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Application of neutron activation analysis to bone samples

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Neutron activation analysis technique, using Au as flux monitor, was applied to determine element concentrations of Ca, K, Na, Mg, Mn and Sr in certified reference material (NIST 1400 Bone Ash). The results were compared with those using comparative INAA and they were compatible. The same results were obtained using the recommended k_0 factors, in order to obtain the activation cross section as input in concentration for the same reference material. Some applications in the health area aiming clinical evaluation in bone samples of medium and small-sized animals were performed and the viability of using this methodology was discussed.

Introduction*

In the last years the Nuclear Structure Laboratory (LEN) at IPEN has been investigated different biological material using NAA aiming to optimize this nuclear procedure for diagnostic application. Based on it, several works have been done using comparative INAA as well as NAA with Au as flux monitor, i.e., a variant of k_0-NAA,[1] or combination of both techniques using the different experimental facilities at IPEN: the IEA-R1 and MB-01 nuclear reactors and neutron irradiator.[1–8] Most of them are part of a research project in the medical area aiming to establish trace elements reference values in body fluids (urine, whole blood and serum) of small and medium sized guinea-pigs as well in human being for checking the similarities.[3–8] Using these data, it is possible to select the convenient animal for investigation of new medicines, vaccines, antibiotics, for example, as well as for organs transplantation before to be tested in human being. Now, we want to extend this analysis to bone samples. The relevancy of studying also this biological material is that bone is one of the most important biological accumulators of a great number of radionuclides, consequently detailed studies of these nuclides in bone are important for radiological prevision.[9] Another important aspect that also could be explored is relate to its use as an alternative for diagnosing anomalies in bone.

For studying bones the biological material is scarce or difficult to obtain, mainly for human being. However, if the knowledge of the elemental composition from several guinea-pigs are well established, these data could be an indicator for human prevision, i.e., these data could gave us some information about the radionuclides transfer to the bones of human beings.[7]

To perform this investigation bone samples of Wistar, Cobb and Beagles were used. These samples came from the facilities of the UNITOX Laboratory from the Universidade Santo Amaro (UNISA). The animals were not sacrificed for this experiment; the biological materials were donated to us when they must be sacrificed.

Particularly small-sized animal such as Wistar are very convenient to perform this kind of investigation due to low cost, easy handling and medico-legal implications,[10] while Beagles are selected for medical diagnostic studies because of their physiological similarities with the humans.[11] Regarding Cobb, it is also interesting to perform the analysis because it belongs to human food habits and it is very consumed by the Brazilian population.

For the development of this investigation, NIST 1400 Bone Ash[12] was first analyzed to verify the accuracy and precision of the results. After that, the analyses of bone samples of the selected animals were performed.

Experimental

The biological sampling procedure was performed at the Universidade Santo Amaro facilities. Each bone sample was calcinated for about 4 hours at 600 °C, ground and homogenized and the ashes, after weighing, were sealed into an individual polyethylene bag to be irradiated. A total of 6 samples of bone ashes for each species (~20 mg, for Wistar and ~50 mg for Cobb and Beagle) were analyzed in replicate. We used different aliquots of the same sample, of each species, to verify its homogeneity. To perform measurements using reference material 3 samples (~150 mg each) of NIST SRM 1400 Bone Ash[12] were prepared and analyzed in replicate. All the results are a mean value and the errors associated represent one standard deviation.

In order to determine the concentration of the elements in the certified biological reference material, the cadmium ratio technique was used for the

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measurement of thermal and epithermal neutron flux distribution.[1] In this technique, Au foils, both bare and Cd covered, are irradiated together with the biological sample in the IEA-R1 nuclear reactor at IPEN/SP (IEA-R1, 2-4MW, pool type), for a few minutes, allowing the simultaneous activation of these materials under the exact same irradiation conditions. Using this procedure the γ-ray activities induced in the Au foils by both the thermal and epithermal neutrons were obtained as well as the activation of biological sample. A γ-spectrometer system with a semiconductor detector connected to an ADCAM multichannel analyzer and to a PC computer was then used to measure the induced gamma-ray activity. The detector was a HPGe of high resolution (FWHM=1.87 keV) calibrated for energy and efficiency through the measurements of standard sources of ^{56}Co and ^{152}Eu. All gamma spectra analysis evaluations were performed using the IDF computer code,[13] which locates peak position, identifies the energies and calculates net areas. The concentration of each element was then obtained by using an in-house software,[14] which correlated the measured parameters, i.e., neutron flux, net area and efficiency of the selected gamma-ray with the constants involved (the decay constant; the atomic mass, the Avogadro's number, the cross section for the selected capture reaction, the isotopic fraction and the intensity of the gamma-ray). In a running of the software, the isotope that will be determined must be selected by the user and all the physical constants are previously defined. After that, the user needs to input data about: irradiation time, counting time and waiting time (the time elapsed between the end of the irradiation and the start of the counting) and the sample mass as well as the measured parameters.

