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Instrumental Neutron Activation Analysis of Rib Bone Samples and of Bone Reference Materials

M. SAIKI,*[,1] M. K. TAKATA,[1] S. KRAMARSKI,[1] AND A. BORELLI[2]

[1]*Instituto de Pesquisas Energéticas e Nucleares, IPEN-CNEN/SP, Radiochemistry Division, P.O. Box 11 049, CEP 05422-970, São Paulo, SP, Brazil; and* [2]*Faculdade de Medicina-USP, Av. Dr. Arnaldo 455, CEP 01246-903, São Paulo, SP, Brazil*

ABSTRACT

The instrumental neutron activation analysis method was used for the determination of trace elements in rib bone samples taken from autopsies of accident victims. The elements Br, Ca, Cl, Cr, Fe, Mg, Mn, Na, P, Sr, Rb, and Zn were determined in cortical tissues by using short and long irradiations with thermal neutron flux of the IEA-R1m nuclear reactor. The reference materials NIST SRM 1400 Bone Ash and NIST SRM 1486 Bone Meal were also analyzed in order to evaluate the precision and the accuracy of the results. It was verified that lyophilization is the most convenient process for drying bone samples because it does not cause any element losses. Comparisons were made between the results obtained for rib samples and the literature values as well as between the results obtained for different ribs from a single individual and for bones from different individuals.

Index Entries: Rib bone; neutron activation analysis, bone reference materials, trace elements.

INTRODUCTION

In the last few years, there has been an increasing interest in determining trace elements in biological tissues in order to elucidate their roles in human beings as well as to diagnose diseases *(1,2)*. Trace element studies have also been undertaken in bones because they are deposits of essential and toxic elements. Mineral contents of bones can be good indicators for the detection of different diseases, such as osteoporosis *(3)*.

*Author to whom all correspondence and reprint requests should be addressed.

Osteoporosis is one of the primary metabolic and degenerative bone diseases characterized by a low mineral bone mass, although the remaining bone is normal. Today at least 10 million individuals in Brazil aged more than 60 yr are suffering from this disease. Saltman and Strause (4) studied the role of trace minerals in osteoporosis and confirmed that adequate amounts of dietary Ca maintains optimal bone mineral density (BMD) in post-menopausal women and Cu, Mn, and Zn are essential to the maintenance of BMD.

Analyses of trace elements in bones are very rare, as they are examples of biological samples presenting difficulties of obtaining representative specimens for chemical analyses. In the case of humans, collecting samples generally presents problems because of medico-legal implications.

The determination of trace elements in bone samples have been carried out using several techniques *(5)* including in vivo and in vitro nuclear methods *(6,7)*.

In this work, the instrumental neutron activation method was applied in order to determine preliminary results on trace elements in ribs. The precision and the accuracy of the results were also evaluated by analyzing the reference materials NIST SRM 1400 Bone Ash and NIST SRM 1486 Bone Meal.

MATERIALS AND METHOD

Sample Collection and Preparation

Human rib bone samples were obtained from autopsies of accident victims. The autopsies were performed at the Institute of Forensic Medicine of the Mogi das Cruzes University, SP. The samples were wrapped in polyethylene foils and stored in a freezer until they were treated for analysis. The ribs were cleaned free of connected soft tissues (periosteum) and were washed with distilled water to remove the blood. The cortical tissues were broken up into small pieces and then freeze-dried for analyses.

