A novel on-line organic mercury digestion method combined with atomic fluorescence spectrometry for automatic mercury speciation

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Abstract

A novel on-line digestion method was developed based on organic mercury (Org-Hg) oxidation at ambient temperature using potassium permanganate in the presence of sulphide. The digestion of Org-Hg was instantaneous and quantitative. Consequently, a simple mercury speciation method was developed to differentiate inorganic mercury (In-Hg) and Org-Hg in water samples with a sequential injection analytical system in conjunction with atomic fluorescence spectrometric detection by using Hg^{2+} as the sole standard for calibration. In-Hg was determined after acidification and decomposition of the organic matter in the sample matrix with $KMnO_4$, while total mercury (T-Hg) was determined after online Org-Hg digestion, and Org-Hg was calculated as the difference between T-Hg and In-Hg. The operational parameters were optimized and the possible role that sulphide played in the digestion of Org-Hg was discussed. A detection limit (3σ criterion) of 3 ng L^{-1} Hg was achieved, which is far below the guideline value of Hg in drinking water set by WHO, viz., 1 μg L^{-1}. The method was applied to the analysis of lake water samples for the determination of In-Hg and Org-Hg. Relative recoveries of 94-97% for In-Hg and 93.5-94.5% for T-Hg with relative standard deviations of 1.1-3.1% were obtained in real samples spiked with 100 ng L^{-1} Hg^{2+} and 100 ng L^{-1} Org-Hg, respectively, indicating the feasibility of the automatic method for the determination of Hg species at the low ng L^{-1} level.

Keywords: Mercury speciation, On-line digestion, Flow analysis, Sulphide, Permanganate.

1. Introduction

Mercury is one of the most prominent legacy pollutants in the environment due to its high toxicity and bio-accumulative potential [1]. Mercury is normally occurring in different chemical forms in the environment and food consumed by humans, including inorganic mercury (In-Hg) and organic mercury (Org-Hg) compounds, such as methyl mercury ($MeHg^+$), ethyl mercury ($EtHg^+$), phenyl mercury ($PhHg^+$), and dimethyl mercury (DMHg). For example, $MeHg^+$ toxicity is mainly related to its ability to cross the blood–brain barrier via the neutral amino acid transport system as a complex with L-cysteine [2]. Compared to In-Hg, Org-Hg species, especially $MeHg^+$, are accumulated in aquatic biota and crops with elevated concentration factors [3, 4] and thus easily enter into the food chain. The main route of human exposure to mercury is consumption of contaminated fish [5, 6]. Because of the high biomagnification factor of mercury from water to top level aquatic predators, small changes in the mercury concentration in surface waters can lead to huge changes in mercury concentrations in fish [7]. As a result, routine analysis of mercury in water is essential for the quality assurance of aquatic ecosystems.

Conventionally, total mercury (T-Hg) in waters is determined by cold vapor generation (CVG) atomic spectroscopic techniques such as atomic absorption spectrometry (AAS) [8], atomic fluorescence spectrometry (AFS) [9], and inductively coupled plasma-mass spectrometry (ICP-MS) [10], after digestion to convert Org-Hg to In-Hg. However, such analytical approaches are unable to distinguish different Hg species and cannot provide sufficient information on their eco-toxicity. For a better understanding of the overall toxicity of Hg in water bodies, it is necessary to perform Hg speciation. Two categories of analytical methods have been developed for such purpose. The first type involves the use of column separation techniques, such as gas chromatography (GC) [11], high performance liquid chromatography (HPLC) [12-14], ion chromatography (IC) [15], and low-pressure chromatography [16] in conjunction with atomic spectrometric detection. This kind of methodology provides detailed information on individual chemical forms of Hg. However, sophisticated instrumentation is needed and the sample throughput is usually quite low. The second type of methods differentiate In-Hg and Org-Hg by use of several simple strategies, such as (i) using different reductants for selective CVG of In-Hg and T-Hg in sequence and calculating Org-Hg as the difference between T-Hg and In-Hg [17, 18], (ii) selective extraction of In-Hg or Org-Hg from the sample [19], and (iii) determining In-Hg and T-Hg before and after digesting the sample [19]. Although no detailed insight into speciation data can be

obtained, non-chromatographic methods can still provide invaluable chemical information because $MeHg^{+}$ is often the predominant Org-Hg species in natural waters [20]. In addition, such methods usually use less complicated instrumentation and hence are more robust and cost-effective [21]. They are also often more advantageous with respect to the use of larger sample sizes to ensure improved sensitivity for samples with low Hg concentrations.

