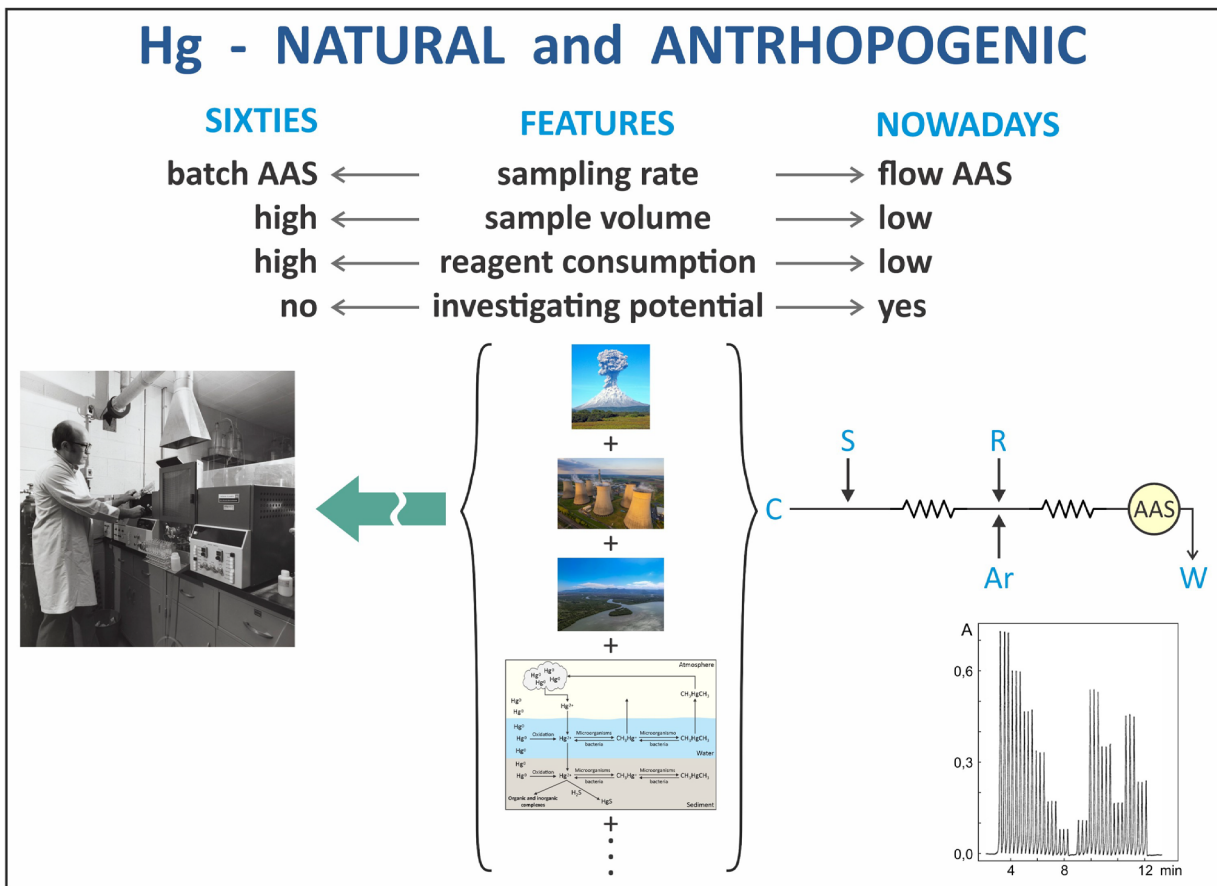


Flow-based determination of total mercury in waters by flameless atomic absorption spectrometry

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Graphical Abstract



Abstract

A flow injection analyzer is proposed for total mercury determination in natural waters by flameless atomic absorption spectrometry. Following a 30-min wet digestion with nitric acid under 90°C, in a closed Teflon vessel, a sample aliquot is inserted into an acidic carrier stream and, at the next confluence site, a 2.0% (w/v) stannous chloride solution and an argon flow are simultaneously added. After the gas/liquid phase separation, the evolved mercury vapor is directed towards a cylindrical windowless glass flow cell (16-cm optical path, 0.5-cm inner diameter). Details of the analyzer design and operation are provided. Mercury contents $\geq 0.25 \pm 0.08 \mu\text{g/L}$ can be effectively determined at a sampling rate of 90/h. Good repeatability (r.s.d. ca 2.0%) was attained for 1.50 $\mu\text{g/L}$ Hg, and results were in agreement with those obtained by the analogous manual procedure ($t = -0.10$; $P = 0.92$; $n = 6$).

