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Determination of Mercury in Fish by Cold Vapor Atomic Absorption Spectrophotometry Using a Multicommuted Flow Injection Analysis System

M. Fátima Silva, Ildikó V. Tóth, and António O. S. S. Rangel†

Escola Superior de Biotecnologia, Universidade Católica Portuguesa, Rua Dr. António Bernardino de Almeida, 4200-072 Porto, Portugal

A flow system was developed for the determination of total mercury concentration in fish samples by cold vapor atomic absorption spectrophotometry (CVAAS), based on the multicommuted flow injection analysis (MCFIA) approach. The system uses independently controlled solenoid valves for the introduction of reagents and samples. When not injected, solutions were recirculating to the reservoir bottles, in this way reducing the waste produced by the analytical system and also the sample consumption. Results were compared to those obtained by the reference flow injection procedure. Accuracy was also assessed by recovery studies using a certified reference material as well as spiked samples; recovery percentages in the range of 90.7% to 99.8% were found. The repeatability of the method was better than 6.0% (RSD, n = 10). A limit of detection of 4.8 μg of mercury per kg of fresh fish sample was achieved. The total waste produced was reduced to 30% of that from the reference flow injection CVAAS procedure.

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Introduction

Mercury and its compounds are considered health hazards; their toxicity depends on the chemical form. The organic mercury is generally more toxic than inorganic mercury. Methylmercury present in fish makes the most significant contribution to human dietary exposure to mercury. The presence of mercury in the aquatic environment could be a result of the anthropologic activities or could come from geological sources. Fish and other aquatic species accumulate methylmercury, primarily produced by microorganisms, which is absorbed directly from water or as a result of their position in the food chain. Big old predatory species usually present high levels of mercury.[1,2]

The EU legislation for total mercury sets a maximum level of 0.5 mg kg^{-1} for fishery products, with exception for certain listed fish species for which 1 mg kg^{-1} applies.[3]

Nowadays, the most widespread method for mercury determination is cold vapor atomic absorption spectrophotometry (CVAAS). One of the early examples of this technique was described by Hatch and Ott in 1968.[4] CVAAS is based on the chemical reduction of mercury, usually by Sn^{2+} or BH_4^- ions, to elemental mercury which is swept from the solution by a carrier gas to a quartz cell placed in the optical path of an atomic absorption spectrophotometer where the absorption of mercury is measured. Since then CVAAS was adopted as a standard method for analysis of mercury in foodstuffs.[5]

The techniques for CVAAS can be classified into batch and flow systems. High sample consumption (usually between 10 and 50 ml), and more time expended in the analysis are the major limitations of the batch systems. The first flow system based on flow injection analysis (FIA) for mercury determination by CVAAS was proposed by Andrade *et al*. in 1983.[6,7] Since then FIA-based automated methods are widely accepted by analytical chemists dealing with the determination of mercury.[8] The high sampling frequency (about 100 h^{-1}), the good precision and the low sample volumes (usually about 500 μl) are the main advantages of these systems. Further development in the area of automation of wet chemistry methods led to the appearance of different flow techniques for the coupling of the vapor generation process to the detection system.[9,10]

Another approach for CVAAS is multicommutation, because it presents some valuable advantages when compared to the flow injection analysis,[11] like reduction of sample/reagent consumption and waste production. Multicommutation[12,13] (MCFIA) is based on the use of individually controlled three- or two-way valves forming a flow network, where the time control of the valves determines the volume of the injected solution. Solutions can be moved inside the manifold by aspiration or by propulsion. One of the most important improvements is the possibility of miniaturization of the flow assembly, allowing the introduction of reduced volumes of reagent and sample solutions with high precision. The flexibility of the method is also clear, since any needed modification in the developed method is easily accomplished by changes in the time-based programming procedures. The potentialities of the method include the implementation of tandem streams or binary sampling by alternately introducing small volumes of different solutions (reagents and samples), then increasing the contact area between them, and therefore improving the mixing conditions.

In this work, a novel multicommuted flow injection analysis system coupled to CVAAS was developed. This methodology

† To whom correspondence should be addressed.
E-mail: aorangel@esb.ucp.pt

was applied to the determination of mercury in fish samples. Results were compared to those obtained by the reference CVAAS FIAS-100 method,[14] in terms of sampling frequency, precision, accuracy, reagent consumption and waste production.

Experimental

Reagents

All solutions were prepared in water with a resistivity of 18.2 MΩ cm^{-1}, obtained from a Milli-Q (Millipore Corporation, Bedford, MA, USA) water purifier system; the chemicals used were of analytical grade or better.

Mercury(II) working standards were prepared daily by appropriate dilution of a mercury certified standard solution 1000 mg l^{-1} (CertiPUR, Merck, Darmstadt, Germany) in H_2SO_4 10% v/v and stabilized by the addition of 1 – 2 drops of a 5% m/v $KMnO_4$ solution.