In both chromatographic and non-chromatographic methods, Org-Hg is often converted to In-Hg (Hg(II) or Hg^0) prior to detection by atomic spectroscopic techniques, especially with AAS and AFS. Two categories of digestion techniques have been applied to break the Hg-C bond, namely, chemical digestion with reagents in aqueous solutions and photo-induced digestion. In chemical digestion, an oxidant, such as potassium persulphate [22-27], hydrogen peroxide [28], potassium permanganate [29, 30], potassium dichromate with Cd^{2+} as a catalyst [31], or bromine [32-34] is applied to decompose Org-Hg prior to AAS or AFS detection. Without heating and/or the use of prolonged reaction times, chemical oxidation of Org-Hg species is often incomplete and standards of the individual Hg species are needed for their respective quantification [23, 25, 31, 33] because the same sensitivity cannot be achieved for all of the Hg species.

In this study, however, we report on the ability of $KMnO_4$ to quickly mineralize Org-Hg in the presence of sulphide (S^{2-}) at room temperature and the use of this approach for the development of a novel automatic on-line method for the determination of In-Hg and Org-Hg based on CVG and subsequent Hg^0 determination using AFS.

2. Experimental

2.1. Reagents and apparatus

All solutions were prepared in deionized water (<18.2 MΩ·cm, Millipore, Synergy 185,). Mercury(II) stock solution (1,000 mg L^{-1}) was prepared by dissolving $HgCl_2$ (Sigma-Aldrich) in 0.4 M HCl (Ajax Finechem) solution. Standard solutions of methylmercury chloride (Sigma-Aldrich) and ethylmercury chloride (Chem Service), containing 200 mg L^{-1} Hg each, were prepared by dissolving 0.0252 g of MeHgCl and 0.0264 g of EtHgCl in 20 mL of 6 M HCl solution with subsequent addition of 100 mL of deionized water. Further dilution was made with 0.4 M HCl solution according to the required final Hg concentration. A 50 mM Na_2S solution was prepared by dissolving solid $Na_2S{\cdot}9H_2O$ (Sigma-Aldrich) in 50 mM NaOH solution. This solution was used for facilitating the digestion of Org-Hg by $KMnO_4$. A purified Na_2S solution was also prepared for a comparative study by acidifying 200 mL of 15% of

Na_2S·solution with 40 mL of 3 M H_2SO_4 (Sigma-Aldrich) solution and absorbing the evolved H_2S gas in a 250 mL of 1 M NaOH solution to obtain 1 M Na_2S solution. This solution was subsequently diluted with 50 mM NaOH solution to obtain a 50 mM Na_2S solution [35]. A $KMnO_4$ solution was prepared by dissolving 1.0 g of $KMnO_4$ (Chem-supply) in 80 mL of deionized water and adding 20 mL of 0.5 M H_2SO_4 solution. A 0.1 M ascorbic acid (AA) solution was prepared by dissolving 1.76 g of the solid reagent (Ajax Finechem) in 100 mL of deionized water. This solution was used for the cleaning of the digestion vial after each sampling as described later. A 0.5 % (w/v) AA solution containing 0.5 mol L^{-1} HCl was used as the carrier stream for the CVG process. A 0.5 mg L^{-1} $NaBH_4$ solution was prepared daily by dissolving 0.1 g of $NaBH_4$ (Scharlab S. L.) in 100 mL of 0.05 M NaOH (Chem-supply) solution. 50 μL of this solution were further diluted to 100 mL with 0.05 M NaOH solution.

