Fresenius J Anal Chem (2000) 368 : 475–479

ORIGINAL PAPER

William E. Doering · Ryan R. James · Roger T. Echols

A sequential injection cold-vapor atomic absorption method for the determination of total mercury

Received: 25 February 2000 / Revised: 17 May 2000 / Accepted: 26 May 2000

Abstract A sequential injection (SI) method for the determination of mercury via cold vapor atomic absorption spectrophotometry is presented. The method differs from flow injection (FI) cold vapor methods for the determination of mercury because of the simplicity of the system required for the method: one pump, one valve, a gas-liquid separator, and an atomic absorption spectrophotometer equipped with a quartz cell. Under optimal conditions, the method has the following figures of merit: a linear calibration range of 1.0 to 20 μg L^{-1}; a detection limit of 0.46 μg L^{-1}; and a precision of 0.90% RSD (8 μg L^{-1}). The procedure allows for a sampling rate of one injection per 80 s (excluding sample pretreatment). Results from the determination of mercury in water and fish specimens are also presented. The figures of merit of the method are compared to two other SI methods for the determination of mercury.

Introduction

Cold-vapor atomic absorption spectroscopy (CVAAS) has become the most widely used method for determination of mercury in environmental and biological specimens because of advantages in sensitivity, selectivity and convenience over other methods [1–4]. Flow injection (FI) methodology has been used in conjunction with CVAAS because FI methods are easily automated and amenable to performing chemistry on-line. Advantages include the ability to precisely control sample and reagent volumes, the coordination of the movement of pumps and valves and a convenient means by which the analyte (mercury) can be separated from the matrix [4]. Moreover, FI methods maintain the sensitivity and selectivity of the cold-vapor technique while providing a high sample throughput and full automation. Recent papers have reported achieving detection limits of 0.1 μg L^{-1} for linear calibration ranges of 0.2 to 20 μg L^{-1} [5, 6].

In the standard FI cold vapor method for the determination of mercury, the analyte is injected into an acidic carrier stream, which is merged with a carrier stream containing tin(II) chloride or sodium borohydride. The reduced mercury enters a short mixing coil and then a gas-liquid separator where the mercury is stripped from solution by argon [2, 7]. Flow rates of 4.0 and 8.0 mL min^{-1} for the sample and reductant carrier streams have been reported [7]. The FI cold vapor method for mercury has been successfully implemented in a variety of systems [5–11] and instruments for FI mercury analysis have been on the market for a number of years. However, the FI method is difficult to run with a basic FI system (one peristaltic pump and one valve). Multiple flow lines on a single pump can be used if each flow line has its own cartridge, but it is difficult in practice to precisely control flow rates of 4–8 mL min^{-1} with this arrangement.

The impetus for this research came from an attempt to set up a FI CVAAS method based on papers in the literature without extensive equipment or a dedicated FI system (e.g., a Perkin-Elmer FIAS-200 system). It was difficult to reproduce results from the literature without expensive hardware (i.e., multiple pumps and valves). Sequential injection (SI) methodology was applied to solve this problem. Sequential injection analysis was introduced in 1990 [12] as a way to reduce the complexity of FI systems and simplify the steps involved in an on-line experiment. Thus, the objective of this work was to develop a SI method for mercury that requires less hardware than the FI methods for mercury (i.e., one peristaltic pump) while maintaining the advantages of the FI cold-vapor method. The SI system was studied and the SI method that was developed was applied to three specimens – natural water, tap water and commercially canned tuna. The validity of the method was confirmed by the analysis of dogfish muscle certified reference material [13].

W. E. Doering · R. R. James
Division of Science and Mathematics,
University of Minnesota Morris, Morris MN 56267, USA

R. T. Echols (✉)
Department of Civil & Environmental Engineering,
University of Michigan, Ann Arbor, Michigan 48109, USA

Three SI methods for mercury have been published in recent years. Brindle and Zheng [14] employed a SI system in a comparative study of gas-liquid separators. Bauza de Mirabo *et al.* [15] optimized and validated (with solid specimens) a system that employs two syringe pumps and an autosampler. Mercury and tin(II) chloride were mixed in a short piece of flow tubing and were stripped by nitrogen that was introduced at a cylindrical gas-liquid separator (GLS). In the most recent work, Ma *et al.* [16] developed and optimized a SI system that employed two pumps (syringe and peristaltic) and a commercial GLS (with membrane filter) of the type often used in FI methods [14]. The mercury sample and reductant (sodium borohydride) were separated by air bubbles; argon was introduced immediately before the sample entered the GLS.

