ELSEVIER

Spectrochimica Acta Part B 60 (2005) 755–758

SPECTROCHIMICA ACTA PART B

www.elsevier.com/locate/sab

Analytical note

Comparison of two digestion procedures for the determination of lead in lichens by electrothermal atomic absorption spectrometry ☆

Fátima R. Moreira[a,*], Renato M. Borges[b], Rosália M. Oliveira[b]

[a]*Laboratory of Toxicology, CESTEH/ENSP/Oswaldo Cruz Foundation, Rio de Janeiro, RJ, Brazil*
[b]*DSSA/ENSP/Oswaldo Cruz Foundation, Rio de Janeiro, RJ, Brazil*

Received 17 September 2004; accepted 6 February 2005
Available online 1 April 2005

Abstract

The efficiency of two procedures for the digestion of lichen was investigated using a heating block and a microwave oven. In the open vessels, concentrated nitric acid was added to the samples, left for 1 h, and the addition of 30% (v/v) hydrogen peroxide completed the digestion. In the closed system, the complete digestion was performed using concentrated nitric acid and hydrogen peroxide, reducing the amount of chemicals, time and contamination risk. Both digestion methods gave comparable results, and recoveries were statistically not different. For a lichen sample spiked with 10 μg Pb, the recovery was 111% and 110% using microwave and heating block digestion, respectively, while it was 100% and 103% for a 100 μg Pb spike. For the determination by electrothermal atomic absorption spectrometry samples were diluted 20 times with water and a volume of 20 μL was injected into the graphite furnace without chemical modifier. Pyrolysis and atomization temperatures of 700 °C and 1500 °C, respectively, were used. The characteristic mass was 8.4 ± 0.6 pg for aqueous calibration solutions and 8.9 ± 0.8 pg for samples. Calibration was against matrix matched standards. The recovery test showed some contamination problem with the lowest concentrations in both procedures. The detection limits were 4.4 μg L^{-1} with microwave oven and 5.4 μg L^{-1} with the heating block in the undiluted blank.

Keywords: Lead determination; Lichen; Heating block; Microwave oven; Digestion; ET AAS

1. Introduction

Bioaccumulation of trace metals from atmospheric deposition is frequently evaluated by analyzing environmental biomonitors, such as mosses, plant leaves or lichens. Lichens are a result of the association among fungus, algae and, unlike plants, without roots or well-developed cuticle. These morphological features emphasize the applicability for monitoring purposes. Lichens are strongly dependent on wet and dry deposition on their surface for mineral nutrients absorption. In addition, their surface, structure, and roughness facilitate the interception and retention of the particles [1]. The advantages of using them as biological monitors of atmospheric deposition in comparison to conventional air sampling techniques is that lichens are present in most terrestrial habitats, are lasting, cheap and of easy reproduction [2].

Lead is one of the most common pollutants in the environment, toxic to the human beings and animals and without any known physiological function, which accumulates in the organism [3]. At moderate levels of exposure, an important aspect of the toxic effects of lead is the reversibility of the induced biochemical and functional changes. In humans, lead toxic action results in a wide range of biological effects depending on the level and duration of exposure. Lead in the environment is a result of anthropogenic activities and when launched to the atmosphere, it does not undergo any degradation process, and remains available to human exposure [4,5].

☆ This paper was presented at the 8th Rio Symposium on Atomic Spectrometry, held in Paraty, RJ, Brazil, 1–6 August 2004, and is published in the special issue of *Spectrochimica Acta Part B*, dedicated to that conference.

* Corresponding author.
E-mail address: fmoreira@ensp.fiocruz.br (F.R. Moreira).