Three water samples were collected from Lake Hyland, Churchill, Victoria. One was from the main body of the lake, the second was from the entrance to the lake, and the third was from a small pond next to the lake which was heavily contaminated with organic matter due to high population density of water birds. The samples were collected with 50 mL Cellstar® tubes (Greiner Bio-One), transported in ice box and stored at 4 ºC. They were filtered through 0.45 μm Nylon syringe filter (Thermo Scientific) before analysis.

The analytical manifold, shown in Fig. 1 and consisting of an FIAlab 3200 sequential injection analyser (FIAlab Instruments) coupled to a CVG unit directly connected to the built-in gas-liquid separator (GLS) of an atomic fluorescence spectrometer (AFS, PSA 10.025 Millennium Merlin mercury analyser, PS Analytical), was used for conducting the online sample digestion and detection of In-Hg and T-Hg at 257.3 nm. The manifold was operated by FIAlab for Windows 5.0 software (FIAlab Instruments) and in the entire analytical procedure, the built-in peristaltic pumps, the sample and reagent channels and the sampling valve of the AFS were idle.

The sequential injection analyser incorporated two syringe pumps (SP), a ten-port selection valve (SV) and a built-in peristaltic pump (PP). One of the syringe pumps with a volume capacity of 5 mL was used for the delivery of all the solutions through the selection valve of the manifold (Fig. 1) with the exception of the HCl – AA solution and the $NaBH_4$ solution, both used in the CVG process. The other syringe pump was idle. The HCl-AA and $NaBH_4$ solutions in the CVG unit were delivered by the built-in peristaltic pump of the FIAlab 3200 analyser (Fig. 1) through Tygon tubing (Elkay Products) of 1.52 mm i.d. and 1.05 mm i.d., respectively. The corresponding solution flow rates, measured gravimetrically, were

4 mL min^{-1} and 2 mL min^{-1}, respectively. A magnetic stirring bar with a length of 10 mm inside the digestion vial (DV, Fig. 1) was run continuously by another magnetic stirring bar attached to an R18 magnetic stirrer (MS, Fig. 1) (Ingenieurbüro CAT M. Zipperer) positioned beside the DV. The DV was a plastic syringe (Shandong Hapool Medical Technology) with a volume capacity of 5 mL. The holding coil (HC, Fig. 1) was of 1.2 mm i.d. and volume capacity of 3 mL. The reaction coil (RC, Fig. 1) was made of PTFE tubing of 0.5 mm i.d. and length of 100 cm.

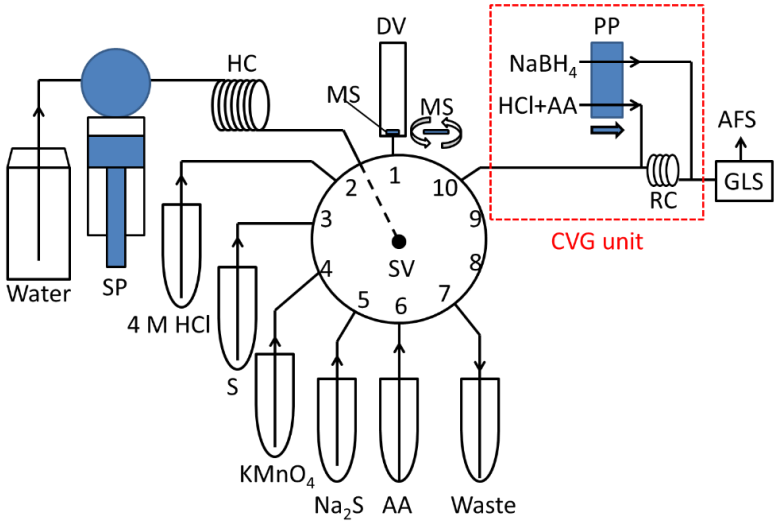

Figure 1. Schematic of the analytical manifold for automatic Hg speciation using the newly developed on-line digestion method (SP: syringe pump, SV: selection valve, HC: holding coil, DV: digestion vial, MS: magnetic stirring bar, PP: peristaltic pump, RC: reduction coil, CVG: cold vapour generation, GLS: gas-liquid separator, AFS: atomic fluorescence spectrometer, S: standards or samples, AA: ascorbic acid solution.