Instrumental Neutron Activation Analysis

Aliquots of about 200 mg of sample weighed in polyethylene envelopes were irradiated in the IEA-R1m nuclear reactor along with the synthetic standards of the elements. The synthetic standards were prepared by pipeting the elemental solutions onto pieces of Whatman No. 41 filter paper. These standard solutions containing one or more elements were prepared from standard solutions provided by Spex Chemical. Two procedures were used for the irradiations. Irradiations of 1 min at the pneumatic rabbit station with thermal neutron flux of about 10^{12} n/cm^2/s were used for determining the elements Ba, Ca, Cl, Mg, Mn, Na, P, and Sr: Longer irradiations of 8 h under thermal neutron flux of 10^{12} n/cm^2/s

Table 1
Elemental Concentrations in Cortical Rib Bone
from Healthy Individuals

Element	This work		Ref (8)
	$X_M \pm s_M$	Range	$X_M \pm s_M$
Br	0.83 ± 0.23	0.55 - 1.27	4.1 ± 4.0
Ca, %	20.5 ± 0.8	19.4 - 21.7	20.0 ± 4.1
Cl	547 ± 183	217 - 716	
Fe	18.7 ± 14.6	3.4 - 44.4	23 ± 11
K	843 ± 200	529 - 1182	
Mg, %	0.30 ± 0.04	0.23 - 0.37	0.26 ± 0.04
Na, %	0.46 ± 0.07	0.37 - 0.56	0.54 ± 0.10
P, %	9.47 ± 1.5	7.4 - 12.1	8.8 ± 2.2
Rb	1.33 ± 0.43	0.84 -2.10	2.1 ± 3.0
Sr	100.4 ± 9.9	84 - 113	62 ± 18
Zn	91.1 ± 14.3	78 - 120	180 ± 44

Note: $X_m \pm S_M$ arithmetic and standard deviation; number of individuals = 6.

Results are given in µg/g, dry weight, unless otherwise indicated.

were used for Ba, Br, Ca, Cr, Na, Fe, Rb, Sr, Sc, and Zn determinations. After appropriate decay times, the gamma activities of the samples and the elemental standards were measured using a EG & G Ortec hyperpure Ge detector with a resolution 0.90 keV for a 122-keV gamma-ray of ^{57}Co and 1.98 keV for 1332 keV of ^{60}Co. This detector is connected via TRUMP card to a microcomputer with EG&G Ortec software (MAESTRO). For P analyses, the beta activity of ^{32}P was measured in a Geiger–Muller detector. The gamma spectra were processed using VISPECT software. The comparative method was used for calculating the content of the respective elements. The radioisotopes used in this study were: ^{139}Ba, ^{131}Ba, ^{82}Br, ^{49}Ca, ^{47}Ca, ^{38}Cl, ^{51}Cr, ^{59}Fe, ^{27}Mg, ^{56}Mn, ^{24}Na, ^{86}Rb, ^{87m}Sr, ^{85}Sr, ^{46}Sc, and ^{65}Zn.

Analysis of Certified Reference Materials

The certified reference materials NIST SRM 1400 Bone Ash and NIST SRM 1486 Bone Meal were analyzed under the same conditions as for the analyses of bone samples. The concentrations of reference materials were evaluated on a dry weight basis, as recommended in their respective certificates. The following values (in percent) of weight loss were found for correcting the final results: 0.26 for Bone Ash and 2.71 for Bone Meal.

Table 2
Comparison of Elements in Two Different Ribs from the Same Individual and Ribs from two Different Individuals

Element	Individual No. 1		Individual No. 2
	Rib 1	Rib 2	Rib 3
Br	0.81 ± 0.06	1.05 ± 0.88	0.77 ± 0.06
Ca, %	19.4 ± 0.5	21.0 ± 0.1	21.2 ± 0.4
Cl	708 ± 37	647 ± 21	508 ± 19
Fe	21.3 ± 2.7	13.4 ± 1.8	14.0 ± 3.9
K	836 ± 75	831 ± 70	1182 ± 212
Mg, %	0.31 ± 0.01	0.29 ± 0.01	0.350 ± 0.016
Na, %	0.422 ± 0.003	0.51 3± 0.007	0.37 ± 0.006
P, %	8.7 ± 0.1	9.1 ± 0.1	9.1 ± 0.1
Rb	1.26 ± 0.70	1.05 ± 0.88	2.10 ± 0.24
Sr	100 ± 10	84 ± 13	113 ± 9
Zn	81.8 ± 0.7	83.8 ± 0.7	83 ± 1

Note: (Results are given in μg/g of dry weight, unless otherwise indicated

RESULTS AND DISCUSSION

Elemental concentrations obtained in rib samples are presented in Table 1 along with literature values. These results indicated a good precision with relative standard deviations varying from 3.8% to 14%. The less precise results were obtained for Fe, probably the result of the rib contamination by blood containing high levels of this element. Besides, most results obtained for human ribs are within the range values for healthy individuals reported in the literature *(8)*.