Keywords: Automated Determination. Total Mercury. Waters. Industrial Effluents. Atomic Absorption Spectrometry.

INTRODUCTION

After the well-known Minamata Bay accident in Japan caused by the release of Hg in wastewater of a plastic and chemical industries¹, the importance and significance of this metal became ubiquitous with regard to environmental and human health. This is justified by recalling that, when present in the environment, the metal accumulates in living organisms, magnifying itself through the food chain, causing damage to the consumers^{1,2}.

The increasing industrialization with potential for the generation and release of this pollutant, as well as the need for knowing the behavior of the metal in the environment, demonstrate the importance of developing accurate, precise and sensitive methods for the Hg determination in different substrates of environmental, clinical and agronomic relevance. All this, coupled with the need for increasing the analytical rate^{3,4}. It should be reminded that, since the pioneer work by Hatch and Ott⁵, flameless atomic absorption spectrometry (AAS) has been widely used for Hg determination.

This trend is increasingly significant, as mercury is commonly linked to various toxicological issues, including neurotoxicity and developmental impairments². As above stressed, flameless AAS addresses the need for accurate and feasible mercury determination in various matrices, ensuring compliance with regulatory agencies. In batch analytical

procedures, however, the AAS measurements are in general laborious, often leading to operator errors, and this tends to be magnified in routine large-scale assays³.

In automated procedures, a significant increase in the analytical laboratory capability as well as improvement in analytical figures of merit, including sample throughput have been noted. This is in adherence with the principles of Green Analytical Chemistry⁶. The number of methods for Hg determination has therefore significantly increased⁷.

Special attention has been given to flow analysis, which exploits the insertion of a small sample aliquot into a continuous flowing carrier stream⁸. In addition, the sample digestion can be carried out inside the analytical path during the sample transport while the sample flows towards detection⁹. In view of the low Hg contents in natural waters, and the sensitivity of flame AAS for Hg determination, numerous attempts have been implemented. The accessory of the Agilent model 95 for cold vapor generation and the formation of hydride elements¹⁰, as well as a dedicated analyzer specific for Hg, known as Flow Injection Mercury System¹¹ should be here mentioned. Furthermore, analytical techniques more sensitive than flameless AAS, such as e.g. atomic fluorescence spectrometry (AFS)¹² and inductively coupled plasma mass spectrometry (ICP-MS)

have been proposed^{9,13}. Although ICP-MS and AFS present similar sensitivity for Hg, the former is more expensive, nevertheless a multi-element analyzer^{13,14}.

It should be emphasized that flameless AAS is still in use for Hg determination and that a dedicated commercially available Hg analyzer with the same configuration as the AFS is attained just by replacing the detection mode¹⁵.

Depending on the organic matter content in the matrices, a previous sample treatment with nitric acid in a digestion bomb¹⁶ is required for total mercury determination. The feasibility of exploiting batch sample solubilization for the analyses of industrial effluents and/or other pollutant sources enhanced

the use of the proposed flow analyzer. A remarkable aspect is that this analyzer is compatible with the maximum concentrations for total mercury in Class 3 fresh water bodies and effluents preconized by the Brazilian National Environmental Council (CONAMA), set as 2.0 and 10.0 µg/L Hg, respectively¹⁷.

The aim of this work was therefore to develop an automated procedure for Hg determination in waters using a classical atomic absorption spectrometer (Perkin-Elmer, model 503) connected to a simple flow injection analyzer comprising a peristaltic pump, an acrylic proportional injector¹⁸ (Fig. 1a), a homemade gas/liquid separation chamber (Fig. 1b) and a windowless flow cell (Fig 1c).

METHODOLOGY

Solutions

All solutions were prepared with water from a Milli-Q system (specific conductivity < 0.1 µS/cm) and chemicals of analytical reagent (AR) grade quality. Nitric acid was purified by isothermal distillation. Glassware cleaning involved washing with tap water and overnight standing into a (1:4 HNO₃:H₂O) solution. Before use, it was plentiful washed with deionized water and dried at room temperature in an air vertical flow hood. High purity argon (99.96%) was used.

The R reducing reagent (R, Fig. 1a) was a 10% (w/v) SnCl₂·2H₂O solution, freshly prepared by dissolving 10.0 g of the salt in about 20 mL of a 1:1 HCl:H₂O solution and filling the volume up to 100 mL with water. The R reagent was purified by 10-min argon bubbling.

The 100 mg/L Hg stock standard solution was prepared by adding 13.54 mg HgCl₂ plus 200 µL of a (1:1) HNO₃ solution to 20 mL of water, and filling the volume up to 100.0 mL with water. Working standard solutions in the 0.00-10.00 µg/g Hg range were prepared in 0.014 mol/L⁻¹ HNO₃.

