PHOSPHORUS AND CALCIUM ANALYSIS IN BONE BY ACTIVATION WITH 14 MeV NEUTRONS.

J. R. Berretta, I. I. L. Cunha
Instituto de Pesquisas Energéticas e Nucleares
IPEN-CNEN/SP Caixa Postal 11049 - Pinheiros
São Paulo - Brasil

SUMMARY. Instrumental procedures for phosphorus and calcium analysis in bone by activation with 14 MeV generated by a Van de Graaff accelerator are presented. The variation of the neutron flux was followed by a BF_3 detector. Mathematical equations used for the normalization of neutron flux during the irradiation are reported. Calcium analysis was performed by cyclic irradiation. Results obtained for the IAEA reference material A3/74 were $(15.6 \pm 1.8)\%$ for phosphorus and $(31.8 \pm 4.1)\%$ for calcium.

key words: phosphorus, calcium, bone, 14 MeV neutrons, instrumental method.

INTRODUCTION

Analysis of phosphorus and calcium in bone is of great interest because they are essential for the body and the role of these elements in the development of bone diseases is of special concern in recent years.

Fast neutron activation followed by gamma spectrometry is an useful technique for the determination of calcium and phosphorus[1-2].

The aim of this work was to develop analytical methods for the phosphorus and calcium analysis *in bone by means of activation with 14 MeV* neutrons. Phosphorus was determined via $^{31}P(n,\alpha)^{28}Al$, while calcium analysis was based on the $^{44}Ca(n,p)^{44}K$ reaction.

The main error sources in the analysis using Van de Graaff accelerator are associated to the low neutron flux produced by the accelerator as well as to the fluctuation of neutron flux during the irradiation[3]. In this work, these problems were overcome by employing cyclic irradiation and by the normalization of all counting rates. The neutron flux fluctuation was followed by a BF_3

detector. The ratio of neutron flux by the counting registered at the BF_3 detector was obtained employing an aluminium monitor. This ratio was used to normalize the counting rates observed during the sample irradiation.

Mathematical equations[4] used in order to avoid errors in the counting rates due to the fluctuation of neutron flux at the bombardment of samples are presented and discussed in details.

Analysis methods were applied in IAEA reference material.

MATERIALS AND METHODS

Ge detector calibration

A efficiency calibration of Ge detector at a short source-to-detector distance was performed using several nuclides emitting gamma rays, according to *KAWADE et al*[5].

BF_3 detector calibration

The neutron flux was determined by irradiation of 99.999% pure aluminium foil (44.3 mg, 7 mm diameter and 1mm thickness) wrapped in cadmium box for 10 minutes and measuring the activity of ^{24}Na produced via $^{27}Al(n,\alpha)^{24}Na$ reaction.

The neutron flux produced during the irradiation was calculated by means of the neutron activation equation and its parameters, such as content of aluminium, nuclear parameters of the reaction above mentioned and the counting rate obtained for ^{24}Na. The value (ϕ_m) was related to the medium counting of neutrons (C_m) registered in the BF_3 detector, being obtained the value of $\phi_m/C_m=3.34 \times 10^4$ n.cm^{-2} per counting of the BF_3 detector with a error of 3.2%[6].

Reference material

Calcined Animal Bone (A3/74) from the International Atomic Energy Agency (15.5% P; 31.3% Ca).

Phosphorus analysis procedure

Calcined Animal Bone (A-104 code) from calves (100 mg) and reference material (77 to 300 mg) were pressed into pellets (7 mm diameter), weighed (mass varied from 30 to 300 mg) and transferred to polyethylene bags. These vials were inserted into cadmium box and irradiated for 5 minutes under a neutron flux of 3×10^7 n.cm^{-2}.s^{-1}. After a decay time of about 60 seconds, samples were counted in a Ge detector for 5 minutes, and the photopeak count rate of the 1778.8 keV line of the ^{28}Al was evaluated by the Maestro II (ORTEC) software.