2.2. Experimental procedure

Prior to starting the analytical measurements the HC and the flow-through sections connecting it to the central port of the SV were filled with deionized water and the flow-through sections connecting Ports 2-6 to the corresponding vials were filled with 4 M HCl solution, sample/standard solution, 1% $KMnO_4$ solution, 50 mM Na_2S solution, and 0.1 M AA solution, respectively.

2.2.1 Calibration procedure

In the calibration process, 3,500 µL of deionized water were aspirated from its reservoir into the SP (Fig. 1) at a flow rate of 500 µL s^{-1}. This was followed by consecutive aspiration into the HC of 200 µL of 4 M HCl solution at 50 µL s^{-1} and 1,000 µL of Hg^{2+} standard solution

at 100 μL s^{-1} via Ports 2 and 3 of the SV, respectively. A metered volume of 1,500 μL solution already aspirated into the HC, consisting of the 200 μL of 4 M HCl solution and 1,000 μL of the Hg^{2+} standard solution together with 300 μL of deionized water, were dispensed into the digestion vial (DV) at 100 μL s^{-1} under agitation with the magnetic stirring bar at 300 rpm. This mixture was afterwards aspirated back into the HC. The peristaltic pump (PP) was turned on and the mixture in the HC was delivered at 150 μL s^{-1} via Port 10 of the SV into the CVG unit where it was merged sequentially with the HCl - AA and $NaBH_4$ streams of the CVG unit (Fig. 1). This reaction mixture was transported to the GLS of the AFS using the remaining in the SP deionized water and the gaseous phase was swept with an argon gas flow into the Hg detection section of the AFS. The fluorescence intensity versus the Hg concentration relationship was used to construct the calibration curves used in determining the concentration Hg in the real samples.

2.2.2 Analytical procedure for Hg speciation in water samples

For the speciation of Hg in water samples In-Hg and then T-Hg were determined sequentially.

In-Hg was determined first by following the calibration procedure described above except that 50 μL of 1% $KMnO_4$ solution were aspirated at 20 μL min^{-1} into the HC via Port 4 after the aspiration of 1,000 μL of a water sample. The $KMnO_4$ solution was required for the decomposition of the organic matter in the water sample that might otherwise consume the CVG reagents. In this case 250 μL instead of 300 μL of deionized water were dispensed into the digestion vial (DV) together with the remaining solutions aspirated into the HC.

For T-Hg determination, 3,300 μL were aspirated at a flow rate of 500 μL s^{-1} into the SP and this was followed by the consecutive aspiration of 100 μL of air at 50 μL s^{-1}, 200 μL of 4 M HCl solution at 50 μL s^{-1}, 1,000 μL of a water sample at 100 μL s^{-1} and 80 μL of 50 mM Na_2S solution at 20 μL s^{-1} via Ports 9, 2, 3 and 5, respectively. A total volume of 1,400 μL, consisting of all aspirated solutions and air together with 20 μL of deionized water, were dispensed into the DV at 100 μL s^{-1} under agitation with the magnetic stirring bar at 300 rpm. Afterwards, 50 μL of air and 100 μL of 1% $KMnO_4$ solution were aspirated sequentially into the HC at 20 μL s^{-1} via Ports 9 and 4, respectively. A total volume of 250 μL, consisting of the air, the $KMnO_4$ solution and 100 μL of deionized water, were propelled into the DV at 10 μL s^{-1}. The resulting 1,500 μL of aqueous mixture in the DV was aspirated back into the

HC at 100 μL s^{-1} and remaining steps leading to the determination of T-Hg were identical to those described above.

Org-Hg was calculated as the difference between T-Hg and In-Hg. The use of air segmentation eliminated the longitudinal dispersion of the sample in the HC.

After each determination, any remaining $KMnO_4$ in the DV was washed with 100 μL of the 0.1 M AA solution, introduced via Port 6, and 1500 μL of deionized water, additionally aspirated into the SP.