0584-8547/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.sab.2005.02.017

The use of lichen as bioindicator requires to be conscious of the basic difficulties in environmental specimen analysis. Electrothermal atomic absorption spectrometry is one of the convenient techniques for this task since it is fast, precise and has low detection limits. The digestion procedures commonly used for plant tissues are dry ashing, open vessel digestion, microwave-assisted dissolution and acid bomb digestion, which generally show good accuracy and precision, although some of them are time-consuming and prone to contamination. Thus several factors should be considered while preparing samples and these include contamination level, sample homogeneity, complete digestion, time consumption for preparation, suitable analysis technique and economic aspects [6].

The aim of this work is to compare the efficiency of two procedures consisting of a heating block and a microwave oven for lichens digestion associated with electrothermal atomic absorption spectrometric determination of lead.

2. Experimental

2.1. Instrumentation

A Model AAnalyst 300 atomic absorption spectrometer with deuterium background correction, equipped with an HGA-800 graphite furnace and an AS-72 autosampler (Perkin-Elmer, Norwalk, CT, USA) was used throughout this work. The operating parameters, such us hollow cathode lamp current, wavelength and slit setting, were as recommended by the manufacturer. All experiments were performed using pyrolytic graphite tubes with integrated platforms (Perkin-Elmer, Part No. B300-1264). Integrated absorbance (peak area) was used for signal evaluation throughout. A typical graphite furnace temperature program is shown in Table 1.

A Prolabo Model 7195 microwave oven (O I Analytical, Texas, USA) and a Tecnal Model TE 040-G40/25 heating block (Piracicaba, SP-Brazil) were used to digest the samples. Table 2 shows the operating conditions for the microwave-assisted digestion procedure adopted for lichen samples.

Table 1
Temperature program for lead determination in lichens

Step	Temperature (°C)	Ramp (s)	Hold (s)	Internal Flow (mL min^{-1})
1[a]	90	1	10	250
2	120	10	10	250
3	700	10	10	250
4	20	1	15	250
5[b]	1500	0	5	0
6	2600	1	5	250

[a] Sample.
[b] Read.

Table 2
Microwave oven program

Step	Time (min)	Power (W)
1	2	240
2	2	360
3	15	480

2.2. Reagents and solutions

All reagents (Merck, Elmsford, NY, USA) were of analytical grade and all solutions were prepared using water previously deionized with a Milli-Q system (Millipore, Bedford, MA, USA). The 1000 μg mL^{-1} Pb stock solution was prepared from Titrisol ampoules and the work solutions were prepared daily by further dilution. The aliquot volume injected into the furnace was 20 μL for all solutions. Prior to analysis, all glass and plastic ware were soaked in 5% (v/v) Extran solution for 24 h and rinsed several times with tap water. Thereafter they were immersed in 10% (v/v) HNO_3 for 48 h followed by rinsing with Milli-Q water.

2.3. Digestion procedures

Two different procedures were applied to digest the lichen samples. In both cases, two digestion blanks were prepared together with each sample batch. All digested samples and blanks were transferred into 50 mL volumetric flasks and stored at 4 °C until analysis.

The accuracy of the analytical technique was checked by adding 10 and 100 μg of lead to lichen samples with low analyte levels. These samples were only digested 24 h after the addition, following the same procedures as the others.

2.3.1. Heating block digestion

A mass of 0.2 g of lichens was weighed in quartz tubes and 5 mL of concentrated HNO_3 was added and left for 1 h. After that, 10 mL of HNO_3 was added and the samples were heated to 90 °C in the heating block for 12 h. Then 10 mL of 30% (v/v) H_2O_2 was added and the samples heated for another 3 h at 90 °C. The elements bound in the silicate structure are not commonly dissolved by this procedure, as they are not mobile in the environment. The residues were filtered and the extracts transferred into 50 mL volumetric flasks with deionized H_2O. The total lead content was determined by ET AAS in the 20 times diluted final solution.

