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SUMMARY

The severe matrix interference on chromium determination in whole blood samples of Southern elephant seals from Antarctica by a GFAAS (ZEISS-5 model) at 357.9 nm is demonstrated. Such interference was due to overlapping absorption spectra of chromium and iron at 357.9 nm and appeared as a systematic constant error, which was not corrected with the application of the standard addition technique. Thus, the interference was eliminated using the second resonance wavelength (359.4 nm). No iron interference was observed in nickel and lead determinations. However, for cadmium a suppression of the signal (15 -20%) was observed.

KEYWORDS:
metals, elephant seal, Antarctica, blood, GFAAS.

INTRODUCTION

The effects of environmental degradation are now being felt on regional and global levels. However, there are "remote" places, like Antarctica, which might be still considered undisturbed by humankind's activities. Data obtained from such areas may be of special importance for comparative studies and for establishing background values in seawater, biota, etc.

lt is known that organic life cannot develop and survive in the absence of metallic ions (1). Many of the heavy metals (Co, Cu, Fe, Zn, etc) are essential to metabolism at low concentrations. However, all of them can be toxic to estuarine and marine organisms when concentrations above the threshold level are reached. Some of them, such as Hg, Cd, and Pb, do not have known biological function and may greatly affect biotic communities (2).

For low-level analyses of metals various techniques are applied, including graphite furnace atomic absorption spectrometry (GFAAS), neutron activation, inductively coupled plasma-mass spectrometry (ICP-MS), and stripping voltammetry. GFAAS and ICP-MS are frequently used in clinical and hydrochemical laboratories and are capable of accurate and precise results. However, it has been demonstrated that for complex matrix samples like brines, geological, and biological samples, interference problems exist (3, 4, 5). The analysis of trace elements like Cr, Cd, Pb, and Ni in biological samples is wrought with difficulties due to low concentrations and interference problems. In such cases, great care should be taken throughout sample processing procedures to prevent contamination. In addition, applying a proper digestion procedure and diminishing the matrix interference are also extremely important. Several authors have reported interference on the determination of Cr, Cd, Pb, and Ni by GFAAS. Attempts to eliminate these interferences have been done by using matrix modifiers and optimising furnace conditions (3, 5, 6, 7). Bannon et al. used matrix standards for diminishing the interference on lead determination in blood samples (8). Quinaia and Nobrega report the using of a calibration curve prepared with Cr spiked urine correcting all potential matrix interference (9). Nevertheless, it is known that the matrix interference is not always avoided by using standard addition method, especially when systematic constant error is present (10).

In light of the above, our aim was to assess the background levels of Fe, Cu, Zn, Hg, Cd, Pb, and Cr in whole blood samples of Southern elephant seals. However, during analysis some troubles were observed especially with Cr when analysed using a ZEISS AAS-5 model. It is known that the absorption signal of Cr is greatly reduced by the presence of Fe when AAS in flame mode is used,

due to the formation of compounds, which are difficult to atomise (11). In whole blood samples of Southern elephant seals, the iron concentration found is about 700-800 µg/ mL. During Cd, Pb, Cr, and Ni determinations, in which levels are less than 15 ng/mL, iron is a likely interfering element when the samples are diluted 5 to 10 times. In this study is shown that during Cr determination by GFAAS, Fe brought about a significant overestimating of Cr concentration, demonstrating the presence of a different interference type compared with the interference encountered in flame AAS. Some data obtained and the difficulties faced are presented and discussed.

MATERIALS AND METHODS

All reagents used were Suprapur™ from Merck. The glassware was subjected to careful cleaning procedure (12). Fe, Zn, and Cu were determined by flame using a CG-AA 7000 model (Instrumentos Cientificos C.G. Ltda., Brazil). For Cd, Cr, Pb, and Ni, a ZEISS GFAAS-5 (Carl Zeiss Jena GmbH, Germany) was used. GFAAS parameters for chromium are presented in Table 1. Mercury was determined using a constructed cold vapour device coupled with the CG-AA 7000 instrument. Deuterium background correction was used in all measurements.

Southern elephant seals (*Mirounga leonina)* were captured at the Elephant Island during the XVII Brazilian Antarctic Operation (1998-1999) and blood samples were collected using plastic syringes containing heparin. After collection, whole blood samples were kept deep-frozen (-20 °C) during transportation and storage until analysis.

For sample digestion, about 2-3 g of whole blood was thawed and treated with 5-6 mL, HNO_3 cc in Teflon vials. The vials were tightly closed and heated on a hot plate at 100 °C. The temperature was then increased to 140 °C until a yellow transparent liquid was obtained. All manipulations were carried out in a laminar flow hood. Magnesium nitrate was used as matrix modifier to permit somewhat higher temperatures on the pyrolises step, particularly for Cd and Pb, but also for Cr (3). For Hg determination, an aliquot of the digested sample was treated with 0.5 mL BrC1 solution.

Quality control included the use of blanks and analysis of a tuna fish-350 reference material (IAEA, Monaco). No reference blood sample was available in our laboratory. Blanks with Milli-Q water and heparin were prepared in the sampling site. Respective blanks were also prepared during digestion procedure. Results obtained for the reference sample were in agreement (± 7 %) with the certified values. The field and laboratory digestion blanks showed concentration values below the detection limit.

TABLE 1 - GFAAS parameters employed for Cr analyses.

Step	Temp (°C)	Ramp °C/sec)	Hold (sec)	Gas flow (argon)
1	105	5	25	Max
2	300	20	15	Max
3	1100	20	15	Max
4	1100	0	6	Stop
5	2600	Max	3	Stop
6	2700	1500	3	Max

TABLE 2 - Chromium concentration (µg/g) in the whole blood of adult male Southern elephant *seals (Mirounga leonina)* measured at 357.9 and 359.4 nm wavelengths.

