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ARTICLE *in* ANALYTICAL CHEMISTRY · SEPTEMBER 2005

Impact Factor: 5.64 · DOI: 10.1021/ac058007s · Source: PubMed

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Atomic Absorption Spectroscopy for Mercury, Automated by Sequential Injection and Miniaturized in Lab-on-Valve System

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Sodium borohydride-based hydride generation was automated by using programmable flow within the lab-on-valve module. Mercury vapor, generated in the reaction mixture, was extracted in a gas/liquid separator. The gas-expansion separator was miniaturized and compared with the performance of a novel gas separator that exploits the combination of Venturi effect and reduced pressure. Cold vapor atomic spectroscopy was used as a model system, with detection of mercury by absorption at 254 nm and limit of detection of 9 μg of Hg/L, using 300 μL of sample and 100 μL of borohydride. This work introduces, for the first time, sequential injection technique for hydride generation, highlights advantages of using programmable flow, and outlines means for miniaturization of assays based on spectroscopy of volatile species.

Hydride generation-based atomic spectroscopy includes atomic absorption spectroscopy, cold vapor atomic absorption spectroscopy (CVAAS), and inductively coupled plasma spectroscopy (ICP), as well as inductively coupled plasma mass spectrometry (ICPMS). All these techniques benefit from advantages of hydride generation: separation of the analytes from complex matrixes, analyte enrichment, fast reaction speed, and ease of automation. While originally designed as manual batch-type assays (as was the cold vapor mercury assay of Hatch and Ott[1]), hydride generation is now almost exclusively automated in continuous-flow format, which was originally proposed by Åström[2] and demonstrated in his pioneering work on the flow injection-hydride generation assay of bismuth. A comprehensive review of flow injection atomic absorption spectrometry by Fang[3] offers a detailed account of hydride generation methodology, including characteristics of gas expansion and membrane-based separators and a critical review of the entire field. It follows that, at present, hydride generation-based spectroscopy is widely and routinely used for trace analysis of As, Bi, Ge, Pb, Se, Sn, and Te, while assay of volatile compounds of Ag, Co, Cu, Ni, and Zn has been reported in research publications.[4] Yet, the most frequently assayed element is mercury, using cold vapor AAS.[3]

Following nearly three decades of research, development, and deployment of commercially available systems, the method has reached maturity, although the hydride generation technique still has some undesirable features: high reagent and gas consumption, generation of large volumes of chemical waste, and nonportability—as the instruments tend to be bulky and heavy. With the advent of miniaturized spectrometers and progress in microinstrumentation, such as plasma on a chip,[5] it is time to focus once again on downscaling the volumes and dimensions of fluidics for hydride generation, with the aim of making this technique compatible with advances in instrument miniaturization.

The key to downscaling is replacement of continuous flow by programmable flow, which will move both liquids and gas when and where they are needed in a "digital" fashion, by stopping, reversing, and accelerating flow rates. This principle is the basis of sequential injection (SI) techniques[6] that has been downscaled and integrated into the lab-on-valve (LOV) platform[7] and used for miniaturization of reagent-based assays, immunoassays, bioligand interaction assays, and ion exchange and affinity chromatography.[8] While the microSI-LOV format has already been used in connection with AAS, mainly for matrix removal and element enrichment,[9−11] the present work introduces the microSI-LOV technique as a tool for hydride generation for the first time and documents advantages of miniaturization: reduced sample and reagent consumption, small size of the apparatus that makes it portable, and advantages of programmable flow.

EXPERIMENTAL SECTION

Apparatus. A FIAlab microSequential Injection System with LOV module was used as purchased (FIAlab Instruments, Bellevue, WA, www.flowinjection.com) and configured as shown in Figure 1, using a 2.5-mL syringe, spectrophotometer model S2000-UV−Vis (Ocean Optics Inc., El Dorado Hills, CA). A standard mercury lamp model 90-0012-01 (UVP, Inc., Upland, CA, www.uvp.com) was mounted inside a homemade aluminum casing, fitted with a thread to a 600-μm quartz fiber-optic cable (model

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10.1021/ac058007s CCC: $30.25
Published on Web 07/12/2005