Experimental

Apparatus

A simple SI system was used to perform all experiments (Fig. 1). Peaks were obtained by measuring absorbance (at 253.7 nm) over time using a Perkin-Elmer 3110 Atomic Absorption Spectrophotometer (Norwich, CT, USA). The spectrophotometer was equipped with a Perkin-Elmer EDL System2 mercury lamp. The SI system was composed of the following components: standard flow tubing (0.76 mm i.d.) and connectors (Global FIA, Gig Harbor, WA, USA), peristaltic pump (Alitea, Medina, WA, USA), multi-position (10-port) valve (C25Z, Valco, Houston, TX, USA), a standard quartz cell for cold vapor work (Perkin Elmer, Norwich, CT, USA) and gas-liquid separator (GLS). Typical dimensions used in mixing coils are noted in Fig. 1. The peristaltic pump was typically operated at a flow rate of 2.25 mL min^{-1}, and the argon carrier gas (Boc Gases, Murray Hill, NJ, USA) was typically operated at 130 mL min^{-1}. Manipulation of the pump and valve and data acquisition were controlled by FlowTEK software (version 1.2) distributed by Global FIA using a microcomputer (DEC Celebris 366).

A modified plastic tee with a 6 mm o.d. (Fisher, Hampton, NH, USA; catalog no. 15-315-26B) was used as the gas-liquid separator (GLS). The tee was oriented (as shown in Fig. 1) such that liquid would enter and fall by gravity and gas would flow to the quartz cell. The section of the tee into which liquid and gas entered from the SI system was cut such that the dimensions of the tee were 55 mm x 18.5 mm; a reducing union (Global FIA) provided the connection between the standard FI connector and the GLS. Large diameter (~4 mm i.d.) rubber hose (Fisher) was used as the transport tubing between the GLS to quartz cell and as the waste line tubing. Gas flow rate through the GLS was measured by connecting the transfer tubing to a soap bubble flow meter.

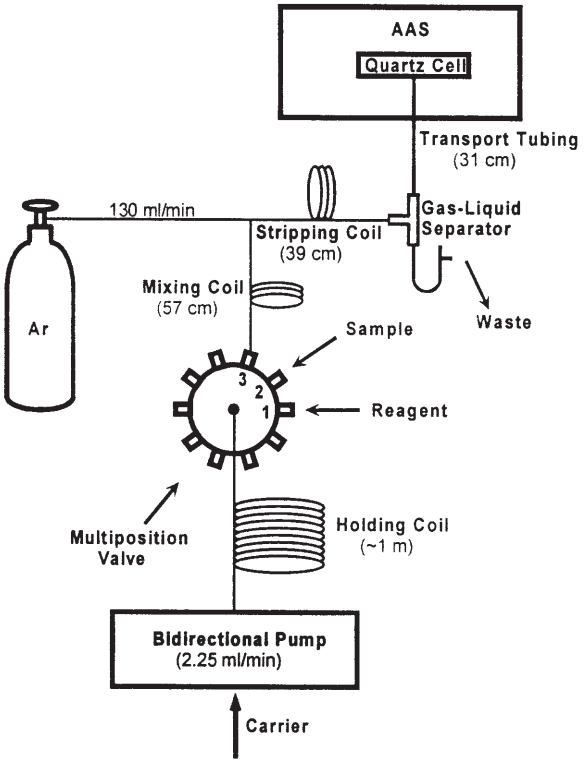

Fig. 1 Schematic of the sequential injection system. Carrier stream is 1.0 M HCl. Reagent stream is 1.1 (m/v) % $SnCl_2$ in 1.0 M HCl. Transport tubing is rubber hose with ~4 mm i.d. All other tubing is 0.76 mm i.d