Natural water samples were collected from rivers in the São Paulo Metropolitan

region using 500-mL polyethylene bottles to ensure minimal contamination and preserved by adding 1.0 mL of a 7.0 mol/L HNO₃ solution. For samples with low contents of organic matter, a simplified wet digestion procedure involving addition of 10 mL HNO₃ and 10 mL of sample to a 60 mL Teflon bomb, 30-min heating at 90°C, and a resting period for attaining room temperature was carried out¹⁹.

Apparatus

A Perkin-Elmer model 503 AAS with a mercury hollow cathode lamp was connected to a REC 61 radiometric recorder with a REA 112 high sensitivity unit, which allowed graphical recording of the obtained signals, proportional to the Hg concentrations in the samples. The damping factor was set as 2s, enough for attaining acceptable levels of analytical noise. The AAS was connected to the flow injection manifold system, which comprised an Ismatec model mp13 GJ4 peristaltic pump with Tygon tubing, which pushed the liquids through the analytical path. These liquids were discarded at the end of the process. The inserted sample aliquot of the input sample was inline processed during

transport towards detection, where the produced Hg^0 was monitored at 253.7 nm. This derivatization is called flow injection atomic absorption spectrometry (FI AAS).

The L sampling loop (Fig. 1a), whose inner diameter and length define the injected sample volume, was a 0.8-mm inner diameter, 2.0-mL volume coiled glass tube. The other components of the flow injection manifold such as proportional injector, connectors, polyethylene tubes, etc. were described elsewhere¹⁸.

The 5.0-cm height, 1.0-cm inner diameter SC separation chamber (Fig. 1b) was made from borosilicate glass, and designed as small as possible. It was an essential component of the flow manifold, where the liquid and vapor Hg phases separation took place. It also attenuated the analytical interferences in the signal, and enhanced the Hg^0 concentration. A silicone rubber stopper, set at the SC top, allowed the inlet and outlet of the liquid phase via y and v holes, respectively. Meantime, the Hg^0 vapor generated after the confluence of the reagent with the dispersed sample was introduced into SC and pushed by the argon through the z-k transfer line towards detection. With this configuration, the Hg^0 vapor reached the flow cell in its lower middle part.

The FC windowless cylindrical glass flow cell (Fig. 1c) was positioned along the optical beam of the spectrometer. It was fixed at the upper part of the burner, in the support usually utilized in the Perkin-Elmer hydride generator kit11 for flameless cold vapor AAS. The original version of this cell was used with the same length, a wider diameter (1.0 cm), and quartz windows in both sides¹¹.

The flow injection analyzer

When the FI AAS (Fig. 1a) rests in the sampling position, the sample is aspirated to fill the sampling loop, and its excess is wasted (W). Manual commuting of the injector to the alternative position introduces the sampling loop into CS, originating a sample zone¹⁴ that is transported towards SC. At the x confluence, the SnCl_2 solution and the argon converge with the sample zone, starting the Hg^{2+} reduction to Hg^0 , which is transferred from the liquid to the gaseous phase. The fast Hg^{2+} reduction occurs inside the x-y line and SC chamber. From there, the vaporized Hg^0 is carried out by the argon through z-k line towards the center of the 16-cm FC (Fig. 1c) to be detected. The reproducible transient absorbance is recorded as a peak with height providing the measurement basis.

The liquid phase, together with some the gas bubbles, is removed from SC by the peristaltic pump and wasted. The Hg^0 vapor flowing through both ends of FC was sucked from the laboratory environment through the equipment chimney, similar to the exhaust system used in flame AAS. The CS flow rate was selected as 11.6 mL/min, by considering the low R pumping rate (0.46 mL/min). In these conditions, sample dilution at the x confluence was minimized. The argon flow was estimated by the soap bubble technique and controlled by a precise gauge. The i-x line was set as short as possible (15 cm), the x-y line (50 cm) was enough to provide good mixing conditions for enhancing the reaction development, and the z-k line was set as short as possible (15 cm) to avoid water vapor reaching the flow cell.