The sodium borohydride alkaline solutions were prepared daily by dissolving the appropriate amounts of the solids $NaBH_4$ (Merck) and sodium hydroxide pellets (Merck). Hydrochloric acid (Pronalab, Lisbon, Portugal) was used to prepare the carrier 3% v/v HCl solution and Argon C-45 was used as the carrier gas.

For sample digestion the following solutions were used: 10% m/v hydroxylamine hydrochloride solution prepared from $NH_2OH{\cdot}HCl$ (Merck); 5% m/v $KMnO_4$ solution prepared from Merck mercury-free salt; concentrated H_2SO_4 (Pronalab). Antifoam 1-octanol (Merck) was employed to avoid foam formation in sample analysis.

Sample treatment

We analyzed fish samples (shellfish, cod, skate, prawn, squid) that were commercially available and a certified reference material NRC DORM-2 (dogfish muscle) from the National Research Council, Canada.

A 5-g portion of fresh fish or 0.5 g of dried fish (NRC DORM-2) was introduced in a digestion flask with 5 ml of water and 10 ml of H_2SO_4. The sample was left overnight at room temperature. Small aliquots of a 5% m/v $KMnO_4$ solution were added to the digest to obtain a permanent pink color, which should persist for at least 4 h. The excess of $KMnO_4$ was removed by the addition of a 10% m/v hydroxylamine hydrochloride solution. Two or three drops of 1-octanol was added to prevent excessive foam during analysis. The sample solution was stabilized by the addition of 1 – 2 more drops of $KMnO_4$ and the mixture was diluted to 100 ml with water. A reagent blank was also exposed alongside the sample.

Apparatus

A Perkin Elmer Model 4100ZL (Boston, MA, USA) atomic absorption spectrometer equipped with a mercury hollow cathode lamp was used with the Model FIAS-100 flow injection analysis system and hydride generation accessories. The analytical wavelength and slit width were 253.7 nm and 0.7 nm, respectively. All the instruments were controlled by Perkin Elmer AA-WinLab software.

The multicommuted flow system developed in this work (Fig. 1) consists of a peristaltic pump (Gilson Minipuls 3 M312, Villiers le Bel, France), tygon pump tubes, three three-way solenoid valves (NResearch 161T031, Cardwell, NJ, USA), an X-type confluence (Omnifit 1060, Cambridge, England), reaction coil and flow lines of PTFE with 0.8 mm internal diameter. The analytical system control was achieved by means of an interface card (Advantech Model PCL-818L, Taipei, Taiwan) and a 486 computer. A homemade power drive based on a UNL 2003 integrated circuit was used to operate the solenoid valves[15] controlled by software written in QuickBASIC (Microsoft). The flow injection valve of the FIAS-100 system was not used in this study. The reaction coil was connected to a three-way connector (Perkin Elmer Manifold Block) where the flow merged with the argon carrier gas (set at 70 ml min^{-1}) and was directed to the gas-liquid separator (Perkin Elmer), with a membrane filter for removal of liquid droplets. The gas-liquid separator and the quartz atomizer cell, heated at 100°C, were connected with a 100 cm, 1.0 mm internal diameter PTFE tube. A single pump channel from FIAS-100 was run continuously for withdrawal of the reaction waste from the gas-liquid separator.

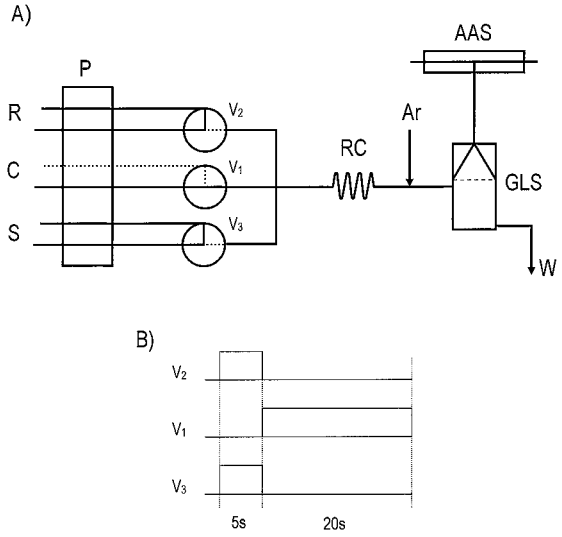

Fig. 1 A) Manifold of the MCFIA system for the determination of mercury; B) timing course for the functioning of the solenoid valves. C, Carrier HCl 3% v/v; R, reducing reagent 0.2% m/v $NaBH_4$ in 0.05% m/v NaOH; S, sample or working standard solutions; V_1, V_2 and V_3, three-way solenoid valves; P, peristaltic pump; AAS, atomic absorption spectrometer quartz cell; Ar, argon carrier gas; RC, reaction coil (30 cm); GLS, gas–liquid separator; W, waste.

