Available online at www.sciencedirect.com
SCIENCE DIRECT®

Talanta 64 (2004) 1053–1057

Talanta

www.elsevier.com/locate/talanta

Development of a sequential injection system for trace mercury determination by cold vapour atomic absorption spectrometry utilizing an integrated gas–liquid separator/reactor

Aristidis N. Anthemidis*, George A. Zachariadis, John A. Stratis

Laboratory of Analytical Chemistry, Department of Chemistry, Aristotle University, Thessaloniki 54124, Greece

Received 10 February 2004; received in revised form 29 March 2004; accepted 7 May 2004
Available online 19 June 2004

Abstract

A simple and robust time-based on-line sequential injection system for trace mercury determination via cold vapour atomic absorption spectrometry (CVAAS), employing a new integrated gas–liquid separator (GLS), which in parallel operates as reactor, was developed. Sample and reductant are sequentially loaded into the GLS while an argon flow delivers the released mercury vapour through the atomic absorption cell. The proposed method is characterized by the ability of successfully managing variable sample volume up to 30 ml in order to achieve high sensitivity. For 20 ml sample volume, the sampling frequency is 25 h^{-1}. The calibration curve is linear over the concentration range 0.05–5.0 $\mu g\,l^{-1}$ of Hg(II), the detection limit is $c_L = 0.02\ \mu g\,l^{-1}$, and the relative standard deviation is $s_r = 2.6\%$ at 1.0 $\mu g\,l^{-1}$ Hg(II) level. The performance of the proposed method was evaluated by analyzing certified reference material and applied to the analysis of natural waters and biological samples.
© 2004 Elsevier B.V. All rights reserved.

Keywords: Mercury; Gas–liquid separator; Cold vapour atomic absorption spectrometry; Sequential injection

1. Introduction

Undoubtedly, cold vapour atomic absorption spectrometry (CVAAS) is the most widely accepted and utilized technique for routine mercury determination in trace concentration levels. For total mercury determination a decomposition procedure for organomercury species is necessary [1]. It is well known that manual methods, which involve a considerable number of reagents and repetitive steps, may lead to operator errors particularly when large number of batches has to be analyzed. Continuous flow (CF) [2] or flow injection (FI) [3–5] and recently sequential injection (SI) [6–8] systems can overcome the above drawbacks because they offer automated handling of sample, improved precision, drastic diminishing of reagents and sample consumption and increased sampling frequency [9].

Although mercury in metallic form has a considerable vapour pressure (0.0016 mbar at 20 °C corresponding to a concentration of ca. 14 mg m^{-3} in air) there is always a need for its efficient separation from the liquid phase in order to ensure that the analyte is transferred to the atomic absorption cell (AAC) and consequently to improve the sensitivity. This is achieved by the gas–liquid separator (GLS) component. Generally there are three approaches for the separation of the gaseous phase: (a) hydrostatic (U-type), (b) with forced outlet, (c) microporous poly-tetrafluoroethylene (PTFE) membrane [10]. In batch CVAAS methods, the GLS is employed also as reaction vessel for mercury reduction [11,12]. On the other hand, various GLS configurations for on-line systems have been presented in the literature, in order to increase peak height of the transient signal [13–16].

In the present work, a simple, rapid, precise and accurate sequential injection system, with high reliability in a wide range of sample volume (5–30 ml) for inorganic mercury determination by CVAAS was developed. For this purpose, a newly on-line gas–liquid separator which functions also in the same time as a reaction chamber was manufactured and

---

* Corresponding author. Tel.: +30 2310 997707;
fax: +30 2310 997719.
E-mail address: anthemid@chem.auth.gr (A.N. Anthemidis).

0039-9140/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2004.05.003

its performance elaborated upon. The proposed manifold was tested by the analysis of certified reference material and was applied to mercury determination in natural waters and biological samples.