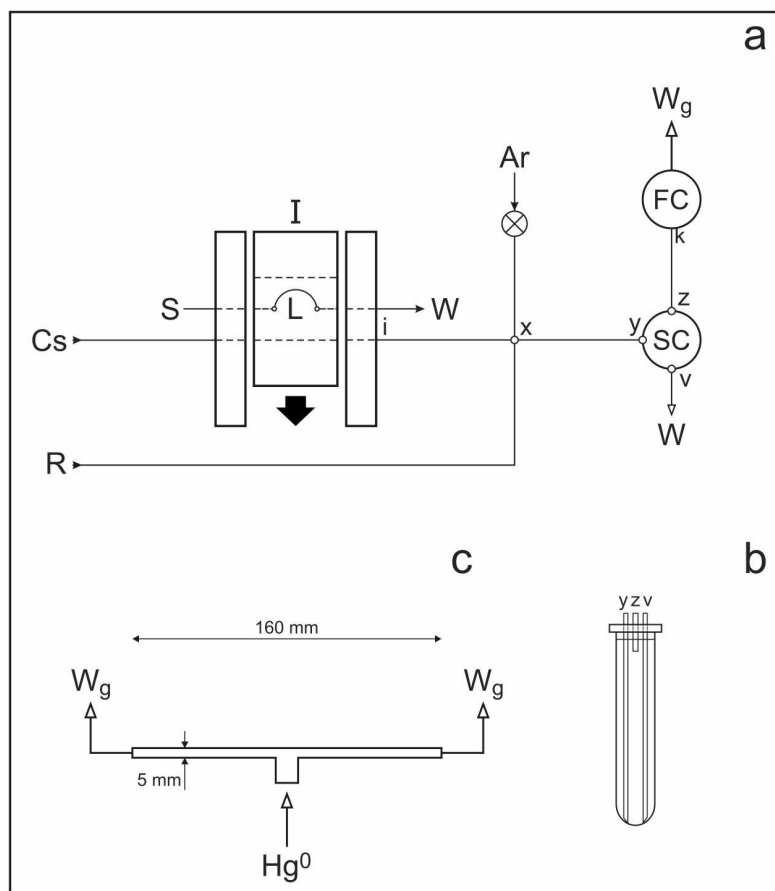


Figure 1 - FI AAS manifold (a) with details of the glass phase SC separation chamber (b) and the FC windowless glass flow cell (c). P: peristaltic pump; S: sample; L: sampling loop; I: proportional injector; CS: sample carrier stream at 11.6 mL/min; R: reducing reagent at 0.46 mL min⁻¹; W: wasted solution; Wg: Hg⁰ waste; Ar: argon at 3.6 L/h; i-x, z-k, x-y: 15, 15, 50-cm transmission lines. For details, see text.

SC was designed with a low dead volume for the liquid phase, and FC was dimensioned by considering the need for a low inner diameter and a long optical path, associated with the facility of assembling. Thereafter, the S injected volume was experimentally selected as 2.0 mL. In this situation, a 5.0 mL/min sample aspiration rate was compatible with the manifold variables, allowing 90 samples to be run per hour.

Procedure

Initially, the SC and FC design and dimensioning were investigated by inserting 1.0 mL of 0.0 – 5.0 µg/L Hg standard solutions into CS, a 0.15% (v/v) HNO₃ solution. The

possibility of water reaching the flow cell, the measurements repeatability, and the recorded peak shape were taken into account, as they may provide relevant information on analytical sensitivity and washing time.

The CS and R flow rates were selected as 11.6 and 0.46 mL/min, respectively. The SC outlet flow rate (Fig. 1b) was slightly higher than CS plus R flow rates. The SnCl₂ concentration was selected as 10% (w/v), as preliminary experiments involving peristaltic pump stopping^{20,21} indicated that an almost quantitative reduction of mercury was already attained. Influence of the argon flow on the recorded analytical signal was investigated within the 2.5 - 5.0 L/h range, and 3.6 L/h

was selected. After varying the SnCl_2 concentration from 1.0 to 10.0 % w/v, a 2.0% w/v solution was chosen. For studying the influence of the CS composition, its nature (HNO_3 , HCl , H_2SO_4), and acidity (0.05, 3.0 and 7.0 mol/L) were varied.

The S volume was selected amongst 0.5, 1.0, 2.0 and 4.0-mL, the sampling loops being made from 0.8 mm i.d. polyethylene tubing or glass coiled tubes with a larger inner diameter. The 2.0-mL glass sampling loop was

chosen.

The stability of the proposed flow system was evaluated by carrying out several standardizations during an entire 8-h period of continuous operation. The analytical precision was estimated as the relative standard deviation of seven Hg determinations in a typical sample (1.53 $\mu\text{g/L}$ Hg), and accuracy was assessed by running five water samples already analyzed by the conventional method¹⁹.

