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Artigo

DESIGN AND EVALUATION OF A CONTINUOUS FLOW, INTEGRATED NEBULIZER-HYDRIDE GENERATOR FOR FLAME ATOMIC ABSORPTION SPECTROMETRY

Miguel Murillo*, Nereida Carrión, Jorge Colmenares, Juan Romero, Gladys Alvarado, Manuel Ríos and Freddy Angulo
Centro de Química Analítica, Escuela de Química, Facultad de Ciencias, Universidad Central de Venezuela, Apartado Postal 47102 Caracas 1041-A, Venezuela

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An evaluation of the performance of a continuous flow hydride generator-nebulizer for flame atomic absorption spectrometry was carried out. Optimization of nebulizer gas flow rate, sample acid concentration, sample and tetrahydroborate uptake rates and reductant concentration, on the As and Se absorbance signals was carried out. A hydrogen-argon flame was used. An improvement of the analytical sensitivity relative to the conventional bead nebulizer used in flame AA was obtained (2 (As) and 4.8 (Se) μg L^{-1}). Detection limits (3σb) of 1 (As) and 1.3 (Se) μg L^{-1} were obtained. Accuracy of the method was checked by analyzing an oyster tissue reference material

Keywords: nebulizer-hydride generation; arsenic; selenium.

INTRODUCTION

The hydride generation (HG) technique represents an excellent method for the determination of gaseous hydrides (As, Se, Bi, Sb, Ge, Pb, Te and Sn) by atomic absorption spectroscopy (AAS). Low detection limits are reached with this technique when compare with conventional nebulization of sample solutions. This is mainly due to the high analyte transport efficiency and efficient atomization of the hydrides.[1-3] Furthermore, the HG technique has a high sensitivity; few atomization interferences are encountered. This is a device friendly used to be adapted to many atomization sources and is also used for preconcentration and speciation studies. The main disadvantages in hydride generation, are the interferences effects caused by transition metals ions in solution[1-7] and the group of elements training of hydrides a high concentrations.[6,7] The interference effect and the mechanisms of analyte signal suppression by transition metals have been widely studied in AAS.[1-5, 8-15] It has been reported that transition metals interfere in the hydride formation process after being reduced to metals[1,4,14] or after being converted to metal borides.[9,15] A catalytic effect of Co^{2+}, Ni^{2+} and Cu^{2+} ions on the aerosol, during the decomposition of $NaBH_4$ in the hydride generation process[12] and transport to the atom cell[14] as well as the deterioration of the tubes during the atomization step[16] have been reported as sources of interferences. The magnitude of the transition metal interference depends on the acid concentration,[8, 9] reductant concentration,[13,14] atomization source[1,17] and on the hydride generation system used.[5,17] Pierce and Brown[17] reported a bath mode hydride generation system which exhibits more marked interferences than the flow injection (FI) mode; the main reason of this observation was the reduction in contact time between the hydride formed and the sample matrix. It has been reported[6,16,18] that the quartz tube atomizer (QTA), which is the most common hydride atomizer for AAS, is prone to interferences by other volatile hydrides due to the low temperatures reached and by the deterioration of the internal surface of the tube. It is known that the thermal breaking of the hydrides is affected by hot surfaces and therefore, it is necessary to clean the silica tubes in order to obtain reproducible measures. Nevertheless, frequent use may irrecoverably modify the surface with the consequence of a reduction in sensitivity. It has also been reported[16,17,19,20] that the original performance is not obtained by cleaning the tube. These interferences are reduced by 2 to 3 orders of magnitude by using either a hydrogen flame in QTA,[6] a miniature argon hydrogen flame,[2,21] a flame-in-gas-shield atomizer,[21] a multiple microflame QTA[22] or a long-path slot burner.[23]

By other hand, it has also been reported HG systems which have the capacity of simultaneously nebulize the acidified sample solution and the reducing agent in ICP OES. The hydride generation reaction is carried out in the aerosol, owing to the droplet collisions between the reductant and sample solution.[24-30] The sample and $NaBH_4$ solutions, are nebulized by means of a concentric nebulizer[24,25] and a V-groove nebulizer[26-28] which have two solution-input channels, or two concentric nebulizers.[29,30] Minor memory effect, shorter contact time of the hydride formed with the first solution which helps to diminish the level of the interferences are some of the advantages presented by these novels generators when compared with other hydride generation systems.