2. Experimental

2.1. Reagents

Working standard solutions of Hg(II) were prepared by appropriate stepwise dilution of a 1000 mg l^{-1} Hg(II) in 0.5 mol l^{-1} HNO_3 stock standard solution (Titrisol, Merck) to the required μg l^{-1} levels just before use. The reductant solution, 2.5% *m*/*v* $SnCl_2$ in 0.6 mol l^{-1} HCl was freshly prepared (a precipitate is formed after few days) from $SnCl_2{\cdot}2H_2O$ (Merck, <0.000001% mmHg). The $SnCl_2$ solution was purified from possible traces of elemental mercury by 30 min degassing with argon prior to use. Doubly de-ionized water was used throughout.

2.2. Instrumentation

A Zeiss PMQ3-MQ3 model UV/VIS spectrophotometer was used as detector, a Perkin Elmer mercury electrode less discharge lamp was used as an intense line source at 253.7 nm, and a laboratory made quartz flow through atomic absorption cell (30 cm length, 8 mm i.d. with quartz windows), heated to ca. 60 °C by an IR lamp in order to prevent moisture condensation. The sequential injection cold vapour generation system and its operation are shown schematically in Fig. 1a. It was consisted of two peristaltic pumps (Watson Marlow model 205U/BA, and Gilson Minipuls-3), two six-port two-position injection valves (Labpro, Reodyne, USA) and a multi-position selection valve (Valco, C25Z). The whole system was controlled by personal computer via LabVIEW 5.1.1 instrument software (National Instrument). The two positions “A” and “B” of the injection valves are presented schematically in Fig. 1a and b, respectively. The central port of multi-position selection valve (SV) was connected to the gas–liquid separator using a PTFE tubing (10 cm length, 1.0 mm i.d.), while the positions 1 and 2 were connected to the reductant and sample solution, respectively. A 20 cm length of PTFE tubing (1.0 mm i.d.) was used for GLS and AAC connection, in order to keep the dead volume of the manifold at minimum level and thus the vapour dispersion at lower level. Argon gas (Ar) was used for the mercury vapour release from liquid mixture. A steel pressurized bomb (Berghof GmbH, Germany), equipped with closed Teflon vessels, was used for the acid digestion of the reference material.

2.3. Gas–liquid separator

The proposed GLS, (Fig. 1c), is characterized by simplicity and small dead volume, facilitating both the reduction of mercury ions as well as the mercury vapour separation operating both as reaction vessel and gas–liquid separator with the minimum vapour dispersion. The separation chamber was constructed of a cylindrical glass tube (100 mm length, 26 mm i.d.) and the push-fit connections at the two ends were made of poly-tetrafluoroethylene. The above connections have internal conical cavities as they are illustrated in Fig. 1c, for effective separation and transportation of the released Hg^0, and increased reproducibility due to the fact that purge gas does not carry liquid droplets into the AAC. The upper connection is a “Tee” type confluence connector with one 1.0 mm i.d. vertical and one 0.5 mm i.d. horizontal channel for the gas mixture (Hg^0 + Ar) outlet and Ar inlet (U), respectively. The lower connection has three parallel inlets,