Reagents

All solutions were prepared using deionized water. Sample preparation and the chemistry performed in the SI system required the following reagents: tin(II) chloride (Aldrich, Milwaukee, WI, USA), potassium permanganate (Aldrich), Tracemetal grade nitric and sulfuric acids (Fisher, Hampton, NH, USA), hydrochloric acid (Fisher), potassium persulfate (Mallinckrodt, St. Louis, MO, USA), sodium chloride (Mallinckrodt), hydroxylamine hydrochloride (Baker, Houston, TX, USA), and mercuric chloride (Fisher). The reagents listed above were approved by the manufacturer for the determination of mercury. Water specimens were obtained from local sources (tap and surface of local lake) and fish specimens were obtained commercially and from the National Research Council of Canada (DORM-2, Ottawa, Ontario Canada) [13]. The tuna specimen was freeze-dried before analysis.

Procedures

Four lines on the multiposition valve were employed in the experiment. The center port was connected to the holding coil and positions 1, 2 and 3 (Fig. 1) were connected to the tin(II) chloride ($SnCl_2$) solution, the sample solution, and the GLS and the spectrophotometer, respectively. Prior to running the method, the 1.0 M HCl carrier stream was aspirated through the holding coil, mixing coil, and stripping coil. Sample and reagent were loaded by reverse action of the pump from valve positions 1 and 2 (Table 1). Two volumes of reagent were aspirated (one in front and one behind the sample) to form a stacked zone of reagent/sample/reagent. Volumes of each solution and pump times for each step are dis-

Table 1 Optimized method used in SI method. Prior to experiments, the entire SI system is flushed with carrier solution. Valve position 1 is connected to the tin chloride solution; valve position 2 is connected to the sample; and valve position 3 is connected to the rest of the SI system (including the GLS and spectrophotometer)

Step	Task	Valve Position	Pump Direction	Time/s	Volume/ mL
A	Load $SnCl_2$ Reagent	1	Reverse	5	188
B	Load Hg Sample	2	Reverse	8	300
C	Load $SnCl_2$ Reagent	1	Reverse	5	188
D	Propel Sample to GLS	3	Forward	52	1950

played in Table 1. After reaction, the valve was actuated to position 3, and the pump was activated to propel the stream to the GLS. When the aqueous solution merged with the argon at the junction of the mixing and stripping coils, the reduced mercury was stripped from solution. At the GLS, the mercury-laden argon was swept to the quartz cell, while the aqueous solution fell to waste under the influence of gravity. A simple U-shaped trap was connected to the bottom of the GLS to prevent any argon or elemental mercury from escaping. The pump was allowed to run well after the necessary data acquisition in order to wash the flow lines. The entire sampling method and washing was complete in 80 s.

Sample digestion of tissue and water specimens was based on established methods [7, 17], which require an acid digestion followed by oxidation by permanganate and persulfate and removal of oxidants by hydroxylamine. The only notable difference in the established methods and the method employed in this work was the proportion of acid utilized for digestion: 4 mL of nitric acid and 1 mL of sulfuric acid were added to approximately 0.2 g of solid specimen or 2 mL of aqueous specimen. It was found that if more sulfuric acid was used, an increase in surface tension led to the formation of bubbles in the GLS and condensation in the quartz cell during the experiment. The digested specimens were diluted by an appropriate factor for analysis. The method was validated by comparison of 95% confidence interval with certified values from the National Research Council of Canada (DORM-2).

