

Determination of Lead in Bone by Electrothermal Atomic Absorption Spectrometry with Zeeman Effect Background Correction

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Um método para a determinação de traços de Pb em ossos por espectrometria de absorção atômica é descrito. Boas precisão e exatidão foram obtidas utilizando-se calibração externa com padrões aquosos e $\text{NH}_4\text{H}_2\text{PO}_4$ como modificador. O limite de detecção alcançado foi de $0,07 \text{ ng mL}^{-1}$ na solução de leitura, correspondendo a $0,022 \mu\text{g g}^{-1}$ na amostra original. Conseqüentemente, o limite de quantificação encontrado foi de $0,23 \text{ ng mL}^{-1}$. Uma precisão de 3,5% foi obtida na leitura de 400 ng de Pb na presença da matriz. A exatidão do procedimento foi confirmada pela boa concordância entre os valores certificado e encontrado na análise de um material certificado de referência (NIST SRM 1846, bone meal).

A simple method for measuring trace levels of Pb in bone by electrothermal atomic absorption spectrometry is described. It is shown that Pb can be accurately measured with good precision and accuracy using external calibration with aqueous Pb analytical solutions containing $\text{NH}_4\text{H}_2\text{PO}_4$ as modifier. The detection limit was found to be 0.07 ng mL^{-1} , corresponding to $0.022 \mu\text{g g}^{-1}$ in the original sample. Consequently, the limit of quantification was found to be 0.23 ng mL^{-1} . The precision of the procedure, expressed by the relative standard deviation, was 3.5% for 400 ng Pb in sample measurements. The accuracy of the procedure was confirmed by the good concordance between found and certified values in the analysis of a bone meal certified reference material, NIST SRM 1846.

Keywords: ETAAS lead determination, $\text{NH}_4\text{H}_2\text{PO}_4$ modifier, bone meal

Introduction

Lead has been one of the most extensively studied toxic elements. Lead in the body comprises 2% in the blood and 95% in bone and dentine. Blood lead may remain elevated for years after cessation of long exposure, due to remobilization from bone.¹ It appears that bone-Pb may be a more accurate and appropriate index of long-term cumulative exposure compared with blood-Pb.² This has led to the development of *in vivo* measurements of Pb in bone by X-ray fluorescence (XRF) including K-XRF³ and L-XRF.^{4,5} However, before these methods become a valuable screening tool for clinical studies, there are still questions that need to be addressed, such as the absence of an adequate certified reference materials for instrumental

calibration and method validation. Nowadays, for validation purposes⁶⁻⁸ most studies of *in vivo* XRF bone-Pb measurements rely on the analysis of bone materials by electrothermal atomic absorption spectrometry (ETAAS) following acid digestion.

The most common and successful technique for measuring Pb in clinical samples is ETAAS⁹⁻¹¹ because of its high sensitivity and favorable detection limits. Moreover, in general, just a simple pre-treatment is required due to the matrix simplification in the pyrolysis step inherent to the method. However, the analysis of bone still poses a challenge due to the complexity of the bone matrix.²

In order to minimize matrix interferences, modifiers have been used to stabilize the analyte at higher pyrolysis temperatures, permitting a more efficient volatilization of the matrix. For instance, Acar¹² showed that a Ni + Pd + $\text{NH}_4\text{H}_2\text{PO}_4$ modifier mixture was preferred for determination of Pd and Cd in biological samples. Iavicoli

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et al.,¹³ in contrast to Parsons¹⁴ used a Pd/Mg(NO₃)₂ mixture as modifier. The authors claimed that it enables a significant reduction of the spectral interferences observed if modifiers based on NH₄H₂PO₄ with either Ca or Mg are used for Ca₃(PO₄)₂ rich samples. Recently permanent modifiers have also been proposed in the determination of Pb by ETAAS.^{15,16} Due to this literature controversy, the present work evaluates the use of NH₄H₂PO₄ as modifier for the determination of lead in bone by ETAAS.

Experimental

Instrumentation

A Perkin-Elmer Model 3030 atomic absorption spectrometer equipped with transverse Zeeman background corrector, a Massmann type longitudinally heated HGA-600 graphite furnace and an AS-60 autosampler was used for the atomic absorption measurements. Perkin-Elmer pyrolytically coated grooved graphite tubes (PN B0109322) containing a L'vov platform (PN B0109324) were used. An Intensitron (Perkin Elmer) hollow cathode lamp was used as line source. Argon (99.99%, Aga, Rio de Janeiro, Brazil) was used as shielding gas, and the stop flow mode used during the atomization step. Injection volume was always 20 μL. Other instrumental parameters are summarized in Table 1.