2.3.2. Microwave-assisted digestion

Approximately 0.2 g of lichen sample were weighed and transferred into Teflon digestion vessels. Four of these vessels were used for the digestion of lichens samples and two of them used for reagent blanks. A mixture of 3.5 mL of 65% (v/v) HNO_3 and 1.5 mL of 30% (v/v) H_2O_2 was added and left for 30 min. Afterwards the vessels were placed into the microwave oven and an optimized program applied. Although this procedure also does not completely dissolve

Table 3
Recovery

Spiked mass	Recovery (%)	
	Heating block	Microwave oven
10 μg	111 ± 12.7	110 ± 2.3
100 μg	103 ± 3.5	100 ± 1.1

Samples diluted 1+19 with water; Tp=700 °C—Ta=1500 °C; sample volume=20 μL. Results are averages of three replicates and their standard deviation.

the samples, the residues here are lesser. The residues were filtered and treated as described above.

3. Results and discussion

3.1. Comparison between the digestion procedures

According to the recovery calculations with respect to the spiked samples (Table 3), both digestion procedures gave comparable results since recoveries were not statistically different (Student's *t* test and 95% of confidence) although some residues were observed with both procedures. However, the recovery for 10 μg Pb was higher than for lichens spiked with 100 μg, showing there is some contamination problem with low concentrations in both procedures. As there was no difference between the two digestion procedures, the microwave-assisted procedure was chosen for all experiments since it is faster.

3.2. Pyrolysis and atomization temperature

Fig. 1 shows the pyrolysis and atomization curves obtained for aqueous standards containing 1.0 ng Pb and a lichen sample spiked with 1.0 ng Pb. Without using a modifier, the optimum pyrolysis and atomization temperatures were 800 °C and 700 °C for the aqueous standard and the spiked lichen sample, respectively. The optimum atomization temperatures found with aqueous standard and lichen matrix were 1500° and 1400 °C, respectively. A pyrolysis temperature of 700 °C and an atomization temperature of 1500 °C was finally used in all situations since the peaks are broader at 1400 °C.

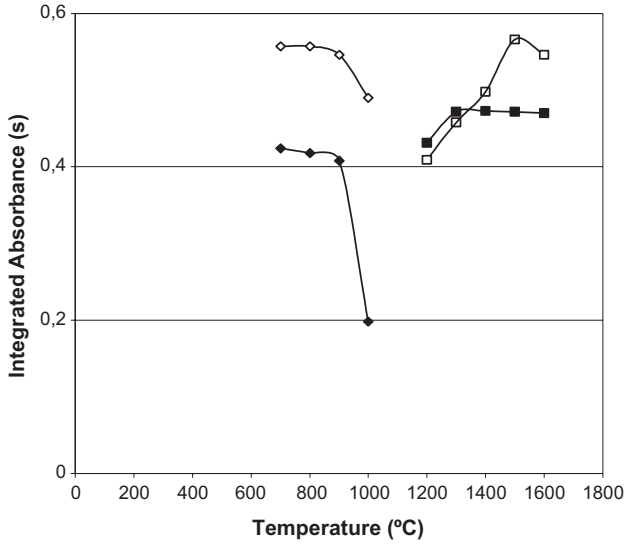

Fig. 1. Pyrolysis (diamond) and atomization (square) curves for 1.0 ng Pb in aqueous solution (open symbol) and lichen spiked with 1.0 ng Pb (full symbol).

Table 4
Recovery of lead from spiked lichen samples

Sample	Experimental values (μg) (matrix matched curve)	Reference values* (μg)
Lichen 1	11.7 ± 0.2	10
Lichen 2	101.4 ± 1.1	100

* CESTEH: spiked lichen; Microwave digestion; Samples diluted 1+19 with water; Tp=700 °C–Ta=1500 °C; sample volume=20 μL. Results are averages of three replicates and their standard deviation.

3.3. Characteristic mass and sensitivity ratio

The average sensitivity ratio between the slope of the analyte addition curve and of the analytical curve obtained in 0.2% (v/v) HNO_3 was 0.94 ± 0.04 (n=3). The characteristic mass values were 8.9 ± 0.8 pg and 8.4 ± 0.6 pg (n=10) for matrix matched and aqueous standards, respectively. According to Student's *t* test and 95% of confidence, the characteristic mass values with and without matrix are different, indicating the presence of matrix effects (sensitivity ratio different from unity), which does not allow the use of aqueous analytical solutions.