As the uncertainty associated to measurement of neutron flux has been estimated around 3 to 4%, and for the efficiency values in less than 3%, then the uncertainty that could be associated to the concentration value at the minimum is near to 4%. But, for some isotopes, there are some parameters such as activation cross section and isotopic abundance that have high uncertainty (e.g., ^{48}Ca, with ~13% for its cross section).[15] In this sense, it is more realistic to hope an uncertainty at minimum around to 4% until 14% in the concentration value, for the elements measured in this report.

Results and discussion

The time optimization used in this experiment to perform these analyses (irradiation time of 2 minutes; counting time of 1 minute for each Au foil and 20 minutes for the biological sample and background radiation) previously established[6] allowed us to conclude the analysis of each sample in about two hours or less, making this nuclear procedure agile. The element concentrations of certified biological reference material by using NAA has Au as flux monitor are shown in Table 1. The results from the comparative method (INAA)[16] and those obtained using activation cross section from recommended k_0-factors[17] are also presented allowing a comparison between these methodologies. The concentration of the elements as well as the detection limit[18] in bone ashes samples of Wistar, Cobb and Beagles are shown in Tables 2, 3 and 4, respectively. The results are a mean value and the errors associated represent one standard deviation (1σ).

Table 1. Analysis of certified reference material NIST 1400 Bone Ash[12] by using Au monitor NAA. The results from the comparative INAA measurements as well as the results obtained using the recommended k_0 factors,[17] in order to obtain the activation cross section as input in concentration for the same reference material, are also presented for comparison

Element	NAA using Au as flux monitor	Comparative INAA[16]	NAA using k_0-factors[17]	Certified value[12]
Ca, %	34.08 ± 4.55	38.08 ± 0.35	33.08 ± 1.25	38.18 ± 0.13
	38.15 ± 1.94[a]			
Cl, μg·g^{-1}	245 ± 23	364 ± 117	235 ± 22	–
K, μg·g^{-1}	173 ± 22[b]	N.D.[c]	183 ± 29	186 ± 8
	180 ± 34			
Mg, %	0.651 ± 0.034	0.647 ± 0.023	0.670 ± 0.034	0.684 ± 0.013
Mn, μg·g^{-1}	16.1 ± 0.8	15.9 ± 0.4	16.3 ± 0.8	(17)[d]
Na, %	0.61 ± 0.04	0.571 ± 0.002	0.62 ± 0.04	(0.6)[d]
Sr, μg·g^{-1}	255 ± 26	256 ± 17	268 ± 20	249 ± 7

[a] Activation cross section adopted from Reference 19.

[b] Time of irradiation was 5 minutes.

[c] Element not detected.

[d] Numbers in parentheses are reported values.

Table 2. The concentration of Ca, Cl, K, Mg, Na and Sr in bone samples of Wistar

Element	Concentration, $g \cdot kg^{-1}$	Detection limit, $g \cdot kg^{-1}$
Ca	321 ± 19[a]	0.26
Cl	0.75 ± 0.06	0.03
K	6.99 ± 0.18	0.50
Mg	0.44 ± 0.04	0.35
Mn	N.D.[b]	–
Na	3.09 ± 0.12	0.01
Sr	0.41 ± 0.05	0.03

[a] Activation cross section adopted from Reference 19.

[b] Element not detected.