The short irradiation procedure has the advantage of presenting a quick response for the analysis of several elements. Even though there is no spectral interference of bremsstrahlung, this irradiation condition does not allow the analysis of elements such as Br, Fe, Rb, Sc, and Zn. The lyophilization was the most convenient process for drying the sample because it does not cause any loss of elements. Samples prepared by calcination showed loss of Br and Cl. Comparison of the results obtained for different ribs from a single individual as well as from different individuals did not show a significant difference. These results are presented in Table 2.

Tables 3 and 4 present results obtained for the reference materials NIST SRM 1400 Bone Ash and NIST SRM 1486 Bone Meal, respectively. In these tables, certified values are also presented for comparison. The mean values found in the analysis of reference materials are in good agreement

Table 3
Concentrations of Elements in Reference Material NIST SRM 1486 Bone Meal

Element	n	This work		Ref. (9)
		X ± s	s_r	
Ba, µg/g	6	251 ± 29	11.7	
Ca, %	9	26.0 ± 1.8	7.0	26.58 ± 0.24
Cl, µg/g	7	194 ± 15	7.8	
Fe, µg/g	6	89 ± 12	13.4	99 ± 8
Mn, µg/kg	6	1008.1 ± 106.6	11.5	1000 (N)*
Mg, µg/g	5	4530 ± 206	4.5	4660 ± 170
Na, µg/g	7	4508 ± 241	5.4	5000 (N)
P, %	5	11.5 ± 0.9	7.5	12.30 ± 0.19
Sr, µg/g	10	233.0 ± 26.1	11.2	264 ± 7
Zn, µg/g	6	133 ± 8	6.2	147 ± 16

Note: n = number of determinations; (N) = informative value.

Table 4
Concentrations of Elements in Reference Material NIST SRM 1400 Bone Ash

Element	n	This work		Ref.(10)
		X ± s	s_r	
Ba, µg/g	6	231 ± 18	7.7	
Ca, %	8	36 ± 2	5.9	38.18± 0.13
Cl, µg/g	6	211 ± 21	10.3	
Cr, µg/g	5	2.9 ± 0.5	17.0	
Fe, µg/g	6	597 ± 27	4.2	660 ± 27
Mn, µg/kg	5	17.3 ±0.7	4.0	17 (N)
Mg, µg/g	5	6384 ± 300	4.7	6840 ± 130
Na, µg/g	4	4925 ± 287	5.8	6000 (N)
P, %	4	17.1 ± 0.6	3.4	17.91 ± 0.19
Sb, µg/kg	4	558 ± 41	7.4	
Sc, µg/kg	5	65.9 ± 7.8	11.9	
Sr, µg/g	8	224 ± 19	8.3	249 ± 7
Zn, µg/g	6	168 ± 11	6.4	181 ± 3

Note: n = number of determinations; (N) = informative value.

with those reported by NIST. The relative errors obtained are lower than 11.7%. The precision of the results was satisfactory for most elements, with relative standard deviations varying from 3.4% to 11.8%. The less precise results were obtained for Fe in bone meal with a relative standard deviation of 13.4% and for Cr in bone ash with a relative standard deviation of 17%. The results obtained for Ba, Cl, Cr, Mn, Na, Sb, and Sc in these reference materials constitute a contribution for their certification.

Results obtained in this work confirm that the INAA is a suitable method for bone analysis because of its multielemental character, the absence of a destruction step, and its good quality results.

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