Various types of flame have been used in hydride atomization, including H_2-N_2,[31] Ar-H_2,[32] H_2-O_2,[6,21,33] H_2-N_2-Air,[34] Air-C_2H_2,[35] Air-acetylene-Ar,[36] as well as the Burners multi-flame, which has been employed with flames of Air-C_2H_2, N_2O-C_2H_2, Air-H_2, Ar (entrained air)-H_2, and N_2 (entrained air)-H_2.[3] The last two flames compositions have some benefits for this study because they do not reach high temperatures and allow low background absorption at wavelengths below 200 nm. These low-temperature flames are useful for atomizing elements such as As and Se.[3] Selenium and As are the elements most studied in HG, due to their low concentration in environmental and biological samples (ng g^{-1}).

The aim of this study was to evaluate the performance of a compact continuous flow hydride generator-nebulizer system for AAS. Hydrides of the elements As and Se are produced *in situ* and analyzed by a hydrogen-Argon flame.

EXPERIMENTAL

Apparatus

Atomic absorption measurements were carried out with an Avanta GBC atomic absorption spectrometer. A gas manual control was adapted for using an Ar-H_2 flame. Two mass flow controllers

*e-mail: mmurillo@ciens.ucv.ve

(Brooks 5850E) replaced the original gas flow meters, one for the nebulizer Ar flow and the other for the H_2 flow (in substitution of the traditional acetylene gas). Figures 1a and 1b show a schematic diagram of the continuous hydride generator-nebulizer (HGN) system and an expanded view of the concentric tubes, respectively. The channels have 3.08, 2.25 and 0.5 mm i.d. for argon, reductant and sample respectively. Essentially, the HGN is a modified concentric-type nebulizer having two solution-input channels (Figure 1c). These channels were used for delivering the sodium tetrahydroborate and the sample solutions. The HGN replaced the original nebulizer of the atomic absorption spectrometer which was inserted into the inlet of the spray chamber. The hydride generation reaction was carried out inside the spray chamber during the nebulization process. Two independent pumps: Ismatec, Model IP and Gilson model Minipuls-3 were used in order to facilitate the optimization of the sample uptake rate and the optimization of the reductant solution rate. Once reached these optimum conditions, only one of these pumps was used throughout the work. A CEM model MDS-2000 microwave digestion system was used for sample digestion. Optimal working conditions are presented in Table 1.

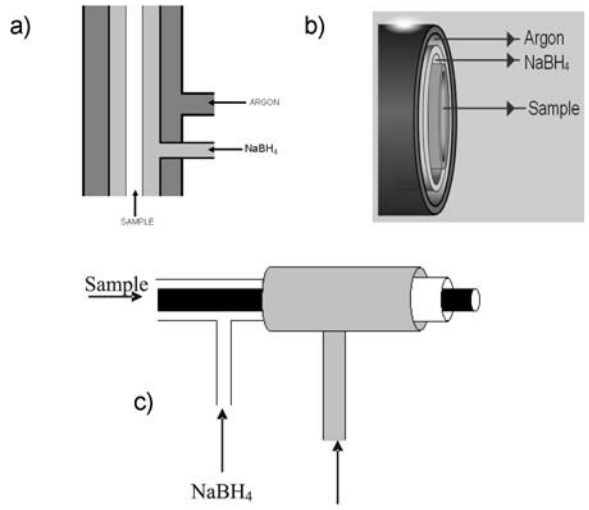

Figure 1. *a) Cross-sectional view, b) top view of the Nebulizer-generator hydrides and c) the whole unit*