RESULTS

The results of this work are summarized in Figures 2, 3 and 4 as well as in Table 1. It should be mentioned that the sample volume (2.0 mL) injected by means of a glass sampling loop and the R reagent concentration (2.0% w/v) were defined in preliminary tests because their values are not critical. With these adjustments, the length of the analytical path and a stable hydrodynamic condition, integrating the liquid (Hg^{2+}) and vapor (Hg^0) phases, were established. Analysis of Fig. 2 reveals that the ideal argon flow was corresponded to 3.6 L/h Ar, achieving excellent linearity and good analytical sensitivity. With this carrier gas flow rate, the best sample-to-confluent reagent flow rates relationship (CS/R) was established for different Hg concentra-

tions, with R equal to 0.46 mL/min and the CS equal to 11.6 mL/min⁻¹ (Fig. 3). The stability of the proposed analytical system can be observed by the behavior of the baseline generated during a routine large-scale analysis, involving two analytical curves, attained with six Hg standards and Hg determinations in five samples (Fig. 4). In addition, the shape of the generated signals is a further indication of the excellent functionality of the FI AAS derivatization system. Under these conditions, a 90/h sampling rate was established. These figures of merit are associated with the accurate and precise results obtained with the proposed system. In fact, they did not statistically differ from those obtained by the reference manual analytical method¹⁹ (Tab. 1).

DISCUSSION

The simplicity of the proposed flow system allows the exploitation of its potential just by modifying the Ar, CS and R flow rates, as well as the sample injected volume. The parameters related to the involved chemistry are not critical, but strongly affected by the established hydrodynamic conditions²⁰. In general, these

conditions are improved in confluence flow analyzers. Here, the argon gas and reagent solution are simultaneously added by confluence to CS where S has been inserted. Results of the related experiments (Figs 2 and 3) permit one to infer that the system response is directly proportional to the sample volume.

The Ar flow was tested in the 2.5, 3.0, 3.6, 4.5 and 5.0 L/h sequence. In general, good linearity of the analytical curves was always observed. However, the best analytical conditions were not observed within the regular ascending or descending order of the gas flow (Fig. 2). This aspect reflects that the sample zone is not homogeneous, but consists of comprising gaseous and liquid phases, and that the mixing conditions are rather dependent on the Ar flow. The best system response, and hence sensitivity, was attained with a 3.6 L/h argon flow. Although less evident at lower Hg concentrations, the analytical signal varied with the CS/R flow ratio, reaching a maximum at about 25 (Fig. 3). The R and CS flows were then selected as 0.46 and 11.6 mL/min, res-

pectively. After this maximum, the absorbance was lessened, with a consequent loss in sensitivity.

Regarding the R concentration, only slight variations (ca. 10%) in the slope of the analytical curve were observed when it was varied within 1.0 and 10.0 % w/v SnCl₂ stannous chloride. This confirms that the R concentration is excessive in comparison with that of the recommended method¹⁹. Since dilute SnCl₂ solutions should be avoided²², a 2.0% (w/v) concentration was selected. Although the CS composition can be varied, the sample acidity should always match that of the standards, in order to avoid detrimental any deleterious matrix effect on sample dispersion, Hg⁰ vaporization or detection.

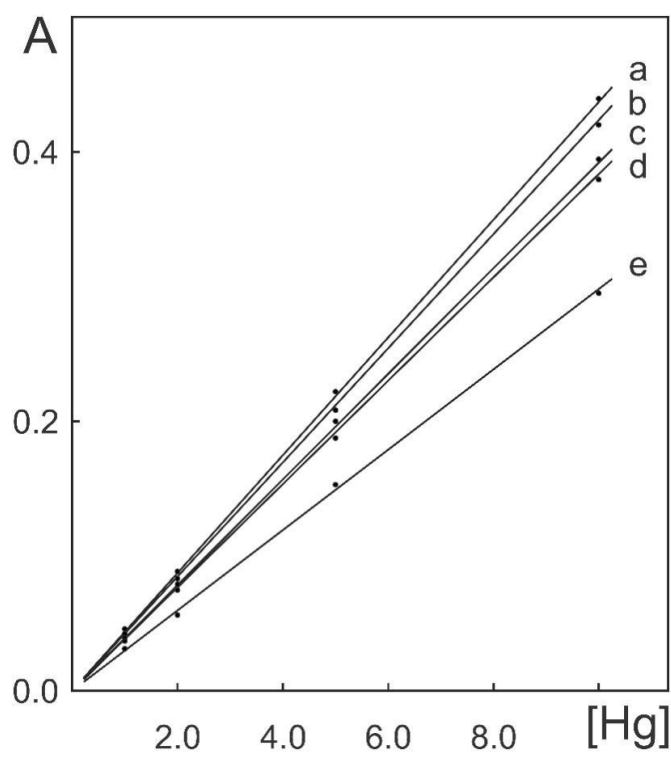


Figure 2 - Influence of the argon flow on the analytical curves; a, b, c, d, e: 3.6, 3.0, 2.5, 4.5, 5.0 L/h Ar; abscissa in µg/L Hg.