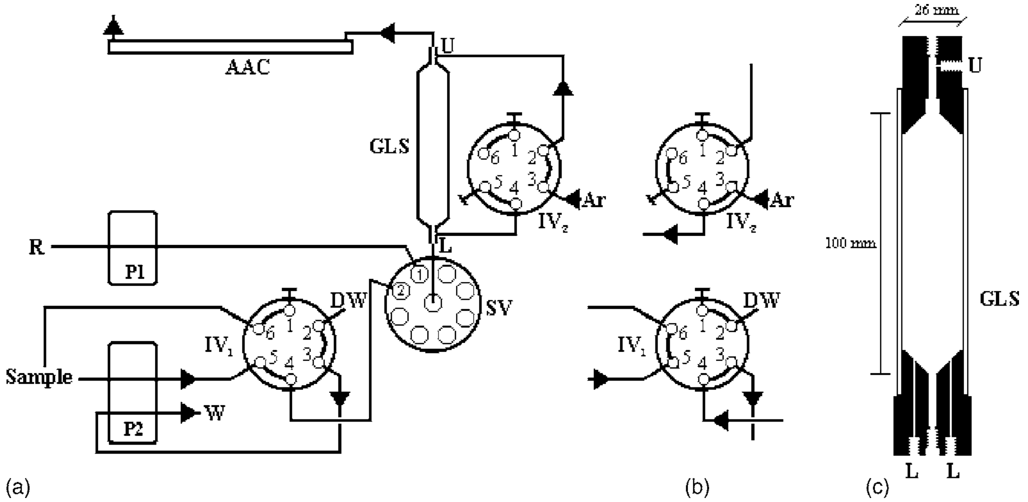

Fig. 1. (a) Optimized manifold (step 2) for mercury determination; R, reductant solution 2.5% *m*/*v* $SnCl_2$; DW, de-ionized water; Ar, argon purge gas 0.20 l min^{-1}; W, waste; P1, P2, peristaltic pumps; IV_1, IV_2, injection valves in “A” position; SV, selection valve; GLS, gas–liquid separator; AAC, atomic absorption flow through cell; U, L, upper and lower inlets of Ar. (b) IV_1, IV_2, injection valves in “B” position. (c) Schematic diagram of the gas–liquid separator. More details in the text.

Table 1
Operating sequences of the proposed method for mercury determination in 20 ml sample volume (for manifold details, see Fig. 1)

Step	Time (s)	SV	Position of		P1	P2	Delivered medium	Flow rate (ml min^{-1})	Operation
			IV_1	IV_2					
1	10	1	A	A	ON	OFF	$SnCl_2$	6.0	Reductant loading
2	60	2	A	A	OFF	ON	Sample	20.0	Sample loading and reduction
3	10	2	A	B	OFF	OFF	Ar	200	Hg^0 vapour separation, transportation and measurement
4	65	2	B	A	OFF	ON	Waste	25.0	GLS evacuation

one 0.8-mm i.d. channel for liquid phase entrance and two 0.3-mm i.d. channels for purge gas (L).

Another advantage of the proposed manifold arises from the combination of the GLS with an injection valve, IV_2. In step 2, IV_2 is in “A” position as it shown in Fig. 1a and the argon flow bypasses the GLS, resulting in that way a positive pressure at point “U”. This pressure traps within the upper region of the GLS the generated mercury vapour, eliminating the analyte dispersion of the vapour, and thus the peak height absorbance is increased. The above GLS can successfully manage a wide range of sample volume (5–30 ml) for varied sensitivity while its performance was stable during all experiments.

2.4. Procedure

The optimized sequences of operation are presented in Table 1.

The use of a time-based injection mode enables the metering of the sample volume as a function of time and is a very advantageous way for sample introduction. Reductant solution and sample were loaded sequentially to the GLS through SV positions 1 and 2, by activating pumps P1 and P2, for appropriate loading time during steps 1 and 2, respectively. Preferably, the reductant solution is loaded prior to the sample in order to assure better mixing and sufficient reduction time. In step 3, mercury vapour (Hg^0) is stripped from the resulting solution and delivered to the AAC by actuating the IV_2 to “B” position (Fig. 1b). In this step, Ar passes through GLS resulting to a flash release and transportation of mercury vapour, which in all other steps bypasses the GLS, purging the AAC. During step 4, IV_1 is actuating in “B” position and the solution from the GLS chamber is evacuated to waste by activating pump P2. The peak height of the absorbance signal was proportional to inorganic mercury concentration in the sample, and was used for all measurements. Five replicates were made in all instances.