Table 3. The concentration of Ca, Cl, K, Mg, Mn, Na and Sr in bone samples of Cobb

Element	Concentration, $g \cdot kg^{-1}$	Detection limit, $g \cdot kg^{-1}$
Ca	261 ± 15[a]	0.51
Cl	9.6 ± 0.4	0.05
K	4.98 ± 0.48	0.98
Mg	16.8 ± 0.7	0.69
Mn	0.072 ± 0.003	0.004
Na	14.14 ± 0.50	0.03
Sr	0.37 ± 0.03	0.05

[a] Activation cross section adopted from Reference 19.

Table 4. The concentration of Ca, Cl, K, Mg, Mn, Na and Sr in bone samples of Beagles

Element	Concentration, $g \cdot kg^{-1}$	Detection limit, $g \cdot kg^{-1}$
Ca	331 ± 17[a]	0.58
Cl	0.29 ± 0.03	0.03
K	0.65 ± 0.10	0.49
Mg	4.2 ± 0.3	0.21
Mn	0.0202 ± 0.0012	0.003
Na	7.39 ± 0.23	0.02
Sr	0.25 ± 0.02	0.05

[a] Activation cross section adopted from Reference 19.

According to Table 1, our results were in general compatible with previous ones obtained from the certified reference material,[12] with those from the comparative INAA[16] (based on the use of standards for each element to be detected) and using the activation cross-section from k_0-factors,[17] suggesting that this nuclear technique can be used to perform analysis in bone. In the particular case of K concentration the comparison between the methods was not possible and for Cl the obtained concentration using this comparative INAA has higher uncertainty if compared with the other methods. This apparently contradictory result, in the case of Cl, could be explained if we take into consideration the experimental procedure used to perform the instrumental analysis. As the comparative method could demand more time (mainly when short-lived radionuclides are involved and usually several irradiation must be done), we decide to check the results from comparative INAA performing these measurement using the time optimization conditions previously established[6] (it means: one irradiation time of 2 minutes, counting time of 1 minute for each Au foil and 20 minutes for the biological sample and background radiation) so, the same experimental procedure was used in the present measurements. This way, Mg and Ca were first analyzed by their short-lived radionuclides and, consequently, when Cl was gamma-counted it had poor statistics. Relating to the result obtained for K, it can be explained if we consider that two different gamma-spectrometers have been used and, although the nuclear instrumentation were very similar, the efficiencies were not the same. Regarding Ca, where the uncertainty associate to the activation cross section is around 13% (1.09±0.14 b),[15] we performed a new determination with more precise value (0.97±0.03 b)[19] and the result was in agreement with the measurements performed by BEER et al.[20] (0.982±0.046 b), so it was also included in Table 1. The Ca concentration calculation using the activation cross-section obtained from the k_0-factors[17] (1.124±0.013 b) is reported in this table too.

The data presented in this study, in connection with other recently measurements involving genetic modified food, using Au as monitor to perform NAA and PIXE technique,[21] confirm its potential for quantitative analyses. At the moment, another important study, that shows its reproducibility, is in progress using the MB-01 nuclear reactor also at IPEN facilities. In this work Al has been investigated in whole blood and serum for clinical diagnostic in patients undergoing long-term dialysis.[22] Particularly, in this study the results from blood and serum analysis by using Au as monitor have been compared with those from AAS and ICP-MS and they are compatible.

In relation to bone investigation we also performed comparative analyses using Au as monitor to perform NAA and also XRF for determination of trace elements in poultry tibia bone[23] and the results were compatible. Although several studies have been performed successfully using this methodology, other reference materials must be analyzed to validate the method. However, the results of SRM 1400 Bone Ash, presented in this paper indicate that this methodology could be useful when the neutron flux is very stable, in the case of neutron irradiator also available for our measurements;[24] when standards are not available and as an alternative to perform studies that involve a large number of samples for quantitative analyses of several elements, such as, e.g., routine analysis in hematology,[6,22] although the results obtained using indirectly the k_0-method for these applications could be used with the advantage to induce small relative uncertainty.

Conclusions

The analyses performed in bone samples of small and medium size-animals indicate that this method could be an alternative for bone diagnostic studies. The results of reference material analysis demonstrate compatibility with results obtained from (single element standards) comparative INAA and results calculated on basis of the activation cross section parameters extracted from the k_0 factors.