3.4. Accuracy

The accuracy of the method was verified by the analysis of samples spiked with known amounts of lead, since reference lichens were not available. Experimental and reference values were in good agreement (Student's *t* test, 95%) only for the highest concentration (Lichen 2), as shown by the results in Table 4. The sample with the low analyte mass presented contamination problems and therefore the results are statistically different. The matrix-matched standard curve was used for calibration since it provided better results for lichen samples based on our previous experiments.

Table 5
Lead concentration found in lichen samples after two digestion procedures

Samples	Digestion procedures	
	Heating block (μg g^{-1})	Microwave oven (μg g^{-1})
1	47.2 ± 1.3	46.3 ± 2.5
2	11.8 ± 0.5	10.4 ± 1.8
3	157.9 ± 1.9	154.4 ± 1.5

Samples diluted 1+19 with water; Tp=700 °C–Ta=1500 °C; sample volume=20 μL. Results are averages of three replicates and their standard deviation.

3.5. Detection limits

Since lead is one of the most common pollutants and naturally present in the environment, it has become difficult to find samples without this metal. So limits (3σ) were obtained from the reagent blank following the same procedures as the samples. The average values found for 10 replicates by calibration against matrix-matched standards were 4.4 μg L^{-1} Pb with microwave oven and 5.4 μg L^{-1} Pb with the heating block in the undiluted blank.

Table 5 shows the results found for Pb levels in lichen samples with different concentrations. These values showed the heating block and the microwave oven gave the same results (Student's *t* test, 95%) as already seen before (Table 3).

4. Conclusions

Both digestion procedures gave comparable results since recoveries were not statistically different, but the recovery test showed there is some contamination problem with the lowest concentrations in both procedures, so that greater attention must be given to this fact. Microwave digestion procedure was faster (20 min) than that using the heating block (20 h).

A pyrolysis temperature of 700 °C was used for all situations and the atomization temperature chosen was 1500 °C since at 1300–1400 °C the peaks are broad and longer integration times are necessary.

The use of aqueous analytical solutions is not possible since sensitivity ratio is different from unity, showing the presence of matrix effects. Thus the use of a chemical modifier is necessary.

The detection limits were adequate for the determination of lead at blank levels with ET AAS. The microwave technique provided a better detection limit according to Student's *t* test and 95% of confidence.

The method is applicable for the lead determination in lichens, which is an important biomonitor for assessing the contamination in the environment.

References

[1] C. Baffi, M. Bettinelli, G.M. Beone, S. Spezia, Comparison of different analytical procedures in the determination of trace elements in lichens, Chemosphere 48 (2002) 299–306.
[2] K. Szczepaniak, M. Biziuk, Aspects of the biomonitoring studies using mosses and lichens as indicators of metal pollution, Environ. Res. 93 (2003) 221–230.
[3] Y. Xie, M. Chiba, A. Shinohara, H. Watanabe, Y. Inaba, Studies on lead-binding protein and interaction between lead and selenium in the human erythrocytes, Ind. Health 36 (1998) 234–239.
[4] L.A. Saryan, C. Zenz, Lead and its compounds, in: O.C. Zenz, B. Dickerson, E.P. Horvath (Eds.), Occupational Medicine, Mosby-Year Book, Inc., Missouri, 1994, p. 506.
[5] ATSDR (Agency for Toxic Substances and Disease Registry), Toxicological Profile for Lead, US Department of Health and Human Services, Public Health Service, Atlanta, 1999, 465 p.
[6] S.G. Tuncel, S. Yenisoy-Karakas, A. Dogangün, Determination of metals concentrations in lichens samples by inductively coupled plasma atomic emission spectroscopy technique after applying different digestion procedures, Talanta 63 (2004) 273–277.