be less than 1%. DMAA content found in CASS-4, SLRS-4, and NASS-5 certified materials was 14.4, 16.1, and 7.8% of total As, respectively.

As a comparison, in the last column, DMAA values found by Matoušek et al. are reported. The discrepancies observed between the data obtained in the present work and those reported by Matoušek are quite marked for seawater samples CASS-4 and NASS-5 (43−55%) and less pronounced for river water SLRS-4 (25%). These differences are not easy to explain and conclusions cannot be drawn in the absence of certified reference samples.

CONCLUSION

The reactivity of inorganic As and methylated As species with the amine−borane complex, AB, and with *tert*-butylamine borane complex, TBAB, has been explored as a function of HCl and $HClO_4$ concentration, with or without L-cysteine, by continuous flow hydride generation coupled with atomic spectrometry. In the presence of L-cysteine, the reactivity of arsenic species toward the formation of corresponding arsanes presents marked differences between HCl and $HClO_4$ reaction media. In particular, $HClO_4$ at a concentration above 1 M depresses the formation of arsanes from the corresponding substrates in the order of As(III) > MMAA > DMAA. In particular, the generation of AsH_3 from As(III) is completely suppressed. This behavior has been assigned to the formation of ion couples between the protonated As substrate and the perchlorate ion. The protonation of already formed arsane does not contribute to the depressive effect, at least in the present employed apparatus.

The reactivity of arsenic species obtained by CF-HG-AS has been usefully employed to individuate conditions for the selective generation of a given species in the presence of the others, without prior separation steps. The most interesting result is the selective determination of DMAA in the presence of As(III) and MMAA, which has been achieved for the first time by using AB as a reducing acid in the presence of Cys and in 0.5 M $HClO_4$ media. Furthermore, AB and Cys properties were suitable to achieve a quite good control of interferences from most common transition metals, with interesting enhancement of DMA signal in the presence of Cu(II).

The method of selective determination of DMAA was tested on CASS-4, SLRS-4, and NASS-5 natural waters, certified for total As, which have been recently analyzed for arsenic speciation by Matousek et al. by selective hydride generation-cryotrapping-ICP-MS.[8] These differences are not easy to

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explain, and conclusions cannot be drawn in the absence of certified reference samples. Nevertheless, the results are encouraging, and the analytical scheme could be useful for those less-favored laboratories which have to face arsenic speciation by using simple and inexpensive equipments. This analytical scheme is useful to quantify not only the estimated concentration of DMAA but also data correction of As(III) concentration for the possible interference of DMAA, as recently described.[30] The two selective nonchromatographic methods for DMAA, herein described, and As(III),[30] both being based on HG in the presence of Cys, presented a good control of interference effects and can be applied to samples of a more complex nature than natural waters, such as foods, biological, geological, and environmental samples.

AUTHOR INFORMATION

Corresponding Author

*E-mail: pitzalis@pi.iccom.cnr.it.

Notes

The authors declare no competing financial interest.

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