Results and discussion

Sequential injection system

Sample volume, mixing coil length, and stripping coil length of the SI system were independently optimized of other variables over a range of values typical for SI experiments using 8 μg L^{-1} Hg^{2+}. Other experimental parameters were chosen on the basis of preliminary work or literature values. A 1.0 M HCl acid carrier stream and a 1.1 (m/v)% $SnCl_2$ reductant solution (in 1.0 M HCl) were used on the basis of previous work [4, 11]. The volume of $SnCl_2$ reagent was found to have little impact on the reaction because of a stoichiometric excess of $SnCl_2$ over mercury. The reductant volume was set in excess of the sample volume and was split in order to place the sample between two volumes of reductant. The length of the transfer tubing (GLS to the quartz cell) was minimized in order to prevent unnecessary dilution. A commercially available glass-bead gas-liquid separator (Perkin-Elmer), which has been used for many years in FI systems [14], was initially used as the GLS. The commercial GLS required that the waste be continually removed by a pump, which was contrary to the goal of creating a simple system with one pump and led to poor precision. The need to have the waste solution fall (under gravity) through a U-tube prompted the use of the simple plastic tee as the GLS.

The sample volume of 300 μL was chosen as a compromise between analytical response and volume of waste per experiment. Mixing coil length (57 cm) and stripping coil length (39 cm) were chosen in a region where the response of the system was optimal and relatively insensitive to changes (no more than a 10% change) in coil lengths. Shorter mixing coils led to poor precision and lower sensitivity because of incomplete reduction of Hg^{2+}. Long stripping coils led to greater dispersion and lower sensitivity, although changes were not substantial because of the presence of the argon gas bubbles. Short stripping coils were advantageous in increasing sampling frequency. The trend toward using short stripping coils has been confirmed in the results recently published by Bauza de Mirabo *et al.* [15] (stripping gas added at the GLS) and by Ma *et al.* [16] (stripping gas added 1 cm upstream from GLS).

Quantitative results

Results obtained for the determination of mercury in various specimens are listed in Table 2. Each water or fish specimen was subdivided and independently digested to provide three or four replicate samples. Each sample was injected into the SI system four times to determine the concentration of mercury. The standard deviation is reported as the deviation of the means for the independent samples. Therefore, the standard deviation not only takes into account the instrumental variations, but those caused by random error in pretreatment of samples. Representative peaks are shown in Fig. 2.

Table 2 Results from the determination of mercury in fish and water specimens. Concentrations, 95% confidence intervals and standard deviations are reported as ppm (mg kg^{-1}) for tuna specimens and ppb (μg L^{-1}) for aqueous specimens. The certified value for DORM-2 (dogfish tuna) is 4.64 ± 0.26 mg kg^{-1} [13]

Sample	Concentration	Standard Deviation	Rel. Standard Deviation, %	*n*
Canned tuna	0.89 ± 0.21	0.13	15	4
DORM-2 (tuna)	4.64 ± 0.16	0.10	2.2	4
Lake water	36.9 ± 2.7	1.1	3.0	3
Tap water	12.4 ± 1.7	1.1	8.9	4

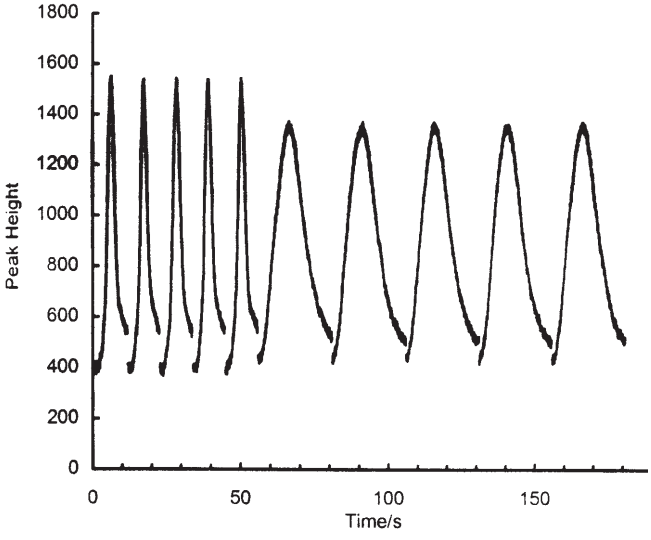

Fig. 2 Representative peaks produced by the analysis of 8 μg L^{-1} Hg^{2+} standard solution. The first five peaks represent those obtained via the "air bubble" method, and the next 5 peaks correspond to those obtained through the typical SI method used in the analysis of tuna specimens

To the 95% level of confidence, the mercury concentration in the commercially canned tuna specimen is 0.89 ± 0.21 mg kg^{-1}. These results are similar to previously reported values of 0.92 ± 0.17 and 1.00 ± 0.16 mg kg^{-1} [8]. Validation of the method was achieved by determination of Hg in the certified reference material (DORM-2) [13]. The experimental result of 4.64 ± 0.16 mg kg^{-1} is statistically equivalent to the certified mercury level of 4.64 ± 0.26 mg kg^{-1}.

