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Investigation of hydride generation from arsenosugars - Is it feasible for speciation analysis?

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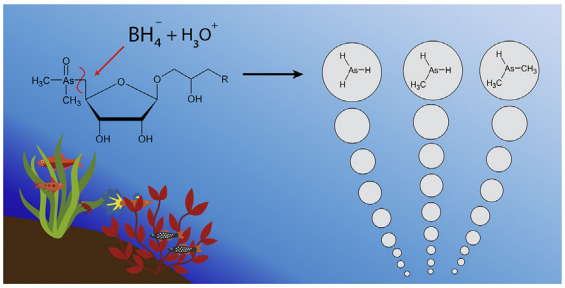
HIGHLIGHTS

- Conditions of hydride generation from arsenosugars were investigated in detail.
- A new design of hydride generation enhanced a generation efficiency to 29%.
- A mechanism of hydride generator from arsenosugars is proposed.

GRAPHICAL ABSTRACT



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ABSTRACT

Hydride generation (HG) from arsenosugars (dimethylarsinoylribosides) in batch and flow injection modes was studied. Its efficiency was found higher in H_2SO_4 medium than in HCl and higher in the batch mode than in the flow injection mode. To increase the efficiency in the flow injection mode a new generator with two inlets of $NaBH_4$ solution was designed. This modified generator was interfaced between a HPLC column and an atomic fluorescence detector. The arsenosugars studied yielded HG efficiencies in the range 13–30% most probably due to a complicated mechanism of HG. While the mechanism included a formation of two structures of the analyte-borane-complexes, only one of them can lead to a formation of volatile arsanes (dimethylarsane, methylarsane, and arsane were identified).

1. Introduction

The determination of arsenic and its species is an important task due to their abundances in the environment and the impact on health. More than fifty arsenic species have been reported in marine organisms [1,2] but their toxicity strongly differs. Arsenite (iAs^{III}), and arsenate (iAs^{V}) are carcinogens of group one. Methylated species, methylarsonate (MAs^{V}), dimethylarsinate ($DMAs^{V}$), and trimethylarsine oxide ($TMAs^{V}O$), are less toxic. Arsenobetaine (AsB) and arsenocholine are considered as non-toxic [3]. The toxicity of arsenosugars (As-sugars) [4] and arsenolipids [5,6] is still a subject of research.

The lack of well characterized standards of As-sugars makes their analysis difficult. Since their chemical synthesis is rather complex involving six to ten reaction steps, reference solutions are usually extracted from various seaweed sources and purified by

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preparative chromatography [7].

The majority of analytical methods for determination of As-sugars (see Fig. 1 for structures of the most common species) are based on high performance liquid chromatography (HPLC) coupled with an inductively coupled plasma mass spectrometry (ICP-MS) [7]. Due to the absence of calibration standards, As-sugars can be quantified according to the calibration curves of the nearest eluting standard species [8,9]. Sensitive detection is not limited only to ICP-MS as element specific detector, but also atomic fluorescence spectrometry (AFS) can be utilized for detection after postcolumn on-line decomposition and hydride generation (HG) [10–12].

HG is a derivatization and sample introduction technique for analytical atomic spectrometry. Nowadays, it is almost exclusively based on the reaction of tetrahydridoborate(1−) (THB) in acidic media with hydride forming elements (As, Se, Sb, Sn, Bi, Pb, Te and Ge) [13]. THB is stepwise hydrolysed to boric acid and hydrogen in acidic media [14]:

$$[BH_4]^- \rightarrow [LBH_3]^n \rightarrow [L_2BH_2]^n \rightarrow [L_3BH]^n \rightarrow H_3BO_3 \quad (1)$$

where L could be one or more groups among H_2O, OH^-, and Cl^- and n is the charge (−1, 0, or +1). THB and its hydrolysed products, *i.e.* hydridoboron species, react with the hydride forming elements to produce an analyte-borane complex (ABC) from which a volatile hydride is formed by the direct transfer of hydrogen from boron to the analyte atom, and fast hydrolysis leading to the final product [14, 15].

In the case of arsenic, the inorganic species $iAs^{III,V}$ and methylated MAs^V, $DMAs^V$, and $TMAs^VO$ species can be converted to volatile hydrides (arsane or mono-, di- and trimethyl substituted arsanes) *via* the reaction with THB in acidic media which is also employed for speciation analysis [16]. It has been believed for a long time that the other organically bound could not be converted to the volatile arsanes because the As-C bond is not cleaved during the reaction with THB. Sánchez-Rodas et al. [11] reported a low but significant HG activity of As-sugars which they explained by a partial degradation of As-sugars to $DMAs^V$ under the acidic conditions. In contrast, Gamble et al. [17] showed that the degradation product of acid hydrolysis of As-sugars was not $DMAs^V$ but aglycone-free As-sugar. Later on, Schmeisser et al. [18] proved that As-sugars were prone to conversion to volatile hydrides with an efficiency strongly dependent on the conditions of HG. The cleavage of As-C bond during HG was subsequently confirmed by Regmi et al. [19] who found 6–9% conversion of As-sugars to $(CH_3)_2AsH$. It was explained by a formation of the ABC making methylene group more susceptible to hydride attack. Very recently, we observed the cleavage of As–C bond during HG even for MAs^V, $DMAs^V$, and $TMAs^VO$ resulting in the formation of non-corresponding arsanes after loss of one or more methyl groups. The mechanism of the As-C bond cleavage was studied in detail to conclude that it proceeded through the action of the second and third hydridoboron species [20].

Inspired by our previous findings [20], the aim of the present work was to investigate HG from As-sugars in detail to disclose its mechanism and to assess its feasibility for analytical applications. Seaweed extracts were employed to substitute nonexistent As-sugar standards. In order to preserve (As-sugar) species integrity and a long time stability we sacrificed high extraction efficiencies of As-sugars from seaweeds by using deionized water (under ultrasonication) as the extractant.