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References

1. L. C. OLIVEIRA, C. B. ZAMBONI, F. A. GENEZINI, A. M. G. FIGUEIREDO, G. S. ZAHN, J. Radioanal. Nucl. Chem., 263 (2005) 783.
2. C. B. ZAMBONI, G. S. ZAHN, O. A. SANT'ANNA, in: Proc. VI SLAFNAP, Argentina, 2005.
3. L. C. OLIVEIRA, C. B. ZAMBONI, J. MESA, in: Proc. XXVIII RTFNB, Brazil, 2005.
4. L. C. OLIVEIRA, C. B. ZAMBONI, P. S. LINS, M. R. AZEVEDO, Brazilian. J. Phys., 35 (2005) 793.
5. L. C. OLIVEIRA, C. B. ZAMBONI, G. S. ZAHN, M. A. MASCHIO, M. P. RAELE, Brazilian J. Phys., 34 (2004) 811.
6. C. B. ZAMBONI, L. C. OLIVEIRA, L. DALAQUA Jr., in: Proc. ANES, USA, 2004.
7. J. D. T. ARRUDA-NETO, M. V. M. GUEVARA, G. P. NOGUEIRA, I. D. TARICANO, M. SAIKI, C. B. ZAMBONI, J. MESA, Intern. J. Rad. Biol., 250 (2004) 10.
8. L. C. OLIVEIRA, C. B. ZAMBONI, A. C. CESTARI, L. DALAQUA Jr., M. V. MANSO, A. M. G. FIGUEIREDO, J. T. ARRUDA-NETO, Rev. Bras. Pesq. Des., 4 (2002) 1035.
9. L. TANDON, G. V. IYENGAR, R. M. PARR, Appl. Radiation Isotopes, 49 (1998) 903.
10. D. RANDALL, W. BURGGREN, K. FRENCH, R. FERNALD, Fisiologia Animal Mecanismos e Adaptações, Ed. Guanabara, Brazil, 2000.
11. N. P. SINGH, M. E. WRENN, Health Phys., 57 (1989) 91.
12. NIST, Certificate of Analysis, Standard Reference Material 1400 Bone Ash, National Institute of Standards and Technology, Gaithersburg, 1992.
13. P. GOUFFON, Manual do Programa IDF, Instituto de Física da Universidade de São Paulo, Laboratório do Acelerador Linear, Brazil, 1982.
14. J. A. G. MEDEIROS, C. B. ZAMBONI, G. S. ZAHN, L. C. OLIVEIRA, L. DALAQUA Jr., in: Proc. 39º CBPC, Brazil, 2005.
15. S. F. MUGHABGHAB, M. DIVADEENAM, N. E. HOLDEN, Neutron Cross-Sections from Neutron Resonance Parameters and Thermal Cross-Sections, Academic Press, London, 1981.
16. A. M. G. FIGUEIREDO, C. B. ZAMBONI, M. SAIKI, L. C. OLIVEIRA, J. A. G. MEDEIROS, in: Proc. V SLAFNAP, Brazil, 2003.
17. F. DE CORTE, A. SIMONITS, At. Dat. Nucl. Tables, 85 (2003) 47.
18. L. A. CURRIE, Anal. Chem., 40 (1968) 586.
19. G. S. ZAHN, C. B. ZAMBONI, L. C. OLIVEIRA, in: Proc. INAC, VII ENAN, Brazil, 2005.
20. H. BEER, C. COCEVA, P. V. SEDYSHEV, Y. P. POPOV, H. HERNDL, R. HOFINGER, P. MOHR, H. Phys. Rev., 54 (1996) 2014.
21. I. M. M. A. MEDEIROS, C. B. ZAMBONI, J. A. G. MEDEIROS, M. A. RIZZUTTO, N. ADDED, M. H. TABACNIKS, Brazilian. J. Phys., 35 (2005) 814.
22. N. SANTOS, C. B. ZAMBONI, F. F. LIMA, E. C. VILELA, in: Proc. XXVIII RTFNB, Brazil, 2005.
23. A. C. CESTARI, J. D. T. ARRUDA-NETO, C. B. ZAMBONI, L. C. OLIVEIRA, A. C. PEREZ, O. D. GONÇALVES, S. D. MAGALHÃES, in: Proc. V SLAFNAP, Brazil, 2003.
24. K. SHTEJER, C. B. ZAMBONI, G. S. ZAHN, J. Y. Z. CHÁVES, Appl. Radiation Isotopes, 59 (2003) 263.