3. Results and discussion

Generally, SI manifolds are characterized by simplicity with a single reaction line [6,17]. However, the performance of those systems is no more valid when the reactions involve gas evolution such as vapour or hydride generation [7]. In these cases, the introduction of small air segment between the sample and reagent zone during the aspiration stage, is a successful technique to prevent the reaction and thus the dispersion within the tubular reactor. Thus, the vapour generation initiates into the GLS, which functions also as reaction chamber. Based on these considerations, in sequential injection systems, it is more convenient and less time consuming, to load the sample and reagents directly into the GLS, especially when large volumes are used. Consequently, the liberated mercury vapour can be delivered with the lower dispersion into the absorption cell. In the proposed manifold, the sample is introduced after the reductant solution in order to start the reaction along with the sample loading.

3.1. Optimization of manifold parameters

The optimization of the manifold parameters (Fig 1a) was proceeded under the above procedure, using 3.0 μg l^{-1} Hg(II) standard solution and fixed reductant loading flow rate (6.0 ml min^{-1}). Sample volume affects significantly the sensitivity of the CVAAS systems, as it has been reported in the literature [7,11,13]. On the other hand, the successful manipulation of a large range of sample volumes is beneficial for a method improving its sensitivity. The effect of sample volume was studied in the range 5–30 ml via two different ways: (i) keeping the absolute mercury amount into the loaded sample volume constant at 15 ng Hg(II) (Fig. 2) and (ii) using a fixed concentration of 3 μg l^{-1} Hg(II) in

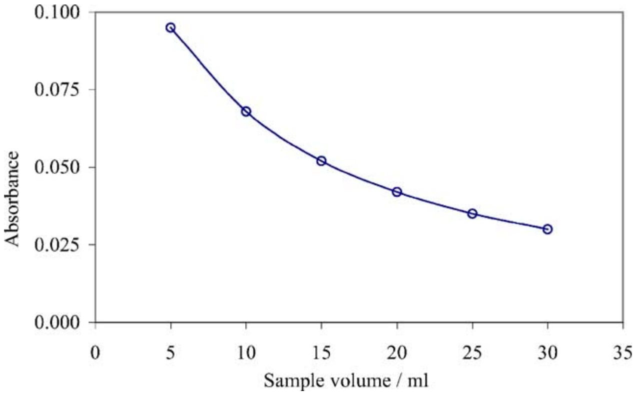

Fig. 2. Effect of sample volume on absorbance of an absolute amount of 15 ng Hg(II) in the GLS. All other parameters as in Table 1.

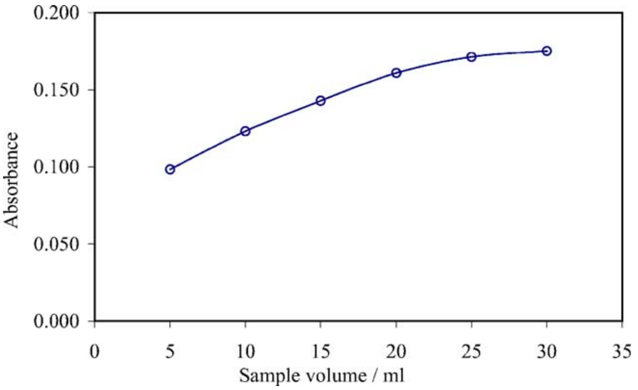

Fig. 3. Effect of sample volume of 3 μg l^{-1} Hg(II) on the absorbance of fixed concentration of sample solution. All other parameters as in Table 1.

the sample solution (Fig. 3). As it is shown in Fig. 2, by increasing the sample volume the absorbance of the fixed amount of Hg was decreased, shown that larger volumes make Hg^0 release more difficult. However, larger sample volumes offer increased overall sensitivity, as it is illustrated in Fig. 3. Therefore the proposed GLS can be used in any sample volume in the range 5–30 ml with proportional sensitivity. For all subsequent experiments, 20 ml sample volume at 20 ml min^{-1} sample loading flow rate was adopted as a compromise between sensitivity and sample consumption.