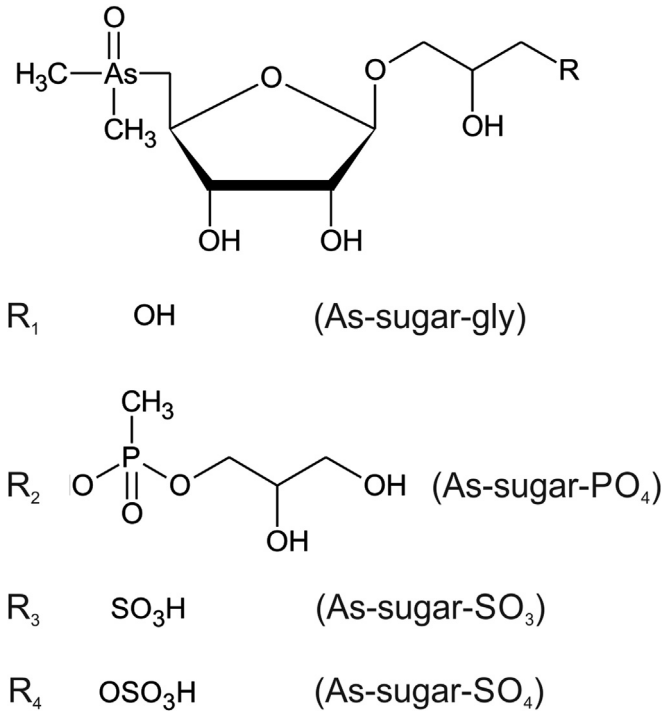

Fig. 1. Structures of four major As-sugars found in marine seaweed.

2. Experimental section

2.1. Standards and reagents

Deionized water (DIW; <0.2 μS cm^{-1}, Ultrapur, Watrex, USA) was used for the preparation of all solutions. A 1000 μg mL^{-1} As standard solution (Merck, Germany) was used as iAs^V stock standard. Stock solutions of 1000 μg mL^{-1} As were prepared for iAs^{III}, MAs^V, $DMAs^V$, $TMAs^VO$, and AsB species in DIW using the following compounds: As_2O_3 (Lachema, Czech Republic); $Na_2CH_3AsO_3 \cdot 6H_2O$ (Chem. Service, USA); $(CH_3)_2As(O)OH$ (Strem Chemicals, Inc., USA); AsB (Sigma-Aldrich, USA); $(CH_3)_3AsO$ was obtained by courtesy of Dr. William Cullen (University of British Columbia, Canada). The total As content of methylated arsenic species standards was confirmed by liquid sampling graphite furnace-atomic absorption spectrometry as described previously [21]. The solution of THB was prepared fresh daily from $NaBH_4$ (Fluka, Germany) in 0.1% KOH (Lach-Ner, Czech Republic). HCl (p.a., Merck, Germany), HNO_3 (semiconductor grade, Sigma-Aldrich, Germany) and H_2SO_4 (suprapure, Merck, Germany) were used for HG.

Mobile phase for anion exchange chromatography was either 20 mmol L^{-1} phosphate buffer prepared from KH_2PO_4 (Lach-Ner, Czech Republic) with pH adjusted to 5.6 with KOH [9] or 20 mmol L^{-1} bicarbonate buffer from NH_4HCO_3 (Lachema, Czech Republic) with pH adjusted to 10.3 with aqueous ammonia (Sigma-Aldrich, Germany) mixed 9:1 with methanol (Sigma-Aldrich, Germany) [8]. Solid NaOH (Lach-Ner, Czech Republic) in the form of 3 mm pearls was used as a filling of a NaOH dryer [22].

2.2. Instruments

The in-house assembled research grade non-dispersive atomic fluorescence spectrometer was employed as the detector. The experimental set up is described elsewhere [21,23]. Briefly, the instrument was equipped with an arsenic electrodeless discharge lamp (EDL system II, Perkin Elmer, USA) as a radiation source, an interference filter (193 nm, full width at half maximum 18.7 nm, CVI Melles Griot, USA) to isolate fluorescence radiation from an atomizer and a solar blind photomultiplier (165–320 nm, Perkin Elmer Optoelectronics, USA) as the detector. A miniature diffusion

flame (MDF) was used as the atomizer. The atomizer optimum observation height (7 mm), flow rates of flame hydrogen (200 mL min^{-1}) and flame argon (600 mL min^{-1}) were optimized previously - see Ref. [23] for details.

An Agilent 7700x ICP-MS spectrometer was used as an element specific detector for comparative purposes and for determination of total arsenic content in seaweed samples. Measurements were carried out in helium gas mode (4.8 mL min^{-1}) in the collision cell. Arsenic was measured directly as ^{75}As. A solution of 10 ng mL^{-1} rhodium (^{103}Rh) was used as an internal standard.

An Agilent HPLC system 1200 (USA) was employed in combination with both the AFS and ICP-MS detectors. 50 μL of sample was injected by an autosampler. PRP-X100 column (250 × 4.6 mm, 10 μm particle size, Hamilton, USA) with a guard column was used as the chromatographic column with a flow rate of 1 mL min^{-1} for phosphate buffer and 0.4 mL min^{-1} for bicarbonate buffer. These chromatographic conditions were taken from Refs. [8,9] with the exception of the column temperature which was ambient.

A HCT-Ultra ETD II Mass Spectrometer (Bruker, USA) with electrospray ionization (ESI, capillary exit voltage was 102.3 V) in positive mode was used for MS^2 measurements. Operation conditions: drying gas N_2 12 L min^{-1}, drying gas temperature 350 °C, nebulization pressure 35 psi (241.3 kPa). MRM method was used for MS^2 measurements. Selected precursor ions were 329.0, 393.0, 409.0 and 483.0 *m/z*, the isolation width was 4 *m/z*, the collision amplitude was 0.8 V, scan 90–490 *m/z*.

2.3. Sample preparation

Dried edible seaweed samples (*Hijiki*, *Nori*, *Kombu* and *Wakame*) were purchased at a local supermarket and milled to the fine powder. A 15 mL polyethylene tubes containing 0.2 g of seaweed powder and 8 mL of DIW were placed in an ultrasonic bath (Elmasonic One, Elma, Germany) at the laboratory temperature for 3 h. The samples were then centrifuged (5 min at 4000 rpm). 6 mL of supernatant was removed into 50 mL vessel and diluted to 25 mL and immediately frozen (−18 °C). The seaweed extracts were thawed before analysis, filtered through a syringe filter (PTFE, 25 mm, pore size 0.45 μm) and spiked with H_2O_2 (to 3% solution) to convert any extracted iAs^{III} species to iAs^{V} before HPLC-HG-AFS or HPLC-ICP-MS analysis.

For total arsenic determination, 0.1 g of seaweed powder was mixed with 3 mL of concentrated HNO_3 in a glass tube (volume 15 mL). Microwave assisted digestion was performed in UltraWave (Milestone, Italy), temperature program was: 15 min ramp to temperature of 220 °C and 15 min hold at 220 °C. Digests were transferred into 50 mL polypropylene tubes and diluted up to 50 mL by DIW.

2.4. Hydride generators

2.4.1. Flow injection and postcolumn mode

The hydride generator in the FI mode (see Fig. 2a) was the same as described previously [24]. The sample was injected into the flow of DIW by a six-port injection valve (Rheodyne, USA) with 0.6 mL sample loop volume. Solutions of THB, acid and DIW were pumped by a peristaltic pump (Ismatec, Switzerland) at the flow rate of 1 mL min^{-1}. PTFE reaction coils of various inner diameters (i.d.) and volumes were tested - 1 mm i.d. for 0.8 mL and 1.85 mm i.d. for 3.5, 5.4 and 8.9 mL. Generated arsanes were separated from the liquid phase in the gas-liquid separator (GLS) described in Ref. [24] as Unit I.