Reagents

All chemicals used were of analytical-reagent grade HCl, HNO_3, $NaBH_4$ from Riedel-de Haën, NaOH (J. T. Baker), L-Cysteine (Merck, Darmstadt, Germany). Distilled de-ionized water (18 MΩ cm) was used throughout, and obtained from a Barnstead Nanopure system (Barsntead/thermolyne, Dubuque, IA, USA). The stock solution (1,000 μg mL^{-1} of As) was prepared with As_2O_3 (British Drug House). Working standard solutions were prepared by dilution of the stock solutions (1,000 μg mL^{-1} Se and As) (Fluka Chem). For the interference studies, solutions of Cu, Ni and Co (Riedel de Haën), 1,000 μg mL^{-1} were used. Low concentration solutions required for calibration purposes were prepared daily by serial dilutions with dilute nitric acid, as required. $NaBH_4$ solutions (Riedel-de Haën) were prepared daily by dissolving NaBH4 powder in NaOH, 0.02% (m/v) (J. T. Baker).

Reference materials

Validation of the method was performed analyzing the oyster tissue (NIST 1566a) certified reference material.

Table 1. Optimal experimental conditions

Parameter	Se	As
AA spectrometer		
Nebulizer Ar Flow rate (L min^{-1})	2.2	0.2
Ar Flow rate (nebulization chamber) (L min^{-1})	–*	0.6
H_2 Flow rate (L min^{-1})	1.5	1.4
Lamp current (mA)	20	20
Measured signal	Integrated absorbance	Integrated absorbance
Measurement time (s)	3	3
Slit width (nm)	1	1
Observation height (mm)	2	2
Analytical lines (nm)	196.0	193.7
Hydride generation		
HCL Concentration (mol L^{-1})	2.5	2.5
$NaBH_4$ Concentration (% m/v)	0.8	1.0
Sample solution uptake rate (mL min^{-1})	3.90	3.90
$NaBH_4$ solution uptake rate (mL min^{-1})	2.44	2.70

* Not necessary for Se since the signal decreases with the addition of argon

Sample preparation

Oyster tissue (0.1000 g) was weighed and poured into a 30 mL Teflon™ microwave digestion vessel, and then 3 mL of concentrated HNO_3 and 1 mL of water were added, and heated following the temperature program recommended by the supplier.[37] The solution was made up to 25 mL with HCl (2M). Addition of 1 mL H_2SO_4 (18M) to all samples was carried out in order to eliminate the fumes of HNO_3. The As(V) was reduced to As(III) with L-cysteine (0.5% m/v) in 3M HCl and heating up to 90 ºC for 20 min. The Se(VI) was reduced to Se(IV) in 4 mol L^{-1} HCl, at 90 ºC, during 30 min.

Serum samples (1 mL) were weighted and 0.8 mL of a mixture of HNO_3 – H_2SO_4 (1:1) and H_2O_2 (100 μL) were added, heated at 90 ºC and stirred in an ultrasonic bath for 1 h till dark fumes disappear. Reduction of Se (VI) to Se (IV) was then carried out by addition of concentrated HCl (0.6 mL), heating up to 90 ºC for 30 min and made up to a final weight of 5 g with deionized water.

Procedure

The acidified samples and $NaBH_4$ solution were simultaneously pumped into the hydride generation system. The hydride generation was carried out during the nebulization process. The aerosol was generated in the convergence region of the solutions and the carrier gas sample where the hydride was produced. It took 1 min to reach a stable steady-state signal.

RESULTS AND DISCUSSION

Optimization of the hydride generation

Preliminary tests were performed using an air-acetylene flame and an Argon-H_2 flame in order to atomize the volatile hydride of As. A characteristic concentration (Co) of 38 and 12 ng mL^{-1} were found for As for air-acetylene and Ar-H_2 flames respectively. This result agreed with those previously reported.[3] An Ar-H_2 flame was then chosen for further experiments.