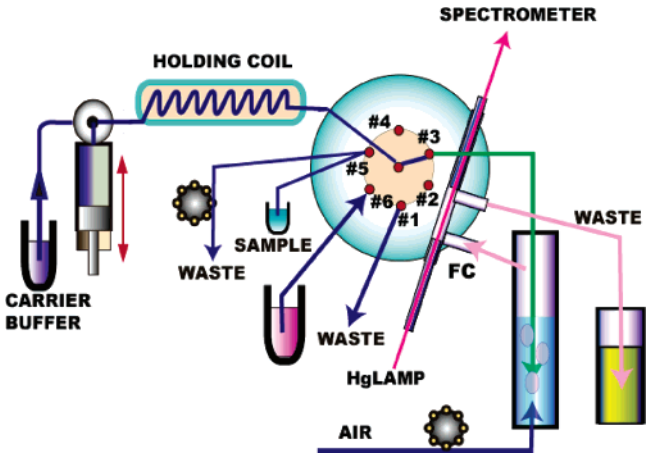

Figure 1. Sequential injection, lab-on-valve system furnished with gas expansion separator. The instrument comprises stepper motor-driven syringe pump, six-position valve, fiber-optic spectrophotometer, and mercury lamp light source (for details, see text). The flow scheme shows injection of reaction mixture into the separator that is purged by an air stream that continues into the flow cell (FC) and from there into absorbing liquid in a waste container.

QP600-2-UV−Vis) that led into a flow cell integrated into the LOV module. The light from the flow cell was collected by a second fiber-optic cable of the same type, leading to the Ocean Optics spectrophotometer. The optical path in the flow cell, defined by the distance between the fibers, was 11 mm long; the volume of the flow cell was 22 μL. All external tubing mounted on the LOV module was 0.8-mm i.d., made of Teflon, and all fittings (Upchurch Scientific, Oak Harbor, WA, www.upchurch.com) were from a kit supplied by FIAlab Instruments with the microSI instrument. The holding coil was 200 cm long (volume, 1 mL) and the connecting line between port 3 of the LOV and the gas separators was 20 cm long.

Gas-Expansion Separator. The expansion separator (Figure 2, top) was constructed along the lines of traditional gas/liquid separators[3] using the following Upchurch components: a PEEK tee (P-712), a low-pressure male union (P-645), an adapter (U-665), and a glass tube, 82 mm long, 3.5-mm i.d., and 6-mm o.d., which formed the body of the separator. Note that the line leading from the LOV module into the separator reached all the way to the bottom of the separator. The internal volume of the separator was 0.8 mL, and the connection between the gas-expansion separator and the flow cell was 12 cm long and made of a 0.5-mm-i.d. tube (volume, 24 μL).

Venturi Gas Separator. The gas separator comprised an external syringe pump (Figure 2, bottom), fitted with a three-position valve and 2.5-mL syringe. (FIAlab Instruments, model micro CSP-3000). The line between separator and flow cell had a volume of 200 μL, since it had to be fitted with an aerosol trap fashioned from a syringe filter (VWR, Brisbane, CA, www.vwrsp-.com) that fitted with a 0.5-mm pinhole.

Reagents and Materials. All chemicals used were of analytical grade. Mercuric chloride (Sigma, St. Louis, MO, www.sigmaald-rich.com) was used for preparing the standard solutions with DI water (17.6 MΩ/cm) and hydrochloric acid (Fisher Scientific, Fairlawn, NJ, www.fisherscientific.com). Carrier solution was 1 $\times$ 10^{-2} M HCl and standard mercury solutions were also made in 1 $\times$ 10^{-2} M HCl, by serial dilution of a stock solution containing 20 mg of $HgCl_2$ in 100 mL of 1 $\times$ 10^{-2} M HCl. Borohydride reagent solution was prepared by dissolving 0.5 g of sodium borohydride