With the flow analyzer system designed as above, good mixing conditions were attained, thus the recorded peak profiles were rather smooth (Fig. 4), demonstrating that the CS/R ratio exerts a significant influence on the system response. Altering this ratio may deteriorate mixing conditions, thus affecting the analytical precision and sensitivity.

The Ar flow was precisely adjusted, as there is an optimal value almost independent of the Hg concentration (Fig. 2). Higher (4.5 and 5.0 L/h) or lower (3.0 and 2.5 L/h) flow rates may decrease the recorded peak height, probably because the volatilized mercury fraction in the SC is reduced, thus Hg⁰ is directly swept towards waste, not reaching the FC. Lower peak heights were also observed for higher Ar flow rates (Figs 2 and 3) due to an increase of the sample plug dispersion in the gas phase.

The Hatch and Ott method¹ required the use of a Mg(ClO₄)₂ powder desiccator column, that should be systematically replaced after four or

five determinations to prevent water entering the FC. In the proposed system (Fig. 1a), no desiccator is required, as water spray reaching the FC was not observed.

A large sampling loop was necessary for minimizing both the sample dispersion in the liquid phase and the Hg⁰ dispersion at the gas phase. To this end, the inner volume of the analytical path (reaction coil plus SC) should be taken into account. With a 2.0-mL sample inserted volume, the dispersion coefficient²¹ was estimated as 0.97. The SC inner volume should be as small as possible, especially with regard to the liquid phase. The volume of this phase inside SC is established by the position of the tube through which the liquid is drained wasted (Fig. 1b). On the other hand, the gas phase volume is not so critical, as it is lower than the FC dead volume. Preliminary tests indicated that the SC inner volume cannot be arbitrarily minimized at will, otherwise the gas/liquid separation would be impaired.

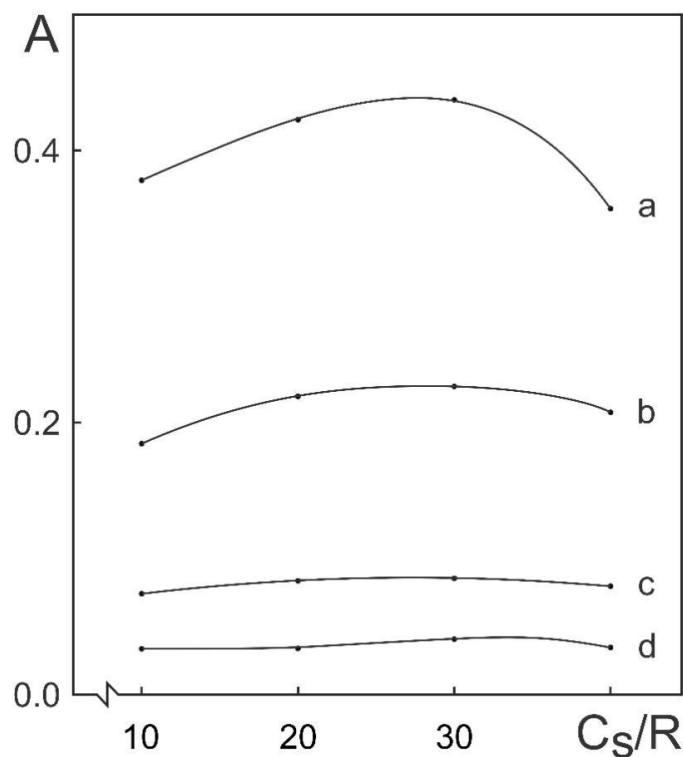


Figure 3 - Effect of the CS/R flow rates ratio on the signal response for 10.00 (a), 5.00 (b), 2.00 (c) and 1.00 (d) µg/L Hg.

The flow cell was designed in accordance to the optical beam characteristics and the available space in the spectrometer. Longer optical paths did not improve sensitivity, because the light beam collimation was a limiting factor in the measurement. In addition, thinner tubes are cumbersome to be adjusted on the spectrometer support, thus less recommended for the routine work.

When comparing the proposed flow cell with that of Perkin Elmer, which has a quartz window at each end, an important remark should be highlighted. Here, the flow cell is windowless. This detail leads to a faster Hg^0 delivery from the detection unit, increasing the analytical speed and reducing inter-peak carry over between peaks. Moreover, a narrower tail is observed in the recorded peak shape. The proposed procedure

allows the analyses of 90 samples per hour. This rate can be increased by using a smaller (1.0 mL) sampling loop, but a slight decrease in the slope of the analytical curve is observed.