The hydride generator employed in the postcolumn mode of HG is shown in Fig. 2b. THB solution was introduced to the generator by two channels. The solution of THB introduced by the first channel was mixed in a T-junction with the acidified mobile phase exiting from the chromatography column. The reacting mixture flew to the reaction coil (3.5 mL) that was connected downstream to another T-junction serving to introduce the additional THB solution. The mixture continued to the second reaction coil (5.4 mL) connected to the GLS described in Ref. [24] as Unit IV. When explicitly stated the outlet from the chromatography column was replaced by a channel for DIW to which the sample was introduced by the injection valve.

2.4.2. Batch generator and cryogenic trap

The batch hydride generator (Fig. 2c) was described previously [20]. It consisted of peristaltic pumps (Ismatec, Switzerland), a GLS with a forced outlet of total volume of 7 mL, a chemifold between the pumps and the NaOH dryer [22].

HCl or H_2SO_4 was pumped by the peristaltic pump at the flow rate of 4 mL min^{-1}. A 0.6 mL of As standard was introduced into the acid channel by a six-port injection valve (Rheodyne, USA). Carrier gas (argon) merged the acid channel downstream the standard introduction point. The acid channel was equipped with an additional T-junction upstream the GLS that served for cleaning of the GLS with DIW by a syringe. Another channel was used to remove liquid waste from the GLS by the other syringe. THB was pumped by another peristaltic pump at the flow rate of 1 mL min^{-1}. The output of the gases from the GLS was connected to the NaOH dryer. The gaseous phase leaving the dryer was mixed with flame hydrogen and flame argon for the MDF atomizer. In some experiments, a cryogenic trap (CT) was implemented between the dryer and the point where flame gases were introduced. The carrier gas was helium in this case. The details on the CT can be found in Refs. [20,21,25].

The procedure was the same as used previously [20]. A standard was injected into the flow of acid introduced to the bottom of the GLS. The total volume of liquid was 1.2 mL. Subsequently, recording of the fluorescence signal was switched on (210 s read time) and 2 mL of THB was introduced into the GLS. After recording had been completed, remaining liquid was removed from the GLS and the GLS was cleaned with DIW. The procedure for HG-CT-AFS is described elsewhere [20].

2.5. Data evaluation

Peak area was invariably employed as the analytical quantity. Since the AFS detector output yields response in μV units specific to the instrument, peak areas cannot be simply related to those yielded by different instruments. Therefore, in the batch mode, we deal with normalized peak areas related to that of 2 ng mL^{-1} iAs^{III} that was generated by 1% THB from 1 mol L^{-1} HCl. In the FI mode, the peak areas are related to the signal of 2 ng mL^{-1} iAs^{III} generated by 1% THB from 1 mol L^{-1} HCl medium using the reaction coil of 3.5 mL. Since arsane from iAs^{III} is generated quantitatively (HG efficiency 100%) in the batch and FI modes under these conditions [20,24], normalized peak area also expresses HG efficiency. HG efficiencies of As-sugars in the FI and batch mode are not corrected for the impurities of hydride active species in the 'standard' (see later) because their total content was lower than 1.5%. Arsenic species concentration or mass is expressed always as elemental concentration or mass. All results are shown as mean ± standard deviations (SD) from three replicates. The uncertainty of arsenic species concentrations in a sample were calculated from propagation of error formula (the relative SD lower than 1% are not presented).

Mathlab 2012b software was used to solve a system of differential equations, OriginPro 9.0 was used for the model fitting.

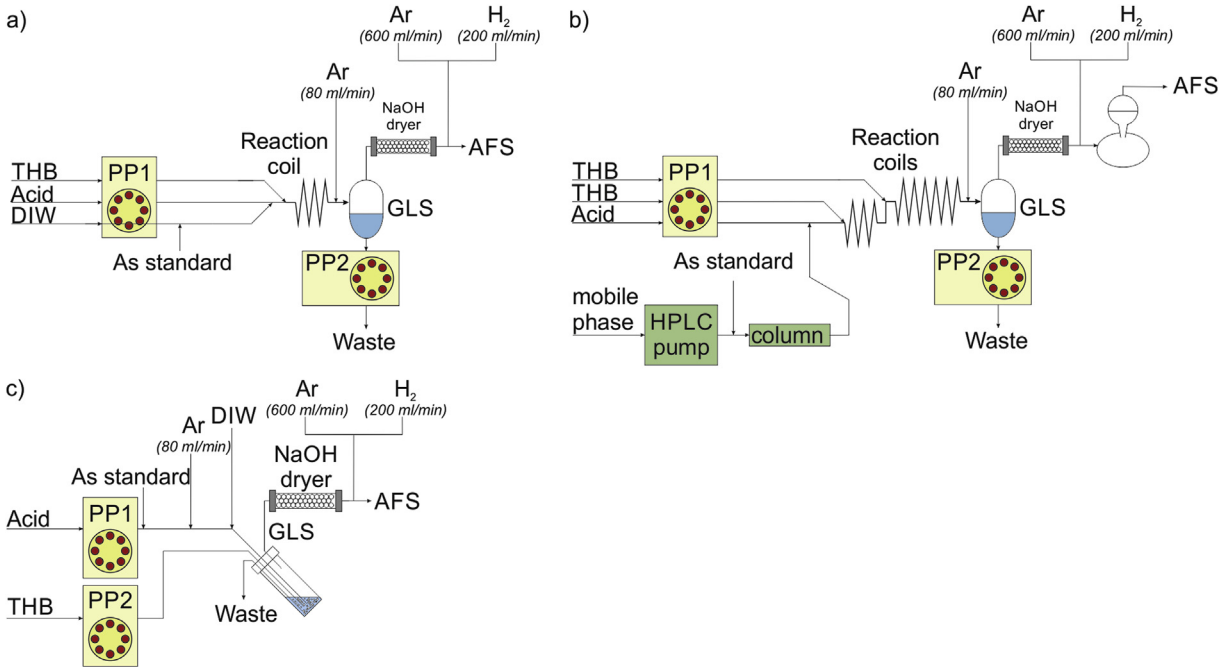

Fig. 2. Scheme of the hydride generator in a) FI mode; b) postcolumn mode; c) batch mode; AFS – atomic fluorescence spectrometer, GLS – gas-liquid separator, PP1/PP2 – peristaltic pumps, THB – solution of $NaBH_4$, DIW – deionized water.