Sample	Wavelength (nm)	
	357.9	359.4
Blood		
1	26.4	6.58
2	24.3	6.48
3	25.5	6.74
4	25.5	4.71
5	35.6	8.76
6	37.2	9.74
Mean ± SD	29.08 ± 5.73	7.17 ± 1.80
Reference Material		
Observed	0.62	0.59
Reported	0.64	0.64

FIGURE 1
Absorption spectra of Cr in a whole blood sample of Southern elephant seal *(Mirounga leonina)* at 357.9 nm (A) and 359.4 nm (B). The small peaks correspond to the background signal.

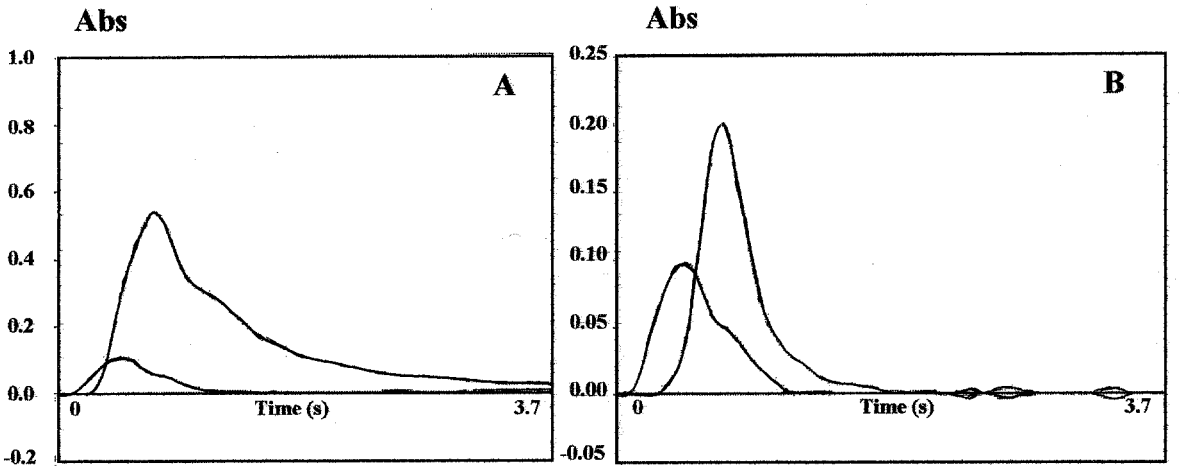

TABLE 3
Metal concentrations in whole blood samples of adult male Southern elephant seals (*Mirounga leonina)* from Antarctica and reference material (tuna fish-350). Data are means ± SD (n=6). Hg, Pb, Cd and Cr in ng/g; Fe, Cu and Zn in µg/g.

Metal	Sample		
	Blood	Tuna 350	
		observed	reported
Hg	99.50 ± 15.40	4.47	4.68
Pb	9.20 ± 2.15	0.092	0.10
Cd	3.79 ± 1.23	0.022	0.020
Cr	7.10 ± 1.79	0.61	0.65
Fe	700.60 ± 28.5	69.8	72.1
Zn	3.13 ± 0.11	17.9	17.4
Cu	1.04 ± 0.04	2.83	2.69

RESULTS AND DISCUSSION

Table 2 summarizes the measurement of Cr performed in some whole blood samples of Southern elephant seals and reference material (tuna fish-350), at two wavelengths (357.9 and 359.4 nm). It is known that the sensitivity obtained at 357.9 nm is slightly better than that at 359.4 nm. However, a significant interference at 357.9 nm seems to occur. At this wavelength, the highest peak that corresponds to the Cr is very big and asymmetric, due to a matrix effeet (Fig. 1A). On the other hand, the peak is rather symmetric and the interference seems to have been removed at 359.4 nm (Fig. 1B). Furthermore, such significant difference was not observed for Cr when the reference material (tuna fish-350) was analyzed (Table 2).

These results indicate that the discrepancy appears only in the blood sample matrix. Considering that Fe concentration in tuna fish sample is relatively low, we believe that the discrepancy observed in blood samples might be due to high iron concentration in the final digested blood solution, which was diluted no more than 5 times. Consequently, the Fe concentration was in the 100-200 ppm range. The observed interference is likely due to spectra overlapping due to the presence of Fe, which absorbs at 358.1 nm, a wavelength very close to 357.9 nm. Such spectral interference produced a systematic constant error on Cr assessment, which was confirmed by the ineffectiveness of the standard addition method.

For Fe interference assessment, synthetic samples were prepared containing 4 µg/L Cd, Pb, Ni, and Cr, while Fe was added in concentrations similar to those found in diluted samples (100 to 200 mg/L) analysed. Unfortunately, the blank of Fe prepared from Fe stock solution showed relatively high Cr contamination (about 2 µg/L Cr). In spite of this, an asymmetric and big peak, similar to that presented in Fig. 1A was obtained at 357.9 nm, whereas at 359.4 nm a similar peak response

was not observed. This result supports the idea that the high Fe concentration in the blood sample is causing interference in the Cr determinations. No interference of Fe was observed during Ni and Pb determination, while for Cd a suppression of the signal was observed (15-20 %). Some data of metal concentrations in the whole blood samples of Southern elephant seals from Antarctica are presented in Table 3.

In conclusion, our results suggest that the second resonance wavelength (359.4 nm) should be used for Cr determination in blood samples when a graphite furnace ZEISS AAS-5 instrument is used. They also indicate that the strong interference in Cr determinations at 357.9 nm is due to high Fe concentration in the blood sample

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