Table 1. Experimental settings for the determination of Pb in bone

<i>Spectrometer settings</i>	
Lamp current (mA)	10
Wavelength (nm)	283.3
Spectral band width (nm)	0.7
Integration time (s)	5
<i>Heating Program</i>	
Drying	
T (°C) / ramp (s) / hold (s)	110 / 10 / 15
Pyrolysis	
T (°C) / ramp (s) / hold (s)	1000 / 10 / 20
Atomization	
T (°C) / ramp (s) / hold (s)	1500 / 0 / 5
Clean out	
T (°C) / ramp (s) / hold (s)	2600 / 1 / 3

Reagents and solutions

All chemicals used were of analytical-reagent grade. The lead stock solution (1000 mg L⁻¹) were prepared from a Titrisol concentrate (Merck, Darmsdadt, Germany). Working solutions were prepared by two consecutive adequate dilutions of the lead stock solution. Nitric acid

(65% m/v, Merck, Darmstadt, Germany) was further purified by sub-boiling distillation in an all-quartz apparatus (Hans Kürner, Rosenheim, Germany). Milli-Q water (18 MT x cm) from a Milli-Q de-ionization unit (Millipore, Bedford, MA, USA) was used throughout.

The modifier solution was prepared by dissolving 2 g of NH₄H₂PO₄ (Merck, Darmsdadt, Germany) in 0.5% v/v HNO₃ and diluting to 100 mL to obtain a 2% m/v solution. The Triton X-100 (0.005% v/v) solution used for rising the autosampler tip, was prepared by a 20000 fold dilution of the Triton X-100 (Merck, Darmsdadt, Germany).

Control of contamination

To avoid possible contamination whilst handling the samples, disposable plastic gloves were worn at all times. Digestion tubes, pipette tips and autosampler cups were always cleaned by immersion in 20% v/v nitric acid for, at least, 24 h. Before use, they were thoroughly washed with Milli-Q water. All sample manipulations and analyte solution preparation were performed in a laminar flow hood (Karl Bleymehl, Rosenheim, Germany). The efficiency of the cleaning procedures were confirmed by the low blank values.

Sample preparation

Bone samples were triturated, homogenized and dried overnight at 110 °C and then left to cool in desiccators. The samples (1000 mg) were weighed directly in the polypropylene screwed cap conical ended tubes (25 mm diameter x 110 mm long; v = 50 mL), used in continuation for digestion. Subboiled nitric acid (10 mL) was added and the tube was heated at 80 °C for two hours in a home-made digestion block. After cooling, the clear solution obtained was transferred to another 50 mL plastic tube and made up to 30 mL with Milli-Q water after the addition of the modifier, representing 33 mg mL⁻¹ of bone and 1% m/v of NH₄H₂PO₄ as final concentrations. This solution was transferred to the autosampler cup for the ETAAS analysis. At least, three blank solutions were analyzed for each sample batch in order to compensate for the reagents contamination. Similar procedure was followed in the analysis of the certified reference material.

Atomic absorption measurements

Calibration was performed with aqueous analytical solutions (5% v/v HNO₃), already containing the modifier (1% m/v NH₄H₂PO₄). Each measurement was performed in triplicate, using the integrated absorbance mode.

Results and Discussion

Method optimization and characterization

In order to optimize the temperature program, pyrolysis and atomization temperatures curves were performed. In this case, an aqueous Pb (40 ng mL^{-1}) analytical solution already containing the 1% m/v $\text{NH}_4\text{H}_2\text{PO}_4$ modifier was first used. Figure 1 shows the results, indicating optimum pyrolysis and atomization temperatures of 1000 and $1500 \text{ }^\circ\text{C}$ respectively. It was also observed that the addition of the modifier solution to the sample was also necessary to reach the same performance in spite of the large endogenous phosphate concentration of its matrix. Taking this into account, similar curves were obtained for a sample solution.

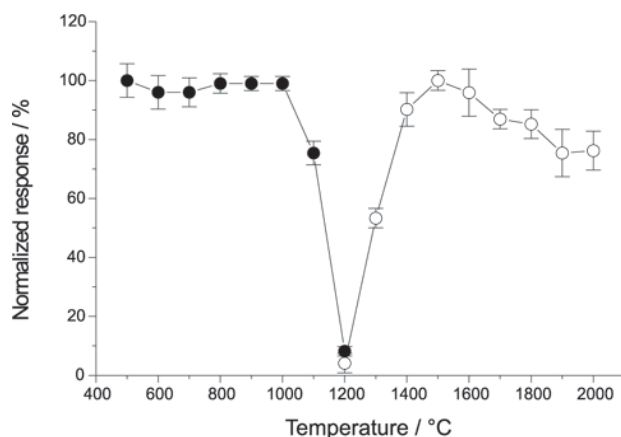


Figure 1. Pyrolysis (●) and atomization (○) temperature curves for Pb (800 pg) in 5% v/v HNO_3 in the presence 0.2 mg of $\text{NH}_4\text{H}_2\text{PO}_4$ as modifier. Atomization temperature, 1600°C for the pyrolysis study; pyrolysis temperature = 800°C for the atomization study.