3. Results and discussion

3.1. Optimization of the Na_2S and $KMnO_4$ volumes for sample digestion at room temperature

Using the operational parameters for CVG, optimized elsewhere [35], the volumes of the 50 mM Na_2S and 1% $KMnO_4$ solutions were optimized for the complete digestion of a $MeHg^+$ standard solution containing 4 μg L^{-1} Hg. Prior to adding 100 μL of 1% $KMnO_4$ solution into the DV, different volumes of 50 mM Na_2S solution were added to the standard $MeHg^+$ solution in the DV. The effect of the Na_2S solution volume on the digestion of $MeHg^+$ in the standard solution mentioned above is illustrated in Fig. 2 where the signal for $MeHg^+$ is shown as a percentage of the signal for the same volume of a 4 μg L^{-1} of Hg(II) standard solution. Figure 2 shows that by adding 80 μL of 50 mM Na_2S solution allowed quantitative digestion of $MeHg^+$ to Hg(II).

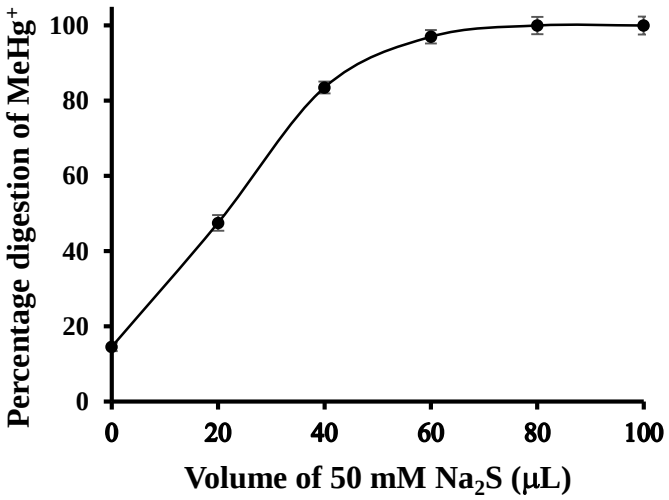

Figure 2. Dependence of the percentage MeHg digestion on Na_2S solution volume. Experimental conditions – Hg concentration in the standard $MeHg^+$ and Hg(II) solutions: 4 μg L^{-1}, volume:1,000 μL, Na_2S concentration: 50 mmol L^{-1}, volume of 1% $KMnO_4$ solution: 100 μL.)

With this Na_2S volume, the decomposition of $MeHg^+$ was investigated by altering the volume of the $KMnO_4$ solution. The results showed that as long as $KMnO_4$ was in excess for the oxidation of sulphide indicated by the persistence of the purple colour of the solution, the dosage of $KMnO_4$ had no impact on the analytical signal for $MeHg^+$. However, a large excess of $KMnO_4$ is expected to deplete the concentration of AA in the HCl carrier stream of the CVG unit and consume $NaBH_4$, resulting in signal depression. In addition, reductive species in water samples will consume a considerable amount of $KMnO_4$. Therefore, the volume of the $KMnO_4$ solution was chosen by considering the complete oxidation of Na_2S and the type of sample analysed. For clear water samples, 100 μL of the $KMnO_4$ solution were sufficient for the oxidation of Org-Hg and the reductive sample matrix. For waters with high load of dissolved organic matter, it was expected to be necessary to increase this volume to compensate for the consumption of $KMnO_4$ by the sample matrix.

It was also observed that the flow rate by which the $KMnO_4$ solution was dispensed into the DV had a considerable effect on the digestion of $MeHg^+$, depending on the purity of the Na_2S solution. By using purified Na_2S, a flow rate below 5 μL s^{-1} for $KMnO_4$ was capable of ensuring 100% digestion efficiency, while the use of a flow rate higher the 5 μL s^{-1} diminished the efficiency. This could be due to the formation of fine particles of elemental S with the slow addition of $KMnO_4$ solution, which facilitated the digestion of $MeHg^+$ as explained later and the formation of coarse S particles with the fast addition of $KMnO_4$ solution, which had lower activity in facilitating the digestion of $MeHg^+$. In comparison, the flow rate had significantly less effect on the digestion efficiency when the original (non-purified) Na_2S reagent was used. These unexpected findings are discussed in the following section.