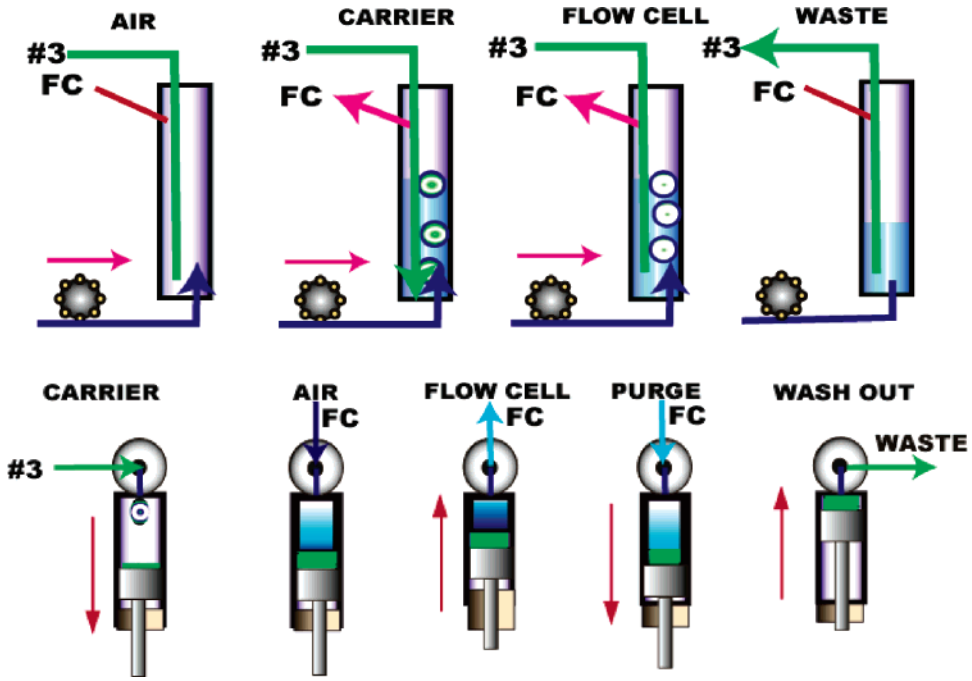

Figure 2. Gas/liquid separators used in this work. Top: The gas-expansion separator operated in four steps: (1) Peristaltic pump starts air flow through the separator. (2) Carrier brings reaction mixture from LOV into the separator. (3) Flow cell is being filled by mercury vapors from reaction mixture. (4) Waste step is initiated by aspiration of spent reaction mixture through LOV module into waste. Bottom: The Venturi separator operated in five steps: (1) Carrier solution expands into previously evacuated headspace, while Venturi effect assists in separation of gas from liquid. (2) Air is let into headspace to reestablish atmospheric pressure. (3) Flow cell is being filled by mercury vapors by upward motion of the syringe. (4) Purge step of flow cell is accomplished by reversed motion of the syringe. (5) Washout is executed by emptying the syringe followed by flush with carrier solution. Line marked 3 leads to port 3; line marked FC leads to flow cell.

Table 1. Assay Protocol for Expansion-Type Separator

step	operation[a]	LOV port, no.	time, s	flow rate, μL/s	vol, μL
air	peristaltic pump on; pump air through gas separator			4600	
	syringe aspirate air into holding coil	4	1	100	100
reagent	syringe aspirate borohydride reagent into holding coil	6	1	100	100
	Syringe aspirate air into holding coil	4	0.5	100	50
sample	syringe aspirate mercury sample into holding coil	5	6	50	300
	reference scan		0		
monitor	start absorbance scanning		0		
carrier	syringe dispense mixture into the gas separator	3	2.8	200	550
flowcell	delay		15		
washout	peristaltic pump off		0		
	syringe aspirate mixture from gas separator into holding coil	3	10	50	500
	stop absorbance scanning		0		
	peristaltic pump on		0		
	syringe aspirate mixture from gas separator into holding coil	3	2	50	100
	syringe aspirate carrier solution		2	200	400
	syringe dispense and carrier solution into waste	1	5	200	1000

[a] Syringe, syringe pump.

Table 2. Assay Protocol for Venturi-Type Separator

step	operation[a]	LOV port, no.	time, s	flow rate, μL/s	vol, μL
reagent	syringe aspirate borohydride into holding coil	6	1	50	50
	syringe aspirate air into holding coil	4	0.1	100	10
sample	syringe aspirate mercury sample into holding coil	5	6	50	300
	syringe aspirate air into holding coil	4	0.2	100	20
reagent	syringe aspirate borohydride reagent into holding coil	6	1	50	50
	reference scan		0		
monitor	start absorbance scanning		0		
air	syringe dispense mixture into the external syringe	3	2.9	150	430
	external syringe produces negative pressure and aspirate reaction mixture		(2.5)	400	1000
flowcell	external syringe dispense gas into the flow-cell		8.5	50	425
purge	external syringe aspirate gas, purge gas by dispensing		8	50	400
washout	external syringe dispense spent solutions into waste		4.9	200	975
	stop absorbance scanning		0		
	syringe aspirate carrier solution		3.5	200	700
	syringe dispense carrier solution into waste	1	1	200	200
	syringe dispense carrier solution into external syringe	3	10	50	500
	external syringe aspirate wash carrier solution		10	50	500
	external syringe dispense wash carrier solution into waste		2.5	200	500

[a] Syringe, syringe pump.