In vapour generation systems, purge gas flow rate affects the height and the width of the transient absorption signal. The Ar flow rate was studied in the range 50–500 ml min^{-1}. The peak height was increased by increasing the flow rate up to 200 ml min^{-1} and over this value it leveled off. In addition, by increasing the argon flow rate, the recorded signal was becoming narrower while the observed deterioration of reproducibility for values higher than 400 ml min^{-1} probably occurs from transported droplets in the AAC.

3.2. *Analytical performance of the method*

The performance data of the method for 10 and 20 ml sample consumption are presented in Table 2. The obtained detection limit could have been further improved by increasing the sample volume up to 30 ml. In Table 3, the figures of merit of the proposed method along with other published SI methods [6–8] are presented for comparative purpose.

Table 3
Figures of merit of the proposed and other reported SI-CVAAS methods

References	Sample consumption	Sampling frequency (h^{-1})	Detection limit (μg l^{-1})	R.S.D. (%)
[6]	800 μl	30	0.34	0.95
[7]	400 μl	90	0.10	2.0
[8]	300 μl	45	0.46	0.9
This work	10 ml	42	0.04	2.2
This work	20 ml	25	0.02	2.6

Table 4
Analytical results of mercury determination in natural waters and biological samples

Sample	Added Hg(II) (μg l^{-1})	Found[a] (μg l^{-1})	Recovery (%)
River-water	0 1.00	0.06 ± 0.02 1.04 ± 0.03	98
Seawater	0 1.00	$<c_L$ 1.02 ± 0.03	102
Urine	0 1.00	0.67 ± 0.05 1.64 ± 0.07	97
Mussel[b]	0 1.00	0.098 ± 0.009 1.048 ± 0.022	95
BCR CRM 278	0.188 ± 0.011[c]	0.185 ± 0.015	98

[a] Mean value ± standard deviation ($n = 5$).
[b] Added and found concentrations are expressed in μg g^{-1} fresh weight (FW).
[c] Certified concentration in μg g^{-1}.

The BCR CRM 278 reference material (Community Bureau of Reference, Trace elements in mussel tissue, *Mytilus Edulis*) was analysed in order to evaluate the accuracy of the proposed method. The reference material was dissolved by a wet-acid digestion procedure as it is described in Section 3.4. The certified concentration was 0.188 ± 0.011 μg g^{-1} ($n = 5$), the concentration found was 0.185 ± 0.015 μg g^{-1} ($n = 5$), thus the calculated recovery was 98.4% (Table 4).

3.3. *Interference studies*

An outstanding advantage of automated vapour generation methods is the improved tolerance to interferences [9]. The effect of potential interferents encountered in natural waters and biological samples on mercury determination was examined using 2.0 μg l^{-1} Hg(II) solution. The results showed that, Al(III), Co(II), Cu(II), Cr(III), Cr(VI), Fe(II), Fe(III), Mn(II), Pb(II) and Zn(II) are tolerated up

Table 2
Analytical performance of the method for inorganic mercury determination

Sample consumption (ml)	20	10
Sampling frequency (h^{-1})	25	42
Linear range (μg l^{-1})	0.05–5.0	0.1–7.0
Regression equation ([Hg] in μg l^{-1})	(0.0576 ± 0.0003) [Hg] + (0.0022 ± 0.0016)	(0.0388 ± 0.0004) [Hg] + (0.0024 ± 0.0014)
Correlation coefficient	$r = 0.9993$	$r = 0.9994$
Detection limit (3s) (μg l^{-1})	$c_L = 0.02$	$c_L = 0.04$
Precision (R.S.D., $n = 10$; 1.0 μg l^{-1}) (%)	$s_r = 2.6$	$s_r = 2.2$

to 10 mg l^{-1} (recovery variation less than three times the standard deviation). Other common matrix cations such as Ca(II), Mg(II), Ba(II) are tolerated up to 1000 mg l^{-1} and NaCl up to 35 g l^{-1}.