Hydride Generation parameters such as the $NaBH_4$ and HCl concentrations were optimized by the one-factor-at-a-time method.

The parameters studied included the $NaBH_4$ and sample solution flow rate. The optimization procedures were carried out by measuring the absorbance of 500 µg L^{-1} of Se and 250 µg L^{-1} of As solutions.

Figures 2a and 2b show the influence of the $NaBH_4$ and HCl concentrations on the As and Se absorption signals, respectively. It can be seen that the absorbance of As and Se increases markedly with increasing the $NaBH_4$ concentration. However, the acid concentration has a negligible effect on the analytes sensitivity, within the range studied. Similar results have been reported by Coelho[38] and Moretto[39] and differ from that obtained when a conventional hydride generator (a separate mixing/reaction coil and gas-liquid separator) is used, where a strong dependency is generally obtained between sensitivity and HCl concentration.[40-42]

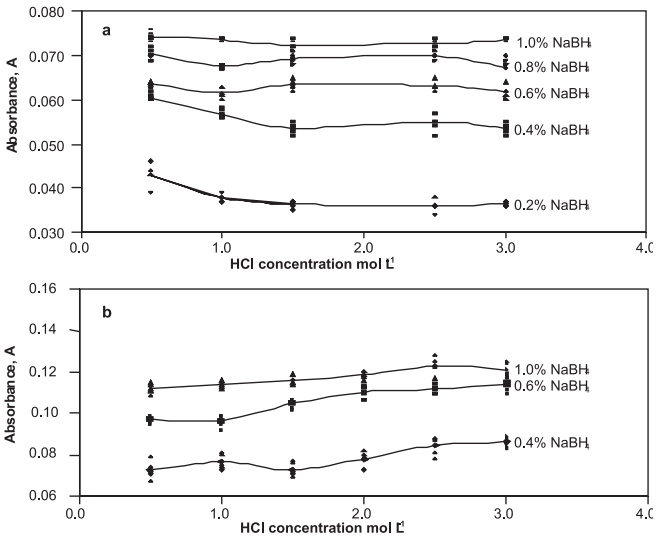

Figure 2. *Effect of $NaBH_4$ and HCl concentrations on a) Se (500 µg L^{-1}) and b) As (250 µg L^{-1}) absorption signals*

Figures 3 and 4 show the effect of reductant and sample solution uptake rates on the As and Se absorption signals respectively. Analytes sensitivity increase as the $NaBH_4$ solution uptake rate increases (Figure 3). A value of 2.5 mL min^{-1} $NaBH_4$ flow rate was chosen as optimum. Reductant reagent is insufficient for complete generation of the hydride forming elements at lower reductant flow rate. Figure 4 illustrates that As and Se signals increase with increasing sample uptake rate. This effect was expected since the amount of analyte reaching the generator system increase with an increase in the sample uptake rate. However, values lower than 4 mL min^{-1} was chosen due to a significant reduction of signal reproducibility.

Figures of merit and characteristics of the system

Transition metals, such as Cu^{2+}, Ni^{2+} and Co^{2+}, interfere severely with the hydride generation process,[1] being selenium the most sensitive element to this type of interferences.[7-9] The effect of nickel, cobalt and copper on the arsenic and selenium absorption signals was therefore studied. Results show that these elements do not interfere in the absorbance signal of As. Among the elements, copper was the only one to interfere with the absorbance signal of Se. A depression of 20% on the Se absorbance signal (100 ng mL^{-1} Se solution) was found at a Cu concentration as low as 1 µg mL^{-1}.