(Fisher Scientific) and 130 mg of sodium hydroxide (J. T. Baker, Phillipsburg, NJ, www.jtbaker.com) in 250 mL of deionized water.

Assay Protocol. For performance comparison of the gas-expansion and Venturi separators, the volumes of reagents, sample solutions, and flow rates within the LOV module were identical for experiments with both types of separators. The modifications of the software protocol were those necessary for an accommodation of the operational differences between the two types of separators. Assay protocols for the gas-expansion separator (Table 1) and for the Venturi separator (Table 2) give details of flow rates, volumes, and timing of all events of the microSI-LOV based assay.

The gas-expansion separator has been designed to combine the salient features of currently used devices. The assay protocol comprised the following steps (Figure 2, top). First, the peristaltic pump started delivering air at a flow rate of 4.6 mL/min while data collection was started. Next, carrier stream composed of the reaction mixture of previously metered volumes of sample and borohydride reagent was pumped from the LOV module into the separator (at a flow rate of 200 μL/s). Next, mercury vapors were swept into and through the flow cell. When the baseline was reached, data collection was stopped. Finally, the peristaltic pump was stopped and the wash out cycle began by aspirating the spent reaction mixture, via flow reversal, through the LOV into the waste.

The Venturi separator was operated in the following way. First, negative pressure was generated within the syringe by downward movement of the piston (1000 μL), while the central port of the LOV module remained connected to port 6. Next, the multiposition valve was turned to connect the holding coil (containing reaction mixture of metered sample and borohydride reagent) with port 3. At the same time, the syringe of the microSI system started to move forward at a rate of 150 μL/s, thus propelling the reaction mixture and the carrier solution into the separator (Figure 2, bottom, carrier). As the liquid entered the chamber at high velocity, the resulting Venturi effect assisted in separation of gas

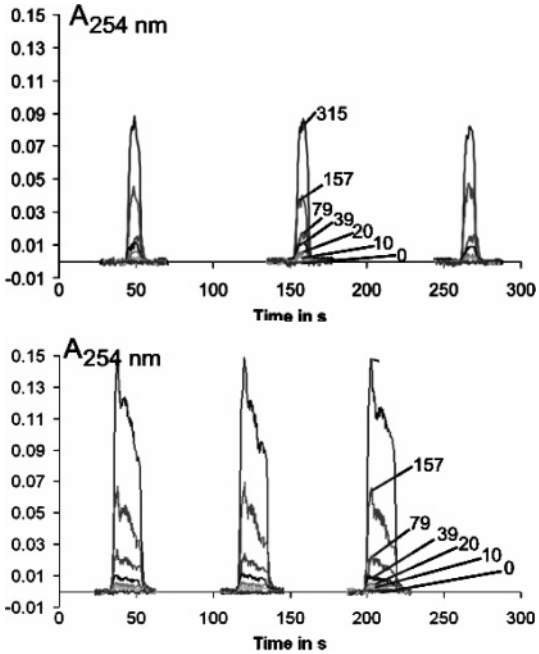

Figure 3. Response curves obtained in three separate runs. Each run comprises seven response curves, shown superimposed, obtained by injecting 300 μL of Hg standard solution, (range 0−315 μg of Hg/L). Lines between individual runs are interrupted since data were not collected between the runs. Top: The Venturi separator. Bottom: The gas-expansion separator.

from liquid, this process being further enhanced by reduced pressure in the separator, where the volume of injected liquid and gas was smaller than the evacuated volume. Next, the three-position syringe valve was opened to the flow cell, allowing air to enter and reestablish the atmospheric pressure within the syringe. In the following step, the piston moved upward, propelling the vapors into and through the flow cell. In the next step, the gas flow direction was reversed, as the piston traveled all the way to full extent of the syringe, thus purging the flow cell of air from outside. Finally, gas and liquid were washed out into the waste, and the syringe was rinsed by carrier stream from the LOV module, which was flushed at the same time.