3. Results and discussion

3.1. Preliminary experiments

Arsenic species were extracted by DIW from dried seaweed samples using an ultrasonic bath [26]. Individual As-sugars in the extracts as well as other As species (iAs^V, MAs^V and $DMAs^V$) were determined by HPLC-ICP-MS using phosphate buffer (1 mL min^{-1}) and bicarbonate buffer (0.4 mL min^{-1}) [8]. There was a sufficient resolution of all eluting As species except for $DMAs^V$ and As-sugar-PO_4 in chromatography using bicarbonate buffer. Structure analysis of individual unknown peaks was carried out with HPLC–ESI-MS^2. Since ESI-MS is not compatible with phosphate buffer, bicarbonate buffer had to be used as the mobile phase. HPLC–ICP-MS chromatograms of seaweed extracts using bicarbonate buffer are shown in Fig. S1 while the ESI-MS^2 spectra are in Fig. S2 of Supplementary Information. *Wakame* extract was not examined with ESI-MS because the detected As-sugars peaks in HPLC-ICP-MS chromatogram were the same as for the other seaweed extracts. Observed fragmentation spectra were similar to the spectra published by McSheehy et al. [27] and by Gallagher et al. [28]. From the knowledge of concentrations of individual identified As-sugars determined by HPLC-ICP-MS using bicarbonate buffer, it was possible to derive the retention times of individual As-sugars in the chromatographic system using phosphate buffer. These elution orders corresponded to those achieved in the other works at similar chromatographic conditions [8,9].

The retention times of As-sugars using phosphate buffer as well as their concentrations are shown in Table 1. The content of As-sugars was quantified according to the calibration curves of the nearest eluting standard species (*i.e.* As-sugar-gly on AsB, As-sugar-PO_4 on MAs^V, As-sugar-SO_3 and As-sugar-SO_4 on iAs^V). Calibration slopes for all arsenic standards were essentially identical (±5%) with the exception of AsB which was about 15% lower, probably because AsB elutes closely to the column's void time. One species, most probably also As-sugar species, eluting at 11.9 min, remained unidentified due to its low content. Total arsenic content in the extracts was determined by liquid sampling ICP-MS, while total arsenic content in the seaweed samples was determined by liquid sampling ICP-MS after microwave assisted digestion (see Section 2.3). The calculated extraction efficiency was the lowest for *Wakame* sample (14%) and the highest for *Nori* (76%) and *Kombu* (80%) – see Table 1, which corresponds to other works that performed extraction in DIW (with or without addition of methanol) [29–36]. Mildly acidic extractions appear to give higher extraction efficiency, but due to acidic hydrolysis this would also lead to degradation of As-sugars [17,37,38]. Also the determined column recoveries are in agreement with other works [12,29].

Nori extract with 114 ± 5 ng mL^{-1} As was properly diluted to yield the total As content of 2 ng mL^{-1}. This solution was further used as 'pure' As-sugar-PO_4 'standard' for investigation of HG 'activity' because 94% of total As in this standard was present in the form of As-sugar-PO_4 (Table 1). Moreover, 98% of As was found in the form of other As-sugars (see Table 1) with negligible content of the classical hydride active species *i.e.* iAs^V, MAs^V and $DMAs^V$. HG in the FI mode carried out from 0.5 mol L^{-1} HCl using 1% THB solution exhibited low HG efficiency corresponding to 9 ± 1%. There was no matrix effect on HG because the recovery of $DMAs^V$ (2 ng mL^{-1}) spiked into this standard was 100 ± 4%. HG carried out from 0.5 mol L^{-1} HNO_3 resulted in even lower HG efficiency corresponding to 1.2 ± 0.1%. This is in a pretty good agreement with the total content of classical hydride active species, iAs^V and $DMAs^V$ (1.4% ± 0.1% - see Table 1) indicating that HG from As-sugars is fully suppressed in HNO_3 medium. This agrees well with our previous finding that the cleavage of As–C bond during HG was dramatically reduced in the medium of HNO_3 [20]. Also Schmeisser et al. [18] observed that As-sugars do not form volatile arsanes from HNO_3 medium. The above experiments proved HG activity of As-sugars in HCl medium and verified the suitability of the prepared As-sugar 'standard' to systematically investigate the conditions of HG from As-sugars.

Table 1
Concentrations in mg kg^{-1} (mean ± SD, $n = 3$) and retention times of arsenic species in seaweed extracts determined by HPLC–ICP-MS (20 mmol L^{-1} phosphate buffer pH 5.6) and total arsenic concentrations in individual seaweeds determined by ICP-MS.

Seeweed	As-sugar-gly	$DMAs^V$	MAs^V	As-sugar-PO_4	U1	iAs^V	As-sugar-SO_3	As-sugar-SO_4	As extract[d]	Column recovery[e]	As total[f]	Extraction efficiency[g]
Hijiki	1.0 ± 0.1	0.75 ± 0.03	<LOQ[a]	0.35 ± 0.03	ND[b]	16.5 ± 0.14	0.19 ± 0.01	1.62 ± 0.04	24.4 ± 0.6	84%	75.9 ± 0.7	33%
Wakame	1.00 ± 0.03	0.083 ± 0.007	<LOQ[a]	0.20 ± 0.02	ND[b]	0.05 ± 0.01	0.16 ± 0.01	<LOD	5.2 ± 0.1	29%	36.7 ± 0.2	14%
Nori	0.45 ± 0.02	0.122 ± 0.004	<LOQ[a]	10.2 ± 0.3	0.05 ± 0.01	0.03 ± 0.01	<LOD	<LOD	14.2 ± 0.6	77%	18.6 ± 0.2	76%
Kombu	2.33 ± 0.04	0.46 ± 0.04	<LOQ[a]	7.77 ± 0.05	ND[b]	<LOD[c]	33.2 ± 0.1	<LOD	56.8 ± 1.0	77%	71.8 ± 0.3	80%
t_r, min	2.7	4.0	6.8	7.4	11.9	13.7	17.7	41.5				

[a] LOD and LOQ for MAs^V were 0.01 and 0.031 mg kg^{-1}, respectively.
[b] Not detected.
[c] LOD and LOQ for iAs^V were 0.005 and 0.016 mg kg^{-1}, respectively.
[d] Total As in extracts determined by liquid nebulization ICP-MS.
[e] Column recovery = sum of As species/As extract.
[f] Total As in seaweed samples determined by liquid nebulization ICP-MS after microwave digestion.
[g] Extraction efficiency = As extract/As total × 100.