The reliability of the procedure was confirmed by spike-tests and by the analysis of the certified reference material. The results of the spike-tests applied to the determination of selenium in human serum are shown in Table 2. Recovery ranged from 95 to 99%, indicating that Selenium can be quantitatively recovered from human serum To further

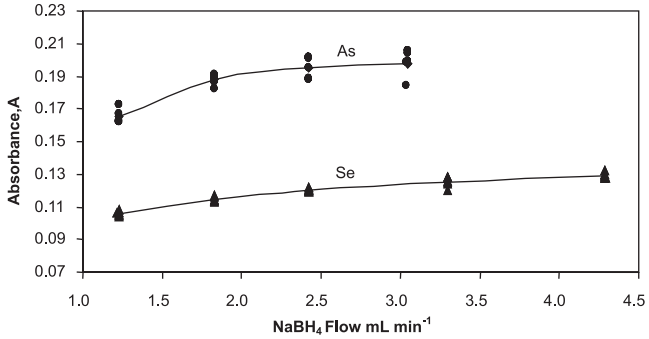

Figure 3. *Absorbance as a function of the $NaBH_4$ flow rate [As (250 µg L^{-1}) and Se (500 µg L^{-1})]*

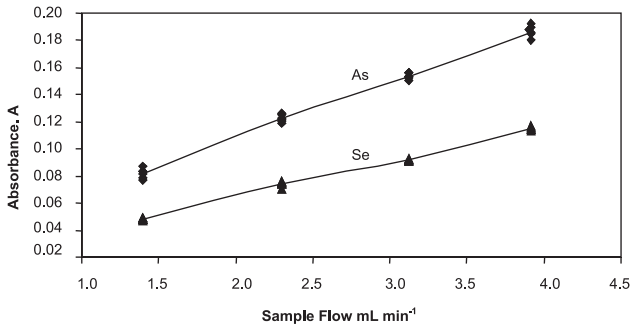

Figure 4. *Absorbance as a function of sample flow rate [As (250 µg L^{-1}) and Se (500 µg L^{-1})]*

confirm the accuracy of the analytical procedure proposed using the designed system, arsenic and selenium were determined in the certified reference material, oyster tissue (NIST 1566a). The results obtained are shown in Table 3. There is not significant difference at the 95% confidence level between the measured and certified values.

The precision of the system was 7 and 3.4% RSD, based on 10 measurements of an aqueous standard solution containing 35 µg L^{-1} As and 20 µg L^{-1} Se, respectively. Similar precision was obtained from both: spike-tests on samples and analysis of CRM (Tables 2 and 3).

Figures of merit achieved with this system were: LOD 1 and 1.3 µg L^{-1}, LOQ 6 and 7.8 µg L^{-1}, and characteristic concentrations 1.3 and 4.8 µg L^{-1} for As and Se, respectively. These results are similar to those best found and previously reported (LOD 0.8 and 1.8 µg L^{-1} for As and Se respectively).[3] The linear range of the proposed system was from 5 to 1000 µg L^{-1} and from10 to 1000 µg L^{-1} , for As and Se respectively.

Table 2. Spike study for Se in serum samples

Se Concentration (µg/L)				
Sample concentration	Added	Found value	F	RSD (%)
42 ± 1	30.5 ± 0.2	72 ± 1	0.99 ± 0.06	6.0
43 ± 1	69 ± 1	106.9 ± 0.7	0.95 ± 0.02	2.0

Table 3. Analysis of reference material NIST 1566a

Elements	Precision (% RSDa)	Found value (µg/g)	Certified value (µg/g)
As	3.8	14.8 ± 0.6	14.0 ± 1.2
Se	7.7	2.3 ± 0.2	2.21 ± 0.24

a: Relative standard deviation of six replicate measurements

CONCLUSIONS

The new hydride generator- nebulizer system is very easy to build and a very economical HG device. It has some potential benefits when compared to conventional HG systems in FAAS: it produces a rapid response and low memory effects. Limits of detection obtained of 1 and 1.3 of μg L^{-1} for As and Se, respectively are similar to those obtained when HG-quartz tube atomization is used with the additional advantage that there is not loss of sensitivity, which is caused by the continuous use of the atomization tubes.

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