Safety Considerations. Mercury vapors are poisonous and mercury compounds are harmful if inhaled or absorbed through the skin. Therefore, it is recommended to operate the analyzer in a hood or under an exhaust. Sodium borohydride solution can release hydrogen upon storage and, therefore, should not be stored in tightly closed glass containers.

RESULTS

The gas-expansion separator performed reliably and efficiently as documented by response curves that were obtained using seven injected mercury standards (blank to 315 μg of Hg/L). The response curves obtained in three separate series of experiments are shown superimposed (Figure 3, top) The assay cycle lasted 83 s, yielding a sampling frequency of 43 samples/h. Since peak width is short (25 s), the sampling frequency could be further increased, if required, by shortening the washout period by means of flow acceleration or by using flow reversal of the peristaltic pump.

The Venturi separator performed as reliably and as efficiently as the gas-expansion separator as documented by response curves,

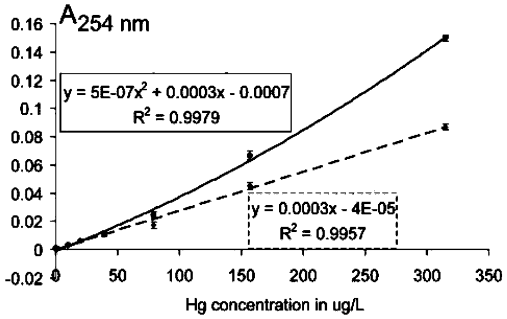

Figure 4. Calibration graph obtained for the gas-expansion separator (solid line) and for the Venturi separator (dashed line). Injected sample volume 300 μL.

obtained in triplicate runs, using seven injected mercury standards (blank to 315 μg of Hg/L) (Figure 3, bottom). The 100-s assay cycle yielded a sampling frequency of 36 samples/h. Considering that the peak width at its base was 12 s long, optimization of flow rates would allow sampling frequency to be further increased. Comparison of calibration curves (Figure 4) shows slightly higher sensitivity for the gas-expansion separator at higher Hg concentrations, while the Venturi separator exhibits a linear response through the entire range of concentrations. The regression coefficients were 0.9957 for the Venturi separator system and 0.9979 for the gas-expansion separator system. These experiments comparing the gas-expansion and the Venturi separators yielded the same: RSD ± 5% and limit of detection of 9 μg of Hg/L.

DISCUSSION

Principal Variables. Sensitivity and detection limit of μSI-CVAAS depends on (1) the injected sample volume, (2) the ratio of the volumes of gas and liquid phases in the separator, (3) the ratio of flow cell volume and separated gas volume, and (4) the volume of gas delivered into the flow cell. Since it has been well established that hydride generation is completed within seconds after reactants have been mixed,[4] the influence of the rate of chemical reaction does not need to be considered.

The ratio of gas/liquid volumes within the Venturi separator was varied in a series of experiments, (Figure 5, top) in the range 1.5:1−4.5:1, by evacuating 600, 1200, and 1800 μL from the syringe prior to injecting reaction mixture into the evacuated space. As the evacuated volume increased, the detector response decreased (Figure 5, top) linearly with increasing volume of the headspace because the mercury vapors were diluted with air entering the syringe during the next step (Figure 2, bottom, air). The volume of headspace gas transferred into the flow cell was 425 μL in all of these experiments.

To ensure that sufficient volume of headspace gas was transferred into the flow cell, the above experiment was repeated with 1200 μL of evacuated headspace, while delivering increasing volumes of mercury vapors (425, 625, 825 μL) into the flow cell. The results (Figure 5, bottom) confirm that delivering more than 425 μL of mercury vapors into the flow cell does not substantially increase the peak height, as the response has almost reached the steady-state level.