Flow manifold and procedure

The developed manifold (Fig. 1) was designed with three solenoid valves; V_1, V_2 and V_3 were responsible for the introduction of the carrier, reducing reagent and sample or standard solutions, respectively. In the stand-by mode, the valves V_1, V_2 and V_3 were switched off; at this time, carrier solution, reducing reagent and the sample or standard were recirculating to their storage vessels.

When the analysis cycle started, valves V_2 and V_3 were switched on at the same time. A single volume of sample solution or standard solution was merged together with an equal volume of reducing reagent. The flow rate and time program of these two valves determined the volume of the slug to be introduced and also the total amount of sample or standard. After sampling, the mixture was transported to the gas-liquid separator by the carrier solution which was controlled by the operation of valve V_1 (V_2 and V_3 are switched off, Fig. 1B). In the reaction coil (RC), $NaBH_4$ promoted the reduction of mercury(II) to mercury vapor. An argon stream was then fed to the reaction mixture before the gas-liquid separator. This gas stream had the function of promoting the transference of mercury vapor from the solution to the gas phase and also of

Table 1 Flow parameters for the cold vapor AAS determination of mercury by FIAS 100 and the developed flow system

	FIAS 100	MCFIA
Reaction coil length/cm	11	30
Carrier flow rate/ml min^{-1}	11	11
Reductant flow rate/ml min^{-1}	6	6
Argon flow rate/ml min^{-1}	70	70
HCl, % (v/v)	3	3
$NaBH_4$, % (m/v)	0.02	0.2
NaOH, % (m/v)	0.005	0.05

carrying the vapor to a quartz cell placed in the light path of the atomic absorption spectrometer. The cell was heated to 100°C to avoid water condensation. The absorbance was monitored at 253.7 nm; the peak area measurement mode was used.

The calibration was performed by using a series of standard solutions (within 0 and 20 μg l^{-1}) analyzed at a fixed volume *i.e.* fixed injection time of 5 s (Fig. 1B).

The results obtained by the developed method were compared to those obtained by the CVAAS FIAS-100 method.[14]

Table 2 Analytical parameters found for the cold vapor AAS mercury determination using FIAS 100 and the developed multicommuted flow system

	FIAS 100	MCFIA
LOD/μg l^{-1}	0.15	0.24
LOQ/μg l^{-1}	0.41	0.65
RSD, %	4.7 (1.6 μg l^{-1})	2.7 (1.6 μg l^{-1})
	1.7 (8.1 μg l^{-1})	5.8 (8.1 μg l^{-1})
Calibration linear range/μg l^{-1}	0 – 20	0 – 20
Regression equation[a] $y = mx + b$		
m, Slope/μg l^{-1}	0.056 (±0.003)	0.068 (±0.005)
b, Intercept	0.014 (±0.014)	0.002 (±0.002)
	(n = 7)	(n = 7)
Correlation coefficient	0.9998	0.9995
Sample throughput/h^{-1}	90	60
Sample consumption/μl[b]	500	500
$NaBH_4$ consumption/mg[b]	0.9	1.0
NaOH consumption/mg[b]	0.23	0.25
HCl conc. consumption/ml[b]	0.25	0.11
Waste/ml[b]	13.3	4.7

a. Mean and standard deviation of the calibration curve parameters of 7 different working days, where y is the analytical signal peak area and x the concentration of mercury in μg l^{-1}.
b. Values per assay.

Results and Discussion

Optimization of the manifold

The flow rates of the carrier solution, the reducing reagent, carrier gas and waste withdrawal and carrier concentration were adopted from Perkin Elmer FIAS 100 conditions.

The concentrations of sodium borohydride and sodium hydroxide in the reducing reagent and the length of the reaction coil were studied in order to maximize sensitivity. The influence of $NaBH_4$ concentration was studied from 0.001% to 0.2% m/v, and the best sensitivity was obtained for 0.2% m/v. When the NaOH concentration of the reducing reagent was varied from 0.05% to 0.5% m/v, no effect was observed. The length of reaction coil was studied in the range 60 – 30 cm and only a slight increase in sensitivity was observed. The selected conditions are shown in Table 1.

Characterization of the analytical procedure

The figures of merit of the developed system using the optimal conditions (Table 1) are shown in Table 2. The calibration curve was studied between 0.5 and 100 μg l^{-1} and linearity was maintained until 20 μg l^{-1}. We conclude that the sensitivities of the compared procedures are similar. The limit of detection (LOD) and the limit of quantification (LOQ) were calculated as $3\sigma_b/S$ and $10\sigma_b/S$, respectively; where σ_b is the blank standard deviation and S the sensitivity of the method calculated as the calibration slope. There was no significant difference between the results from the developed manifold and those from FIAS 100.