3.2. HG from batch mode

HG efficiency from As-sugar-PO_4 'standard' was investigated in the range from 0.1 to 4 mol L^{-1} HCl at THB concentrations of 1% and 2%, respectively (Fig. 3). HCl was chosen because it is the most common acid used for HG [13]. The maximum efficiency was achieved at 1 mol L^{-1} HCl and 1% THB. The inherent property of the batch generation is that pH of the reaction mixture increases in time with THB supply. For 1% THB solution, there was a lack of acid for full decomposition of THB at HCl concentrations below 1 mol L^{-1}. For example, 0.25 mol L^{-1} HCl was completely consumed by THB hydrolysis within 32 s of THB solution introduction. Since the THB solution was added over a period of 120 s, almost 75% amount of THB was not hydrolysed. For 2% THB solution, the minimum concentration of HCl for complete THB hydrolysis was 2 mol L^{-1}. The observed decrease of HG efficiency at higher concentrations of HCl indicates that keeping strong acidic conditions during the whole period of HG cannot enhance HG activity of As-sugar-PO_4.

As-sugar-PO_4 signal shape in HCl medium (see Fig. 4a) was entirely different from the other arsenic species (see below). For 1% THB solution, the rising edge of the As-sugar-PO_4 peak was relatively steep reaching the maximum at $t_{max} = 42$ s but the falling edge was rather slow. For 2% THB solution, the peak maximum was reached even sooner ($t_{max=}$25 s), then signal was slowly decreasing to the minimum but at about half of the peak maximum it started slowly increasing again (Fig. 4a). This increase is probably due to increase of pH during the reaction since this 'second' maximum disappeared when 4 mol L^{-1} HCl was used. It appears that the optimum pH for cleavage of As–C is at higher values.

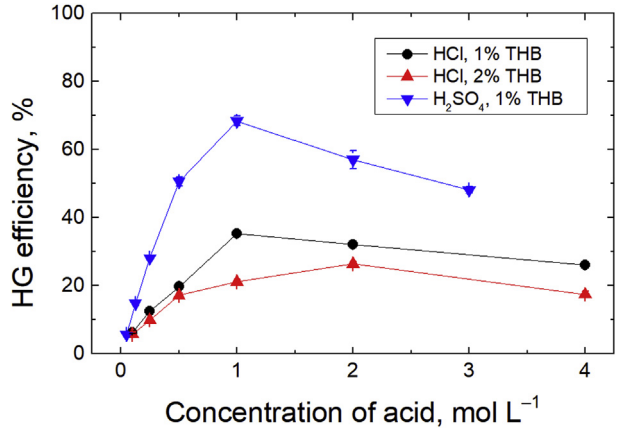

Fig. 3. Dependence of HG efficiency from As-sugar-PO_4 on concentration of acid in the batch hydride generator; ● (black) – HCl, 1% THB solution; ▲(red) – HCl, 2% THB solution; ▼ (blue) – H_2SO_4, 1% THB solution. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

The typical signal shape of As species can be demonstrated by $TMAs^VO$. At lower HCl concentration (0.05 mol L^{-1}) the peak was high and narrow (HG efficiency was 100% [20]). However, increasing HCl concentration led to lower, wider peaks and to a decrease of HG efficiency (see Fig. 4b). At 4 mol L^{-1} HCl, the HG efficiency of $TMAs^VO$ was around 35 ± 1% [20] which was similar as for the As-sugar-PO_4 (1 mol L^{-1}, 1% THB solution) but a completely different signal shape was recorded (Fig. 4a). This difference in signal shapes clearly suggests a different mechanism of HG (see chapter 3.5 for details).

H_2SO_4 was also tested as the medium for HG of As-sugar-PO_4 with 1% THB solution in the concentration range from 0.05 to 3 mol L^{-1} (Fig. 3). The maximum HG efficiency was obtained at 1 mol L^{-1} H_2SO_4 not at 0.5 mol L^{-1} which would correspond to the same acidity as 1 mol L^{-1} HCl. Furthermore, the HG efficiency at optimum was almost twice higher (68 ± 1%) compared to HCl medium (35 ± 1%). This is in agreement with our previous results that the cleavage of As–C bond during HG is more efficient in H_2SO_4 medium than in HCl. The reaction of HCl and THB probably leads to a formation of hydridoboron species with chloride ligand having lower capability to cleave As–C bond [20]. Also the signal shape was different from that obtained with HCl (Fig. 4a).

The identity of formed volatile arsanes was investigated by coupling the generator in the batch mode to the CT (HG-CT-AFS) allowing for their simple separation in the gaseous phase. It was found out that under conditions of HG with the highest yield of arsanes (1 mol L^{-1} H_2SO_4 and 1% THB solution), 66 ± 3% of arsanes was in the form of $(CH_3)_2AsH$, 32 ± 3% as CH_3AsH_2, and 3 ± 1% as AsH_3. No traces of $(CH_3)_3As$ were detected. This observation is quite in line with the statement by Regmi et al. [19] who reported that $(CH_3)_2AsH$ with traces of CH_3AsH_2 were the volatile products formed from As-sugar-SO_3 and As-sugar-SO_4.

3.3. HG in FI mode

HG efficiency from As-sugar-PO_4 'standard' was examined in the FI generation mode in the range from 0.25 to 3 mol L^{-1} HCl and from 0.5 to 2.5% (0.13 to 0.66 mol L^{-1}) of THB solution. The surface plot is shown in Fig. 5a. The highest HG efficiencies were observed when the molar ratio HCl/THB was about 2. Generally, HG efficiency increased with more concentrated THB solution up to 2% but at 2.5% there was a slight decrease. It also decreased with HCl concentration.

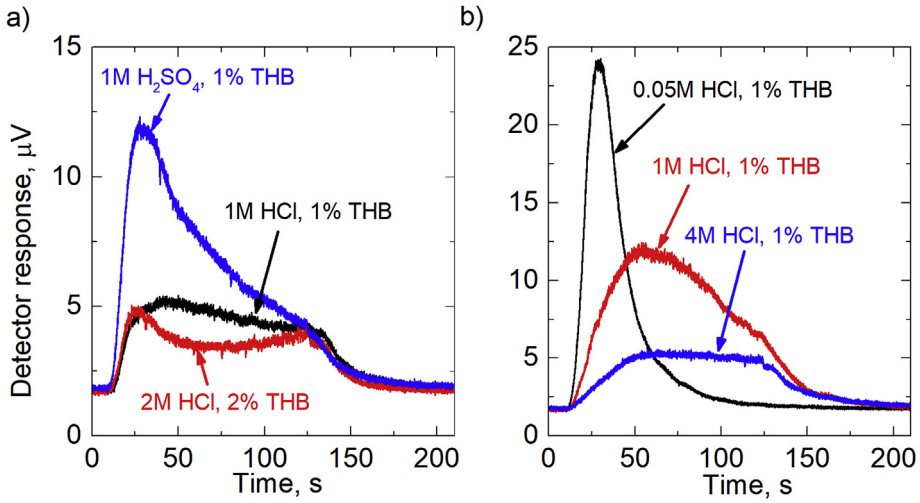

Fig. 4. Signal shapes in the batch hydride generator of a) As-sugar-PO_4; (black) – 1 mol L^{-1} HCl, 1% THB solution; (red) – 2 mol L^{-1} HCl, 2% THB solution; (blue) 1 mol L^{-1} H_2SO_4, 1% THB solution; b) $TMAs^{V}O$; (black) – 0.05 mol L^{-1} HCl, (red) – 1 mol L^{-1}; (blue) – 4 mol L^{-1}; all with 1% THB solution. Concentration of As was 2 ng mL^{-1}. THB solution was introduced to the generator from time 4–124 s. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