Under the optimized conditions for $MeHg^+$ digestion, $EtHg^+$ was also completely digested. This is in agreement with the fact that $EtHg^+$ is less stable than $MeHg^+$ [35]. Digestion of $PhHg^+$ was not investigated in this study due to the lack of an analytical standard. However, it has been established that PhHg is less stable than $MeHg^+$ [36] and its digestion is thus easier than that of $MeHg^+$ [22, 23, 27, 30]. It was also observed that when cysteine was used in place of Na_2S, the digestion of $MeHg^+$ was less efficient. The percentage digestion was only 40% when cysteine, containing the same amount of S as the S amount in 80 μL of 50 mM Na_2S solution, was added.

As mentioned above the residual of $KMnO_4$ in the $MeHg^+$ digest should be eliminated prior to the CVG step to prevent depletion of the concentration of $NaBH_4$ which might lower

the method sensitivity. Therefore, AA was added to the HCl solution used in the CVG step. It was observed that in this way residual $KMnO_4$ was quantitative eliminated within a couple of seconds when mixing between the digest stream and the HCl-AA stream was efficient. As expected, the mixing efficiency was found to depend strongly on the length of the reduction coil (RC, Fig. 1) which had 0.5 mm i.d. It was established that a length of 100 cm was sufficient for quantitative reduction of the residual $KMnO_4$.

3.3. Interpretation of the sulphite mediated Org-Hg digestion

Potential mechanisms for the decomposition of CH_3HgCl and $(CH_3)_2Hg$ with the aid of thiol-bearing enzymes were investigated by quantum mechanics approaches (density functional theory) and atoms-in-molecules calculations [37] using H_2S and HS^- as models for thiol and thiolate groups (RSH and RS^-), respectively, to obtain transition states and energy barriers for possible decomposition routes to $Hg(SH)_2$. Demethylation of MeHgCl was found to be a multistep process that involved initial substitution of Cl^- by RS^- with the subsequent coordination of Hg with thiolates leading to increased negative charge on the methyl group and thus facilitating the protonolysis of the Hg-C bond by H-SH. This was also found to be the case for $(CH_3)_2Hg$ [37]. Similarly, Wand et. al. [38] studied L-cysteine induced degradation of Org-Hg in the formation of Hg^0 with KBH_4. They suggested that cysteine could weaken the C-Hg bond in the Org-Hg species by forming complexes. Compared to the present study these authors used a much higher KBH_4 concentration (0.5%) for the CVG process. This high reductant concentration seemed to be important for the efficient reduction of Org-Hg [18]. However, it should be noted that the high H_2 concentration evolved from KBH_4 can significantly quench the fluorescence of Hg and therefore is not a suitable means for achieving high sensitivity [39].

Based on the results outlined above, it could be assumed that sulphide (as H_2S) in the acidified Na_2S solution may play a role similar to that of thiol and thiolate groups in the cleavage of the Hg-C bond in Org-Hg. However, given the high reduction power of H_2S, the sulphur species which plays the major role in the digestion of Org-Hg seems more likely to be elemental sulphur (S_8). Several facts seem to support such an assumption. Firstly, the optimal quantity of Na_2S needed for complete digestion of $MeHg^+$ was much higher than the stoichiometric equivalence, i.e., 1:1. Secondly, Na_2S without prior purification had higher efficiency than purified Na_2S. Thirdly, the flow rate of $KMnO_4$ solution had significant effect on the digestion efficiency with purified Na_2S but such effect was much less significant in the case of Na_2S without prior purification. It is well known that impurities, including $Na_2S_2O_3$,

Na_2SO_3, and elemental sulphur, occur in the commercial Na_2S reagent [40]. When acidified, these impurities and S^{2-} form S_8 through a series of chemical redox reactions [35]. As a consequence, when Na_2S without prior purification was used, S_8 formed quickly once the solution was acidified and the association between S_8 and MeHg most likely took place immediately so that the digestion efficiency was not affected by the flow rate of $KMnO_4$. In comparison, when purified Na_2S was used, there was no S_8 formed when the solution was acidified and S_8 was formed only when S^{2-} was oxidized by $KMnO_4$ [41]. It can be assumed that the *in-situ* formed S_8 associates with $MeHg^+$ and thus facilitates its decomposition by weakening the C-Hg bond. With purified Na_2S, the addition of $KMnO_4$ must be slow enough to ensure the formation of fine and highly reactive particles of S_8 which can subsequently react with $MeHg^+$. In comparison, fast addition of $KMnO_4$ may result in the formation of coarse S particles which might be less reactive regarding Org-Hg.