3.4. Analysis of aqueous and biological samples

The proposed method was applied to the analysis of aqueous samples seawater and river-water collected from Northern Greece which were filtered through 0.45 μm membrane filter, and acidified to ca. pH 2.5 with dilute HNO_3. Urine sample (250 ml of a healthy person) and a composite sample of mussel tissues (*Mytilus galloprovinciallis* from Thermaikos Gulf, Northern Greece) were also analyzed for total mercury determination. The above biological samples were spiked and digested at 80–90 °C using a mixture of HNO_3–H_2SO_4–H_2O_2 in closed Teflon beakers of the pressurized bomb. After cooling, the digests were diluted and the pH was adjusted to ca. 2.5. The diluted solutions were used for mercury determination. The obtained results are presented in Table 4. The recoveries in spiked solutions were varied in the range 95–102%.

4. Conclusions

The vapour generation atomic absorption system with a newly designed reactor/GLS has been evaluated and demonstrated for mercury determination. The advantages of the proposed GLS are: (i) it operates both as reaction vessel and gas–liquid separator resulting thus to minimum vapour dispersion, (ii) it facilitates the successful manipulation of large range of liquid phase affecting the sensitivity of the method, (iii) it is convenient for sequential injection systems, which make use of time-based technique for large sample volume loading (up to 30 ml). The obtained results proved that although larger sample volumes give rise to decrease of the absorbance, the sensitivity of the method is increased. The proposed manifold is capable for any sample volume in the range 5–30 ml with rational sensitivity depending on the sample volume used. The performance of the GLS was stable during all experiments, while the manifold is ideal for mercury vapour generation. However, to the best of our knowledge this is the first sequential injection manifold managing high sample volume instead of using preconcentration technique, in order to achieve higher sensitivity. Although the system was studied for cold vapour, it could be applied to the determination of other hydride generation forming elements.

References

[1] B. Welz, M. Sperling, Atomic Absorption Spectrometry, Wiley-VCH, New York, 1999.
[2] A. Menendez-Garcia, M.L. Fernandez Sanchez, J.E. Sanchez Uria, A. Sanz-Medel, Mikrochim. Acta 122 (1996) 157.
[3] T. Guo, J. Baasner, Anal. Chim. Acta 278 (1993) 189.
[4] O. Wurl, O. Elsholz, R. Ebinghaus, Talanta 52 (2000) 51.
[5] S.R. Segade, J.F. Tyson, Spectrochim. Acta Part B 58 (2003) 797.
[6] F.M. Bauza de Mirabo, A.Ch. Thomas, E. Rubi, R. Fortza, V. Cerda, Anal. Chim. Acta 355 (1997) 203.
[7] H-B. Ma, Z-L Fang, J-F. Wu, S-S. Liu, Talanta 49 (1999) 125.
[8] W.E. Doering, R.R. James, R.T. Echols, Fresenius J. Anal. Chem. 368 (2000) 475.
[9] Z. Fang, Flow Injection Atomic Absorption Spectrometry, Wiley & Sons Ltd., West Sussex, England, 1995.
[10] A. Sanz-Medel, Flow Analysis With Atomic Spectrometric Detectors, vol. 9, Analytical Spectroscopy Library, Elsevier, Amsterdam, The Netherlands, 1999.
[11] T.R. Gilbert, D.N. Hume, Anal. Chim. Acta 65 (1973) 461.
[12] B. Welz, M. Melcher, H.W. Sinemus, D. Maier, At. Spectrosc. 5 (1984) 37.
[13] C.P. Hanna, J.F. Tyson, Anal. Chem. 65 (1993) 653.
[14] I.D. Brindle, S. Zheng, Spectrochim. Acta Part B 51 (1996) 1777.
[15] J.C. Wuilloud, R.G. Wuilloud, J.A. Salonia, R.A. Olsina, L.D. Martinez, J. Anal. Chem. 57 (2002) 799.
[16] C.P. Hanna, P.E. Haigh, J.F. Tyson, S. McIntosh, J. Anal. At. Spectrom. 8 (1993) 585.
[17] J. Ruzicka, G.D. Marshall, Anal. Chim. Acta 237 (1990) 329.