With the simplified sample preparation using nitric acid inside the digestion bombs¹⁶, only a low blank value was observed (Fig. 4). On the other hand, The recommended digestion procedure¹⁹ involves strong oxidizing agents such as potassium permanganate and/or persulphate, which usually contain high levels of Hg impurities. The blank values are then increased, impairing the analytical sensitivity. The disadvantage can be minimized by using high purity and expensive chemicals and/or cumbersome purification steps. The applicability of the procedure to routine analysis would then be limited.

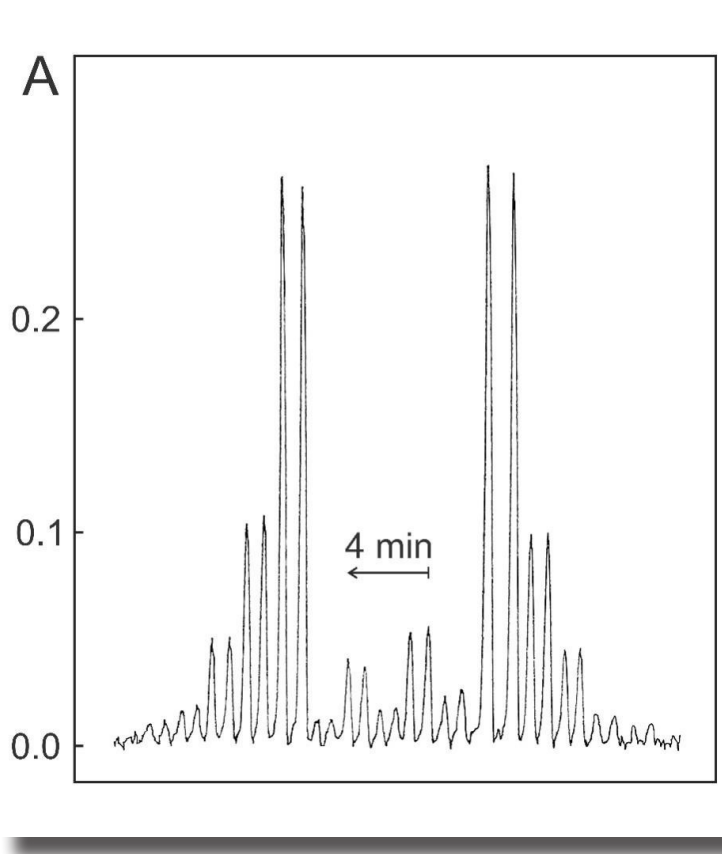


Figure 4 - Recorder output of part of a routine run for Hg determination in natural waters. From left, duplicated peaks refer to six standard solutions (0.00, 0.25, 0.50, 1.00, 2.00 and 5.00 $\mu\text{g/L}$ Hg), five samples, and the standard solutions again.

Linearity of the analytical curve up to 10 µg/L Hg, good measurement repeatability, often better than 97% within 0.25 and 5.00 µg/L Hg, as well as good system stability reflecting itself in the baseline tracing are inherent in

the proposed procedure. Regarding accuracy, analysis of Table 1 reveals that the results obtained with the proposed FI AAS procedure are consistent with those obtained with a batch manual flameless AAS19 ($t = -0.1$; $P = 0.92$).

Table 1 - Total mercury (µg/L Hg) in river waters as determined by the manual and automated procedures.

Sample	Procedure	
	FI AAS	Reference ¹⁹
1	18.3 ± 0.2	21.1 ± 0.8
2	16.1 ± 0.1	14.1 ± 0.2
3	5.8 ± 0.1	5.2 ± 0.3
4	9.1 ± 0.2	11.7 ± 0.2
5	3.3 ± 0.1	2.7 ± 0.3

The potentialities and figures of merit of the proposed FI AS¹⁹ analyzer for the determination Hg were highlighted. Due to the abundance of methods and procedures for this determination^{7,23}, important information and elucidation need to come to light for explaining the past and current state of the art in the analytical methodology associated to this metal.