The use of $Na_2S_2O_3$ or Na_2SO_3 in place of Na_2S did not afford 100% $MeHg^+$ decomposition efficiency (<60%), possibly due to the formation of insufficient amount of reactive elemental S. In conclusion, Na_2S without prior purification exhibited the best effectiveness in facilitating the digestion of $MeHg^+$ with $KMnO_4$ under the operational dynamic conditions used in this study.

3.4. Analytical figures of merit and method validation

Under optimal experimental conditions the newly developed method is characterised by linear calibration curves for Hg(II) and $MeHg^+$ ($Y=77.34\times C_{Hg} - 0.26$, R=0.9996 and $Y=77.31\times C_{MeHg} - 0.30$, R=0.9997, where Y is the fluorescence signal and C is the Hg(II) concentration in $\mu g\ L^{-1}$) in the concentration range of 0 to 10 $\mu g\ L^{-1}$ Hg(II) for both Hg species. Also, identical detection limits (3σ, n=11) of 3 ng L^{-1} were obtained for both Hg species thus indicating virtually identical responses and the possibility of method calibration based on Hg(II) only for both Hg species. A sample frequency of 10 h^{-1} was attained for the simultaneous determination of In-Hg and T-Hg.

The lack of bias of the proposed method was confirmed by determining In-Hg and T-Hg in lake water samples before and after spiking with Hg(II) and $MeHg^+$ at levels far below the WHO guideline value for Hg in drinking water, i.e., 1 $\mu g\ L^{-1}$ [42]. It should be noted that there are no certified reference materials for Hg species in surface waters [43] due to the instability of organic mercury forms at the low abundance levels expected in real samples.

The analytical results, presented in Table 1, show relative recoveries ranging from 93.5-

97% thus indicating that the proposed method was reliable for the speciation of Hg in water samples even with high chemical oxygen demand (COD). An obvious advantage of this method over previously reported methods [22, 30, 32, 44] is the fast and complete digestion of Org-Hg at room temperature even with high dissolved organic matter concentration.

Table 1. Analytical results for the determination of In-Hg and T-Hg in lake water samples.

Sample	COD (mg L^{-1})	Hg species	Measured (ng L^{-1})	RSD[a] (%)	Spiked (ng L^{-1})	Found (ng L^{-1})	Recovery (%)	RSD[a] (%)
1	10.1±0.2	In-Hg	ND		100	94	94	1.7
		T-Hg	29	2.1	200[b]	216	93.5	2.2
2	16.9±0.3	In-Hg	ND		100	96	96	3.1
		T-Hg	33	1.7	200[b]	221	94	2.0
3	140.6±1.1	In-Hg	ND		100	97	97	1.1
		T-Hg	24	2.4	200[b]	213	94.5	1.5

ND: not detectable, [a] n=3, [b] 100 ng L^{-1} Hg(II) + 100 ng Hg L^{-1} as MeHgCl.

4. Conclusions

This work has demonstrated that sulphide can facilitate the digestion of Org-Hg by $KMnO_4$ almost instantaneously at room temperature in on-line flow-based methods. Thus, a facile automatic speciation method has been developed for the determination of In-Hg and Org-Hg. The promoted digestion could be attributed to the coordination of S_8 with the Hg atom in Org-Hg molecules and the increased electron density on the alkyl group that enhances its reduction capacity. Application of the method to lake water samples with elevated COD has confirmed its reliability and the lack of bias in the results. The mechanism by which sulphide triggers the digestion of Org-Hg is under investigation.

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