In summary, the sensitivity and detection limit of the μSI-CVAAS technique can be improved by (1) increasing volume of the flow cell (currently 25 μL), (2) decreasing the volume of the

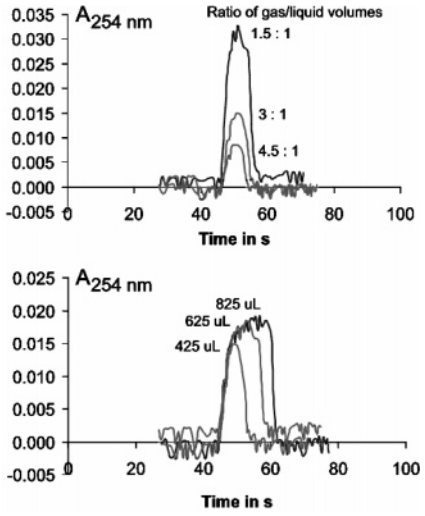

Figure 5. Optimization of experimental conditions for Venturi separator. Sample volume, 300 μL; mercury concentration, 115 μg/L. Top. Changing ratio of gas/liquid volumes within the separator chamber. Bottom: influence of volume of gas delivered to the flow cell.

conduit between the headspace and flow cell, (3) decreasing the volume of headspace, and (4) increasing the volume of injected sample.

First, two variables can be improved by integrating the flow cell with separator and by increasing the flow cell volume. Since the flow cell volume presently constitutes only 4.5% of the headspace volume for the Venturi separator, there is room for a 10-fold improvement in the detection limit if half of the headspace volume will be used to fill the flow cell, while the other half will be used for flushing the air from flow cell and from the connecting conduit. Further improvement of sensitivity and the detection limit could be achieved by using a membrane separator integrated within the flow cell, as used by Fang for a continuous-flow method.[3,12]

Injecting a larger sample volume required a modification of the holding coil length, which was shortened to 50 cm (volume 100 μL) to allow the sequentially injected borohydride (100 μL), sample (1200 μL), and borohydride (100 μL) zones, to react within the syringe. Next, the evolved gas and reaction mixture was moved by flow reversal through the LOV module, into the Venturi separator, and monitored in the way described above. As expected, peak height increased linearly with injected sample volume, reaching the detection limit of ~2 μg of Hg/L for a 1200-μL sample volume. Since a 5-mL syringe can be mounted on the microSIA-LOV system, further increase of injected sample volume is feasible.

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CONCLUSION

From a user's viewpoint, the microSI-LOV method for assay of traces of mercury has the drawback of higher detection limit and lower sampling frequency than reported for the most advanced laboratory system (LOD of 0.06 μg of Hg/L at a sampling frequency of 200 samples/h[3,12]), which used an integrated membrane−separator cell and argon as carrier gas. On the other hand, advantages of microSI-LOV technique are the small size of the instrument and unprecedented reagent economy. The apparatus is smaller than a laptop computer. If deployed in the field, it can be powered by 24 V dc and thus used for assay in situ. Since the microSI-LOV system is based on programmable flow, it consumes reagents only when sample is being processed. In its present, not yet fully optimized mode, only 300 μL of sample, 100 μL of reagent, and 4 mL of air are consumed per assay. In comparison, laboratory-type instruments for hydride generation are based on continuous flow, consume reagents at all times, and operate at carrier flow rates of 6−10 mL/min and argon flow rates of 50−100 mL/min, thus producing waste even when samples are not being processed.[3]

The gas/liquid separators used in this work are robust and maintenance free. Disappointingly, the Venturi-type separator did not yield better sensitivity or detection limit than the expansion-type separator, although it provides better control of gas flow. Future work should include integration of membrane gas separators with the microSI-LOV system, with the aim to improve detection limit and sample throughput.

In addition to the novelty of the present work, such as the first use of the microSI-LOV system for assay of an analyte in gaseous form, the use of programmable flow for hydride generation documented improvement of reagent economy, demonstrated decrease of waste generation, and allowed system miniaturization. For the present generation of AA and ICPMS instruments that rely on continuous gas flow, the gas-expansion separator will serve well. For future generations of miniaturized, inductively coupled microplasma spectrometers on a quartz chip,[5] which operate best on very low gas flow rates, the use of a microSI-LOV system with a membrane-type separator may become a feasible solution.

ACKNOWLEDGMENT

We are grateful to Elo Hansen for his kind guidance through the literature in atomic absorption spectroscopy and to Andrea Carroll for her critical comments in preparing the manuscript.

Received for review February 11, 2005. Accepted May 16, 2005.

AC058007S