The concentration of mercury in the tap water specimen is high, at 12.4 ± 1.7 μg L^{-1}. The EPA recommends inorganic mercury levels less than 2 μg L^{-1} in drinking water [18]. The 36.9 ± 2.7 μg L^{-1} mercury level found in the environmental water specimen is also high, which has been attributed to the large amount of sediment containing organomercury species in the water. Both water specimens were near the detection limit after digestion and dilution, which led to decreased precision versus the tissue specimens. However, even at low mercury concentrations, the RSD between samples is less than nine percent.

Figures of merit and comparison of SI methods

A calibration of the system was performed with standard solutions of Hg^{2+}. The calibration curve had a linear regression given by the equation $y = 0.14x + 0.074$, ($r^2 = 0.998$; y = peak height in arbitrary units; x = concentration in μg L^{-1}). Based on three times the standard deviation of a blank, the detection limit for the SI method was 0.46 μg L^{-1}. An RSD of 0.90% was obtained from 5 injections of 8 μg L^{-1} Hg^{2+} standard solution. These figures of merit and other important experimental parameters of the proposed SI method are summarized in Table 3, along with the same information from the papers by Bauza de Mirabo *et al.* [15] and Ma *et al.* [16].

Although the three SI methods are similar in many respects (e.g., the chemistry of the system and the use of atomic spectrophotometric detection), the three SI systems are different in a number of ways: the number of valves and pumps in the SI system, the place in the system where the stripping gas is introduced, the types of GLS employed, the reductant employed and the order in which reagent and sample are aspirated. Despite these differences, the figures of merit shown in Table 3 do not vary greatly. The differences are often a result of features that were not optimized for that particular method. For example, the method presented in this paper, the reductant and acid concentrations for this work were not optimized to simplify method development.

Table 3 Figures of merit and experimental parameters for the proposed method for mercury in comparison to SI methods developed by Bauza de Mirabo *et al.* [15], Ma *et al.* [16]

	Bauza de Mirabo *et al.* [15]	Ma *et al.* [16]	This Work
Working range / μg L^{-1}	2–50	0–20	1–20
RSD/%	0.95	2.0	0.9
Detection limit/μg L^{-1}	0.34	0.10	0.46
Throughput/h^{-1}	30	90	45
Sample volume/μL	800	400	300
Reagent volume/μL	100	30	376
Acid concentration/M	0.60	0.05	1.0
Reductant	$SnCl_2$	$NaBH_4$	$SnCl_2$

Three figures of merit from the method developed by Ma *et al.* [16] stand-out as compared to the method developed by Bauza de Mirabo *et al.* [15] and the method presented in this paper: (1) The detection limit (0.1 μg L^{-1}) is slightly lower than those of the other two methods and is close to the detection limit of 0.06 μg mL^{-1} achieved by some FI methods [4]; (2) the throughput (or sampling frequency) of 90 samples per hour reported by Ma *et al.* [16] is similar to the throughput of many FI methods; and (3) the volume of reductant and concentration of acid are lower than those used in the other two methods. However, the method presented by Bauza de Mirabo *et al.* [15] and the method presented in this work are more precise as measured by the RSD and were validated by certified digests of biological samples.