In its natural cycle, Hg moves through the lithosphere, atmosphere, hydrosphere and biota compartments²⁴. It stays in the atmosphere during a considerable residence time, which allows its transport to long distances from the sources of origin²⁵. This phenomenon is similar to the “flying rivers” formed in the Amazon rainforest and precipitated in the southern region of Brazil²⁶. The Hg return to the earth surface does not occur discretely, point to point, but rather as gradients, establishing the most diverse concentrations of the metal along the surface (soil, water) where it precipitates. The direction in which this cycle operates establishes that the lithosphere is its largest reservoir^{24,25}. It is in the soils and sediments that Hg undergoes transformations into its inorganic and organic forms²⁵. This dynamic, carried out by microorganisms in the environment²⁷, re-

sults in the formation of e.g. ethyl, phenyl, and methyl mercury, which is considered as chemical speciation. Methyl mercury is the most toxic form to organisms, and the one that gives it the property of concentrating and magnifying in food chains²⁸. It is worth recalling that Hg in the atmosphere undergoes also another form of speciation, where it occurs as Hg⁰, Hg²⁺ and Hg²⁺, in the free and particulate forms, when it is adsorbed to atmospheric particles known as aerosols. In the case of Hg, the anthropogenic contribution to the environment (burning of fossil fuels, gold mining) cannot be neglected. It is believed that approximately 30% of the Hg present in the atmosphere comes from this source²⁹. It should be emphasized that this occurrence is noted at different concentration levels (ppt, ppb, ppm).

With recent advances in analytical chemistry, ppt fractions can also be detected with a high level of reliability in environmental matrices, such as water³⁰, soil, sediments, plants³¹ and biological fluids (blood, urine)³². Nowadays, there are instruments and resources to perform studies of human and environmental health monitoring.

As toxicity depends on the chemical form, the proposed method has restricted applica-

tion, as it operates in concentrations of the order of ppb and does not consider the Hg chemical speciation. The analytical facilities mentioned here are only available in research centers of excellence and in the private sector, due to the high cost and complex infrastructure required for their implementation. The above aspect does not diminish the merit of this proposal, since it still has its role in determining total Hg at acceptable levels for the chosen matrices, within the limits established by environmental policy makers. In addition, it is a robust, economical system that is in line with the objectives of Green Chemistry, especially with regard to reduced amount of reagent and consequent liquid waste lessening.

This article reports an important reality, and clearly demonstrates that the method for Hg determination should be selected as a function of the considered matrix and the

aimed objectives. Other more sensitive methods are often critical in terms of the sample collection, transport, preservation and pre-treatment steps²³.

Emphasis on chemical speciation, whose criteria in sample handling are crucial to ensure that the oxidation states and chemical forms of the species of interest are not altered^{33,34}. This proposal utilizes an old technique for determining Hg in water and industrial effluents, satisfactorily achieving the above-mentioned purposes.

A simple and immediate improvement of this proposal, significantly expanding its applications, is attained simply by replacing AAS by AFS detection¹². This subtle and economical innovation would allow to expand the working range of the proposed system to the ppt-ppm concentration range, including the current working range of the presented system.

CONCLUSION

Given the relevance of mercury determination in agriculture, environment and human health, as well as the robustness of flameless AAS, the determination of this element has undergone continuous evolution. The original procedure, manually carried out, was widely used in the 1960's and has been continuously replaced by automated ones. Corroborating with this trend, other techniques such as AFS and ICP-MS have emerged, mainly due to the increasing need for improving sensitivity and analytical rate, as well as for achieving Hg speciation. Nowadays, interest on these aspects continues to grow, mainly for assessing mercury toxicity.

Results of the present research evidenced that it is possible to determine mercury using an old atomic absorption spectrometer coupled to a traditional and simple flow analyzer. This coupling permits leads to a drastic reduction in sample volume, and is making it relevant in the analysis of e.g. natural and wastewaters. In fact, it tolerates relatively high levels of organic matter and, above all,

is in adherence to the levels recommended by CONAMA for Class-3 waters and industrial effluents, set as 2.0 and 10 µg/L Hg, respectively. It can therefore be concluded that, after so many years, AAS still occupies a prominent place in the determination of this chemical element.

Analytical automation in the Hg determination is very important, as this species represent an essential tool for monitoring water resources in institutions that carry out this activity, in research and in stakeholders. In practice, it allows the analysis of a high number of samples with low reagent consumption and low waste generation, and this aspect is in line with the principles of green chemistry.

Furthermore, efforts to increase the concentration range for Hg determination bring more possibilities of application to different matrices of interest for health and environment. In this regard, use of the proposed flow manifold with AFS detection is an immediate and low-cost action. In fact, novel improvements should always be encouraged. With

the same objectives and principles, the idea could be extended to other toxic metals such as As, Bi, and Sn, which can also be determined by flame AFS. Not only for Hg, but also for the other elements mentioned, these measurements are consistent with the excellent properties of AFS, namely the wide linearity range and the high sensitivity.

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All authors have read and agreed to the published version of the manuscript.

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