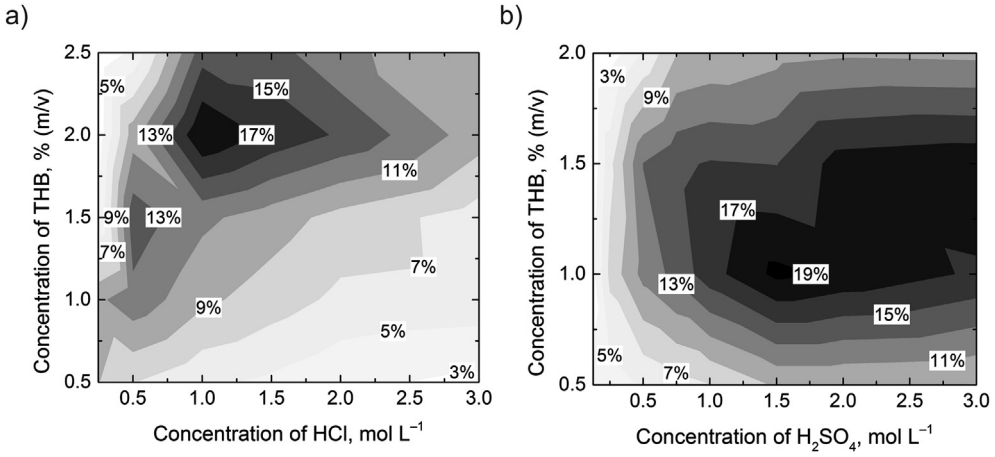

Fig. 5. Dependence of HG efficiency of As-sugar-PO_4 on THB concentration and on concentration of (a) HCl and (b) H_2SO_4 in the flow injection mode; 3.5 mL volume of the reaction coil; concentration of As was 2 ng mL^{-1}.

In H_2SO_4 medium volatile arsanes can be generated from As-sugar-PO_4 in a wider range of reagent concentrations (Fig. 5b). HG efficiency is not so impaired at higher concentrations of acid compared to HCl (Fig. 5a) indicating that HG is suppressed by a higher concentration of Cl^- ions. The reason appears to be that hydridoboron species with chloride ligands are formed which cannot cleave As–C bond or cleave it only with lower probability. The reaction time which can enhance the HG efficiency can be controlled in the FI mode by the volume of the reaction coil [24]. Three different volumes of the reaction coil were tested (0.8, 3.5, and 8.9 mL) using 1.5 mol L^{-1} H_2SO_4 and 1% THB solution (maximum HG efficiency in Fig. 5b). Reduction of the reaction coil to volume 0.8 mL caused a decrease in the HG efficiency to 13 ± 1%. On the other hand, increasing the volume to 8.9 mL did not significantly increase the HG efficiency.

The HG efficiency achieved in the FI mode with H_2SO_4 was much lower (19 ± 1%) than in the batch mode (68 ± 1%). This can be explained by the previously observed phenomenon that only the first two hydrolytic products of THB hydrolysis ($[LBH_3]^n$ and $[L_2BH_2]^n$) are capable of cleaving As–C bond [20] which is a necessary condition in the case of As-sugars for the formation of volatile arsanes. Since the fresh solution of THB is continuously supplied in the batch mode, the first two hydrolytic products of THB are available during the whole process of HG. On the other hand, in the FI mode the analyte can interact with the first two hydrolytic products only in the front part of the reaction coil. In the downstream sections of the reaction coil these products are consumed, converted to different hydridoboron species, less active or inactive in the cleavage of As-C bond. This was demonstrated by the insignificant influence of increased reaction coil volume on HG efficiency.

Theoretically, there are two ways to maintain the effective concentration of first two hydrolytic product of THB in the whole length of the reaction coil: (i) to use more concentrated solution of THB or (ii) to introduce additional solution of THB. The former way resulted in a decrease of the HG efficiency (Fig. 5a and 5b). Therefore, the hydride generator was modified to use two channels to introduce THB solution to acidified sample. The first reaction coil volume was 3.5 mL while the volume of the second one (situated downstream of the introduction of additional THB solution) was 5.4 mL. The HG efficiency from As-sugar-PO_4 thus increased to 29 ± 3% (using 1.5 mol L^{-1} H_2SO_4 and 1% THB solution in both

channels). HG efficiencies from the other 'hydride active' As species were also investigated in this generator arrangement for a comparison and they were 102 ± 2%, 96 ± 2%, 100 ± 2%, 93 ± 2%, and 20 ± 1% for iAs^{III}, iAs^{V}, MAs^{V}, $DMAs^{V}$, and $TMAs^{V}O$, respectively. The HG efficiency from $TMAs^{V}O$ strongly depends on pH - it can be almost completely suppressed at low pH [39] and even the second introduction of THB solution cannot increase its HG efficiency markedly.

3.4. HPLC–HG–AFS

The modified design of the hydride generator was connected to HPLC for postcolumn HG. Phosphate buffer was chosen as the mobile phase due to good resolution of all As-sugars from typical 'hydride active' species (iAs^{V}, MAs^{V} and $DMAs^{V}$). The seaweed extracts were analyzed without further dilution except *Hijiki* which was twice diluted due to high content of iAs^{V}. Obtained chromatograms are shown in Fig. 6. For HPLC–HG–AFS, the concentration of individual As-sugar can be derived from the sensitivity of MAs^{V} offering 100% HG efficiency as was shown in the paragraph above. If we relate this calculated concentration to the concentration of individual As-sugar in the seaweed extracts determined with HPLC-ICP-MS (Table 1), we get the HG efficiency. The accuracy of the HG efficiency obtained in this way is much better than in that obtained from the FI mode measurements because it is not influenced by the purity of As-sugar 'standard'. Furthermore, HG efficiencies for all the other As-sugars in the chromatograms can be easily derived. They were 13 ± 1%, 28 ± 2%, 31 ± 3%, and 17 ± 1% for As-sugar-gly, As-sugar-PO_4, As-sugar-SO_3, and As-sugar-SO_4, respectively.