Conclusions

All three methods discussed above demonstrate that SI methodology offers some advantages over FI methodology for determination of mercury by atomic absorption spectrometry – the reduced consumption of reagents, the reduced generation of waste and the simplicity of required hardware. The SI method proposed in this work requires 300 μL of sample, less than 400 μL of reagent and no more than 2.0 mL of carrier solution per injection. A recent FI experiment [6] used over 4 mL of sample and carrier along with 2.5 mL of reagent per injection. The SI system presented in this work demonstrates that a standard SI system with one peristaltic pump and one valve is sufficient for the method. Moreover, a simple tee-piece GLS has been demonstrated to be suitable for the SI system (which eliminates the need for the second pump). Having only one inexpensive pump in the system is in keeping with the initial philosophy of SI systems, namely, that a basic system can be reconfigured for a variety of analyses without additional hardware [12]. The similarities in the figures of merit shown in Table 3 underscore the fact that the SI method for mercury can be effectively implemented regardless of the differences in hardware and methodology.

The interesting means by which the sample and reagent were separated by Ma *et al.* [16] was also investigated: the separation of fluids in the flow tubing by an air bubble. While Ma *et al.* [16] used an air bubble between the sample and reagent to prevent reaction prior to the GLS, the air bubbles in this work were employed to keep the sample from dispersing into the carrier stream from one direction. Under this scenario, the reducing agent was used as the carrier stream and a small volume of air was aspirated prior to the sample. The presence of the air bub-

ble "behind" the sample and reagent prevented dispersion in the flow tubing in one direction and resulted in larger peak heights. Sample peaks for this alternative method are shown in Fig. 2 along with peaks from the regular method. The "sharper" peaks resulted in an improvement in sensitivity of 20% versus a method with no air bubble. In addition, the analysis time was decreased to 60 s. These preliminary results using an air bubble to increase sensitivity and reduce sample and reagent consumption form the basis for future work.

Acknowledgements The donation of instrumentation from the Perkin-Elmer Corporation to the University of Minnesota Morris (UMM) and funding and support from the Division of Science and Mathematics and UMM undergraduate research programs are gratefully acknowledged. The contributions of Erik Mottl and Charlie Kavaloski are also gratefully acknowledged.

References

1. Clevenger WL, Smith BW, Winefordner JD (1997) Crit Rev in Anal Chem 27:1
2. Fang Z-L (1995) Flow Injection Atomic Absorption Spectrometry, Wiley, New York
3. Navarro M, López MC, López H, Sánchez M (1992) Anal Chim Acta 257:155
4. de Vargas MC, Romero RA (1992) Analyst 117:645
5. Gallignani M, Bahsas H, Brunetto MR, Burguera M, Burguera JL, de Peña YP (1998) Anal Chim Acta 369:57
6. Tao G, Willie SN, Sturgeon RE (1998) Analyst 123:1215
7. McIntosh S, Welz B (1992) Perkin-Elmer Method 245.1A: Determination of Mercury in Drinking Water and Wastewaster by Flow Injection Atomic Absorption Spectrometry, The Perkin-Elmer Corporation, Norwich CT
8. Murphy J, Jones P, Hill SJ (1996) Spectrochim Acta Part B 51:1867
9. Hanna CP, Tyson JF, McIntosh S (1993) Anal Chem 65:653
10. Guo T, Baasner J, Gradl M, Kistner A (1996) Anal Chim Acta 320:171
11. de Andrade JC, Bueno MIMS (1994) Spectrochim Acta Part *B* 49:787
12. Ruzicka J, Marshall GD (1990) Anal Chim Acta 237:329
13. DORM-2 dogfish muscle: Obtained from Institute for National Measurement Standards, National Research Council of Canada, Montreal Road, Bldg M-12, Ottawa, Ontario Canada K1A 0R6
14. Brindle ID, Zheng S (1996) Spectrochim Acta Part B 51:1777
15. de Mirabo FMB, Thomas AC, Rubi E, Forteza R, Cerdà V (1997) Anal Chim Acta 355:203
16. Ma H-B, Fang Z-L, Wu J-F, Liu S-S (1999) Talanta 49:125
17. Lobring LB, Potter BB (1991) EPA Method 245.6: Determination of Mercury in Tissues by Cold Vapor Atomic Absorption Spectrometry (Revision 2.3), U.S. Environmental Protection Agency, Cincinnati
18. Water on Tap: A Consumer's Guide to the Nation's Drinking Water (EPA Document number 815-K-97-002) (1997) U. S. Environmental Protection Agency, Washington DC