Comparison of the absorbance profiles (Figures 2a and 2b) reveals a close match between the profile related to the bone matrix and that related to the aqueous Pb analytical solution both containing the $\text{NH}_4\text{H}_2\text{PO}_4$ modifier. This match encompasses not only the peak shape but also similar appearance and maximum absorbance times. This behaviour indicates the feasibility of using aqueous analytical solutions for calibration. Moreover, the low background absorbance observed is an indicative of the absence of spectral interferences.

The limit of detection was calculated from $3s/m$ where s is the estimated standard deviation of 10 consecutive measurements of the blank solution and m is the slope of the analytical curve. A value of 0.07 ng mL^{-1} was obtained, corresponding to $0.022 \text{ } \mu\text{g g}^{-1}$ in the original sample. This also corresponds to a 0.23 ng mL^{-1} limit of quantification in the sample solution, well below the range indicated by

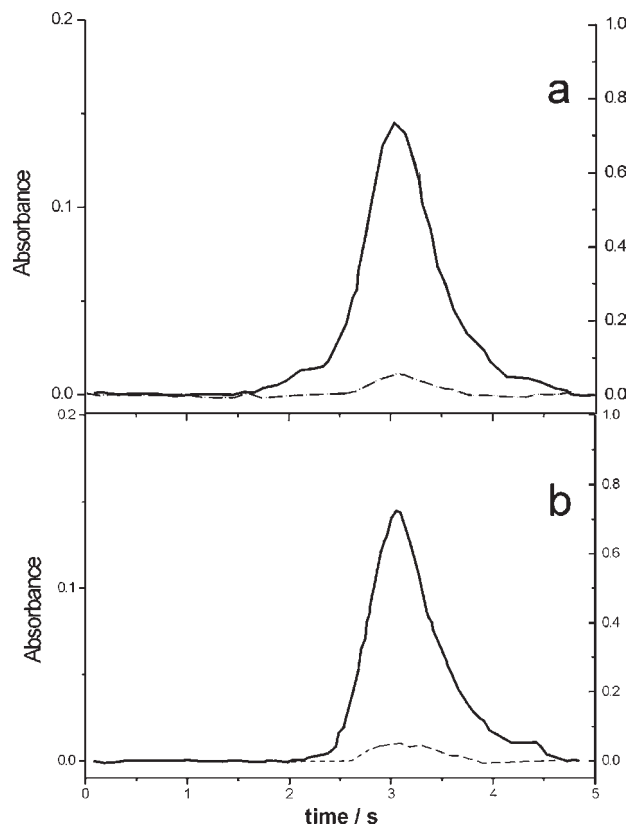


Figure 2. Atomization pulse profiles (— atomic, background) for (a) an aqueous analytical solution and (b) a bone matrix containing solution, using $\text{NH}_4\text{H}_2\text{PO}_4$ as modifier. Left scale: atomic absorption values and right scale: background values.

Pais and Benton Jones¹⁷ ($4 - 30 \text{ } \mu\text{g g}^{-1}$) for Pb in human bone. The precision of the procedure, expressed by the relative standard deviation, was 3.5%, taken in the measurement of 400 ng Pb in a sample solution.

Validation and application of the method

The method was validated by the analysis of a certified reference material (NIST SRM 1846 Bone Meal). This analysis (in triplicate) showed a good concordance between the obtained experimental value ($1.36 \pm 0.03 \text{ } \mu\text{g g}^{-1}$) and the certified value ($1.335 \pm 0.014 \text{ } \mu\text{g g}^{-1}$), confirming the accuracy of the proposed procedure. The procedure was used in the analysis of dolphin bone samples, aiming the assessment of anthropogenic environmental impact on marine mammals. The results ranged from 3 to $6 \text{ } \mu\text{g g}^{-1}$, and will be discussed in detail in a further work.

Conclusion

A phosphate ($\text{NH}_4\text{H}_2\text{PO}_4$) based compound was confirmed as a good modifier for the determination of Pb

in bone by ETAAS. External calibration with aqueous analytical solutions was possible, provided the presence of the same modifier concentration in all solutions. No additional Ca or Mg compound was necessary for a good modification action, confirmed by the high pyrolysis temperature and similarity of the peak profiles for sample and aqueous analytical solutions. The whole analytical procedure is extremely simple, providing accuracy and an adequate limit of detection for Pb-bone studies. The procedure could be made even simpler by avoiding the solution transfer to the second plastic tube. However, this would lead to less accuracy in the final volume adjustment, once the volume of the first tube is irreversibly affected due to the heating.

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