The relative standard deviations (RSD%) corresponding to ten replicates calculated at two different mercury levels (results in brackets) are also presented in Table 2. The values found were similar for the systems studied.

The waste production and the HCl consumption per assay can be reduced to about one-third and a half, respectively, in the developed system.

Analysis of fish samples

The proposed methodology was applied to the determination of mercury in fresh fish samples (shellfish, cod, skate, squid) that are commercially available. Mercury levels of the analyzed samples were very low; thus, to assess the accuracy of the developed method over a wider application range some samples were spiked. The results obtained in the analysis of the samples are presented in Table 3.

A linear relationship ($C_{MCFIA} = C_0 + SC_{FIA}$) was established, and the values for intercept (C_0), slope (S) and the correlation coefficient were 2.99 (±8.33), 0.975 (±0.058), and 0.996, respectively, where the values in parentheses are the limits of the 95% confidence intervals.[16] These figures demonstrate agreement between the developed and the reference procedures. A paired t-test also demonstrated that the results obtained by the developed MCFIA method were not different from those obtained by the comparison procedure at a 95% confidence level; the calculated $|t| = 0.012$ was lower than the critical t value ($p = 0.05$, $n = 12$) of 2.20.[16]

An analysis of a certified reference material NRC DORM-2 (dogfish muscle) from the National Research Council Canada was carried out to assess the accuracy of the developed method.

The results obtained for the reference material for total mercury agree with the certified range (Table 4).[16] The values presented in Table 4 show no statistical evidence that the results are different at 95% confidence level.

Two fresh fish samples with low mercury concentration were also spiked with known amounts of mercury. The obtained results are summarized in Table 5. The recovery percentages for the complete analytical procedure were between 90.7 and 99.8% for all spiked samples, which confirmed the validity of the developed method.[17]

Conclusion

The developed multicommuted flow system has proved to be simple, rapid and accurate for the determination of mercury in fish samples. One of the main advantages of using a multicommuted flow injection system for mercury determination by CVAAS concerns the reduction of waste

Table 3 Comparison of the results obtained for the cold vapor AAS mercury determination in fish samples by the developed multicommuted flow injection analysis and by the reference FIAS systems

Sample	Reference method (FIAS)[a]/μg kg^{-1} fresh mass	Developed method (MCFIA)[a]/μg kg^{-1} fresh mass	Relative deviation RD, %
Shellfish	9.4 ± 0.3	9.8 ± 1.0	4.2
Shellfish	8.1 ± 0.9	9.2 ± 0.4	12.6
Cod	59.0 ± 2.2	60.6 ± 2.4	2.8
Cod	55.2 ± 1.0	57.4 ± 3.8	3.9
Prawn	278.6 ± 9.0	264.3 ± 4.2	–5.1
Skate	156.8 ± 1.0	152.6 ± 6.8	–2.7
Skate	158.0 ± 0.7	157.4 ± 1.5	–0.4
Cod[b]	141.4 ± 5.3	133.4 ± 10.8	–5.6
Cod[b]	204.3 ± 1.5	213.1 ± 0.4	4.3
Shellfish[b]	101.8 ± 1.9	109.5 ± 3.7	7.6
Shellfish[b]	176.3 ± 1.0	185.6 ± 2.2	5.2
Squid[b]	99.0 ± 3.0	94.9 ± 1.7	–4.1

a. Mean and standard deviation of 3 replicates.
b. Spiked samples.

Table 4 Results for certified reference material found for the CVAAS mercury determination using the developed multicommuted flow system

	Certified value/mg kg^{-1} dry mass	MCFIA/mg kg^{-1} dry mass[a]
Dogfish (DORM-2)	4.64 ± 0.26	4.37 ± 0.13

a. Results are average value ± standard deviation of the results obtained on 2 working days with 3 replicates analyzed each day (n = 6).

Table 5 Results for the recovery study found for the CVAAS mercury determination using the developed multicommuted flow system

Sample	Without spike/ μg kg^{-1} fresh mass	Spiked/ ng	MCFIA Recovery, %[a]
Cod	57	200	98.6 ± 0.8
		500	99.8 ± 0.3
		1000	96.9 ± 2.5
Shellfish	≤ LOQ (9 μg kg^{-1} fresh mass)	200	90.7 ± 5.5
		500	94.1 ± 2.7
		1000	95.4 ± 1.2

a. Mean and standard deviation of 3 replicates.

production and reagent consumption, which is a contribution to a greener chemistry. Another advantage is that the dilution of the samples can be easily adjusted by changing the sample volume, *i.e*. the injection time. The methodology proposed in this paper can be extended to the AAS determination of other hydride-forming analytes in fish samples.

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