The HG efficiency of As-sugar-PO_4 determined from these measurements using HPLC correlated very well with the value of 29 ± 3% found in the FI mode for diluted As-sugar-PO_4 'standard'. Although the achieved lower column recoveries (76% for *Nori*, see Table 1) could evoke the presence of As species different from those studied, *i.e.* As species unable to elute from the column and/or to form volatile arsanes, this result fully supports the statement that As-sugars-PO_4 was really the dominant arsenic species (94%) in the As-sugar 'standard'. It clearly justifies using the diluted *Nori* extract as the As-sugar 'standard' for HG optimization. The HG efficiency of 33 ± 12% was found for the unknown species (retention time of 11.9 min). The high uncertainty is due to its low concentration in the extract. The achieved HG efficiencies are similar to those published by Schmeisser et al. [18] (21–28%) although they calculated

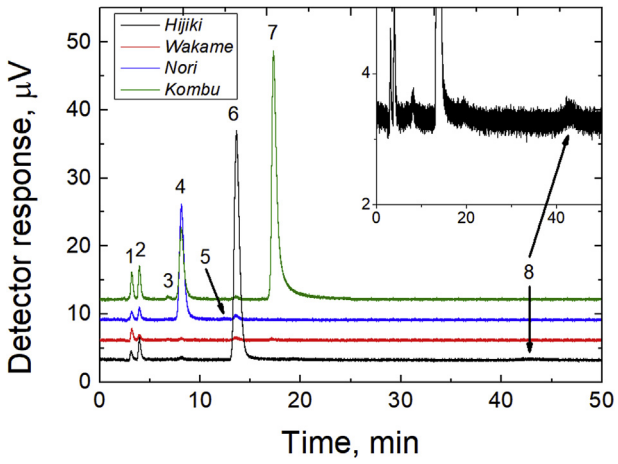

Fig. 6. HPLC–HG–AFS chromatograms of four seaweed samples; HG conditions: 1.5 mol L^{-1} H_2SO_4, two inlets of 1% THB solution; 1– As-sugar-Gly, 2–$DMAs^{V}$, 3–MAs^{V}, 4–As-sugar-PO_4, 5–unknown, 6– iAs^{V}, 7– As-sugar-SO_3, 8– As-sugar-SO_4.

HG efficiency as a relative signal to iAs^{III} without the actual knowledge of the efficiency of HG from iAs^{III} under experimental conditions they employed. Nevertheless, HG efficiencies for As-sugar-SO_3 and As-sugar-SO_4 achieved in our work are about three times higher than those published by Regmi et al. [19].

3.5. Mechanism of HG of As-sugars

Under several simplifying assumptions, the shape of the peak produced by a batch hydride generator can be expressed as [13]:

$$S(t) = A \cdot \{exp(-k_1 t) - exp(-k_m t)\} \quad (2)$$

where *S(t)* is the observed AFS signal. The constant *A* includes all experimental parameters [13]. The most important assumption for the validity of eq. (2) is that concentration of THB and its hydrolysis products are constant during the signal evolution. Hydride release from the solution is thus a pseudo-first order process controlled by the analyte concentration in the reaction mixture with the rate constant k_1. Constant k_m is the first order rate constant of hydride removal from the GLS [13,40].

Fig. 7a shows a result of the non-linear regression of the AFS signal of iAs^{III} to the function (2). To fulfill the assumption of the pseudo-first order hydride release, the curve fitting does not start at the beginning of THB solution addition but by 5 s later. There is a very good correlation between this simple model and the experiment.

However, signal shapes for As-sugar-PO_4 (Fig. 4a) suggest that they cannot be described by the function (2). The obvious reason is that the hydride release from the reaction mixture cannot be considered to be the simple pseudo-first order process.

Two reaction mechanism schemes leading to the hydride release from the reaction mixture (Fig. 8) could be taken into account.

(A) As-sugars are firstly cleaved by the action of THB (and/or its intermediates) to hydride active arsenic species (*e.g.* $DMAs^{V}$). In the second step, it is converted to hydride which is released to gaseous phase.
(B) There are two competitive reactions between the As-sugars species and THB (and/or its intermediates). The first reaction is analogous (but with different rate constant) with that considered for derivation of eq. (2). The second reaction leads to a formation of an ABC which is in an equilibrium with the As-sugar species but from which a hydride cannot be formed.

In the both reaction mechanism schemes, the gaseous hydride is removed from the GLS by the first order process with the rate constant k_m identical to that in eq. (2). Taking the same assumption as to derive eq. (2) (constant concentration of THB and its hydrolysis products during the signal evolution) all reactions defined in Fig. 8 are of the first or pseudo-first order with the corresponding rate constants. Consequently, we get a system of differential equations. Its solution describes the signal shape corresponding to HG of As-sugar-PO_4 (see details in Supplementary Information). Both solutions describe well the signal shape of As-sugar-PO_4 generated from 4 mol L^{-1} HCl with the addition of 1% THB solution (Fig. 7b). However, in the case of 4 mol L^{-1} HCl and addition of 2% THB solution (Fig. 7c), and of 2 mol L^{-1} H_2SO_4 and addition of 1% THB solution (Fig. 7d), there is much better correlation with mechanism (B).

Mechanism B also explains the decrease of the signal for more concentrated THB (Fig. 3) because according to this mechanism the reaction yield depends not only on THB concentration but also on the ratio of hydridoboron species which are capable or incapable of

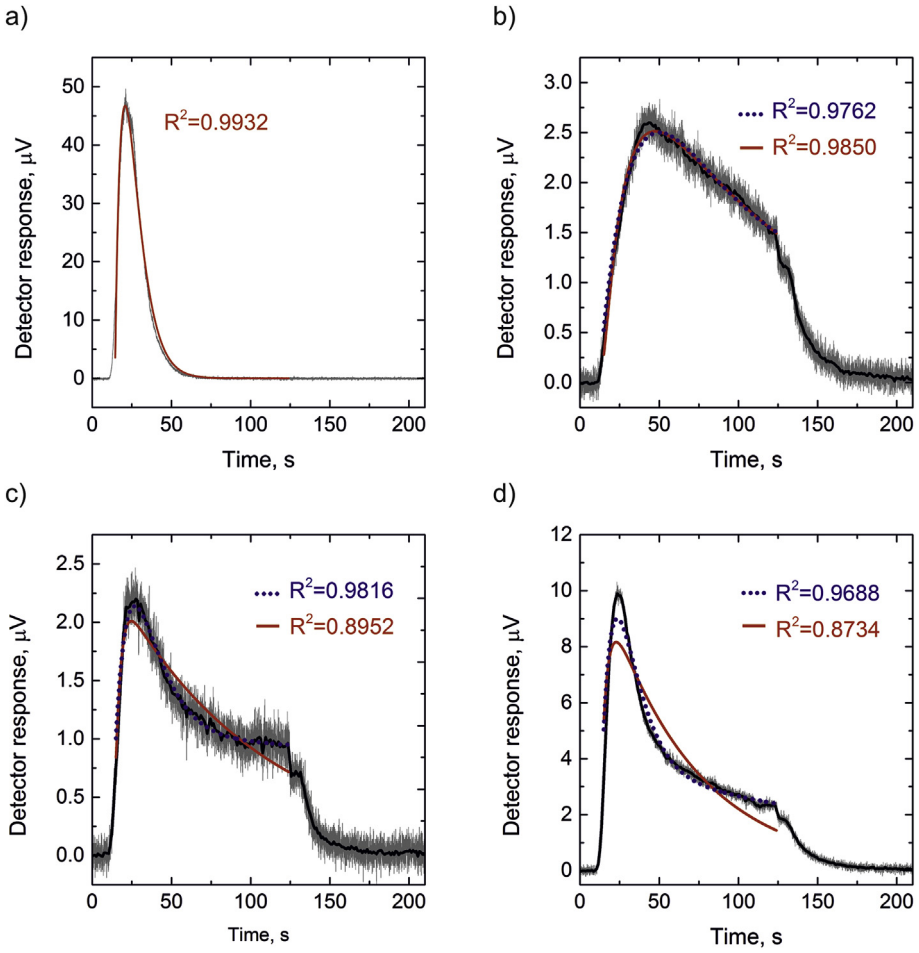

Fig. 7. Signal shape (grey) of (a) iAs^{III} in 4 mol L^{-1} HCl, addition of 1% THB solution in the batch hydride generator fitted to equation (2) (red); (b) As-sugar-PO_4 in 4 mol L^{-1} HCl, (black line represents smoothed data), addition of 1% THB solution in the batch hydride generator fitted with equations which correspond to mechanisms A (red – solid line) and B (blue – dotted line); (c) As-sugar-PO_4 in 4 mol L^{-1} (black line represents smoothed data), addition of 2% THB solution in the batch hydride generator fitted with equations which correspond to mechanisms A (red – solid line) and B (blue – dotted line); (d) As-sugar-PO_4 in 2 mol L^{-1} H_2SO_4 (black line are smoothed data), addition of 1% THB solution in the batch hydride generator fitted with equations which correspond to mechanisms A (red – solid line) and B (blue – dotted line). Concentrations of As were 2 ng mL^{-1}. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

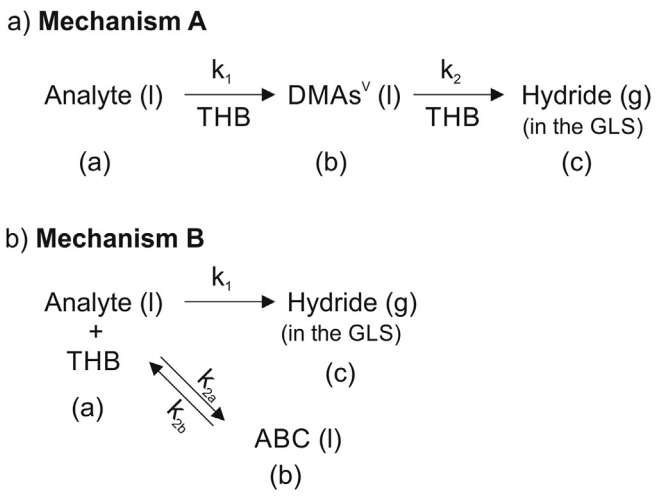

Fig. 8. Schemes of the proposed mechanisms for HG of As-sugars.

cleaving the As–C bond. This ratio can be affected by the total concentration of THB. However, this ratio is probably affected also by concentration of Cl^- ions. This could be due to the different behavior of chloride ions which, in contrast with other ions, may compete with H_2O and OH^- ligands in forming complex hydridoboron intermediates (*i.e.* $[LBH_3]^n$, $[L_2BH_2]^n$ and $[L_3BH]^n$) [41]. HG efficiency is therefore much higher in H_2SO_4 than in HCl medium and it dramatically decreases at higher concentrations of HCl in the FI mode.

Although the reaction of hydride formation is shown schematically as one step reaction (with the rate constant k_1) in mechanism B (Fig. 8), the reality is much more complicated. A possible mechanism has been already proposed by Regmi et al. [19] and it includes a formation of ABC (which is capable of cleaving the As–C bond) which results in formation of dimethylated (or even less methylated) arsenic species which are subsequently converted to the volatile arsanes. However, the formation of ABC from which a hydride cannot be formed has not been considered in the reaction schemes yet. The formation of this complex is a plausible explanation of the low efficiency of HG from As-sugars generally observed.

4. Conclusion

Hydride generation efficiency from As-sugars as high as almost

70% can be reached under properly chosen conditions in the batch mode. The mechanism appears to be rather complex involving formation of two kinds of analyte-borane complex: the first kind results in formation of volatile arsanes and the second kind decomposes back to the analyte. The knowledge of the mechanism of HG from complex organo-arsenic compounds is important because HG method is a common derivatization step in arsenic speciation analysis scenarios. Especially, the possibility of determination of inorganic arsenic by selective HG became popular nowadays [42,43] or when HG is used as postcolumn derivatization in order to increase selectivity of determination of inorganic arsenic – eliminating risk of a positive interference from co-eluting organo-arsenic compounds in seafood samples [44].

From the analytical point of view, the HG from As-sugars offers little applicability for two reasons: (i) the efficiency in the flow mode of HG is not equal for all species and below 100% and (ii) As-sugar standards are not easily available, let alone commercially. For determination of As-sugars, the postcolumn HG after on-line decomposition is currently a better solution.

It should be highlighted that the increase in HG efficiency from 19 ± 1% to 29 ± 3% with the additional introduction of THB solution indicates that the second introduction of THB solution yields efficiency of hydride formation from the reacted mixture present between both reaction coils (Fig. 2b) similar as efficiency of hydride formation from the “fresh” sample introduced upstream the first reaction coil (19 ± 1%). Consequently, increasing number of introduction points of THB solution could gradually increase the HG efficiency but it would lead to a very complicated apparatus and to a high signal noise given by high fluctuations of analyte hydride supply from the generator. However, a further research of THB hydrolysis or the use of other borane compounds may improve this situation in near future.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.aca.2018.01.009.

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