The neutron counting spectrum of the BF_3 detector obtained during the irradiation period was divided in small time intervals (10 seconds). It was possible to calculate the neutron flux of each

interval (ϕ_t) by means of the counting registered in each one of these intervals (C_t), and the value ϕ_m/C_m obtained in the BF_3 calibration, according to the equation 1

$$\phi_t = \frac{C_t}{C_m}\phi_m \quad (1)$$

The fraction of phosphorus in the sample (F) was determined using the following relation:

$$F = \frac{C_{obs}\,\lambda\,M}{N\,m\,\sigma\,f\,\varepsilon\,f_\gamma\left(1-e^{-\lambda t_i}\right)\left(1-e^{-\lambda t_c}\right)\sum_{t=1}^{n}\phi_t e^{-\lambda t_{ct}}} \quad (2)$$

where:

C_{obs} = Total number of counts under the photopeak;

λ = decay constant;

M = atomic weight;

N = Avogadro's number;

m = mass of the irradiated element;

σ = cross section of the reaction;

f = isotopic abundance;

ε = detector efficiency;

f_γ = absolute transition probability by gamma decay;

t_i = irradiation time;

t_c = counting time, and;

t_{ct} = waiting time between the end of irradiation of the interval t and beginning of the counting at the gamma system.

Blank analysis

Samples of blank consisted of polyethylene bags, that were irradiated and counted at the same conditions of the bone samples. The value of phosphorus found in the blank sample by means of equation 2 was discounted from the level of phosphorus determined in the analyzed samples.

Calcium analysis procedure

Calcium analysis was carried out by cyclic irradiation. Sample preparation was similar to phosphorus analysis. Samples inserted into cadmium box were irradiated for 10 minutes. After a decay time of 2 minutes, gamma counting was performed for 10 minutes. The irradiation cycle was repeated 5 times and the counting spectrum of each cycle was accumulated in the

multichannel analyser. Calcium was analyzed by the photopeak of ^{44}K of 1157 keV.

From equation 2, the counting obtained at the end of the first irradiation cycle is:

$$C_{obs_1} = k \sum_{t=1}^{n} (\phi_1)_t \, e^{-\lambda t_{et}} \tag{3}$$

where:

$$k = \frac{N \, m \sigma f \, F \varepsilon f_\gamma \left(1 - e^{-\lambda t_i}\right)\left(1 - e^{-\lambda t_c}\right)}{\lambda M} \tag{4}$$

For the second cycle of irradiation;

$$C_{obs_2} = k \left[\sum_{t=1}^{n} (\phi_2)_t \, e^{-\lambda t_{et}} + \left(\sum_{t=1}^{n} (\phi_1)_t \, e^{-\lambda t_{et}} \right) e^{-\lambda T} \right] \tag{5}$$

where:

t_e = waiting time;

t_r = return time of the sample to the next cycle irradiation, 2 minutes;

T = the total time of the cycle irradiation; $T = t_i + t_e + t_c + t_r$.

For the third irradiation cycle;

$$C_{obs_3} = k \left[\sum_{t=1}^{n} (\phi_3)_t e^{-\lambda t_{et}} + \left(\sum_{t=1}^{n} (\phi_2)_t e^{-\lambda t_{et}} \right) e^{-\lambda T} + \left(\sum_{t=1}^{n} (\phi_1)_t e^{-\lambda t_{et}} \right) e^{-\lambda 2T} \right] \tag{6}$$

For the m^{th} irradiation cycle, the sum of the countings at every cycle C_{obs_t} is:

$$\begin{aligned} C_{obs_t} = k \Bigg\{ & \sum_{t=1}^{n} (\phi_1)_t \, e^{-\lambda t_{et}} + \left[\sum_{t=1}^{n} (\phi_2)_t \, e^{-\lambda t_{et}} + \left(\sum_{t=1}^{n} (\phi_1)_t \, e^{-\lambda t_{et}} \right) e^{-\lambda T} \right] + \\ & + \left[\sum_{t=1}^{n} (\phi_3)_t e^{-\lambda t_{et}} + \left(\sum_{t=1}^{n} (\phi_2)_t e^{-\lambda t_{et}} \right) e^{-\lambda T} + \left(\sum_{t=1}^{n} (\phi_1)_t e^{-\lambda t_{et}} \right) e^{-\lambda 2T} \right] + \ldots \Bigg\} \end{aligned} \tag{7}$$

Then, from equation 7,

$$C_{obs_t} = k \, \Phi \tag{8}$$

Therefore, the fraction of calcium in the sample (F), for m sucessive irradiation cycles is given

by the following relation.

$$F = \frac{\lambda M C_{obs_t}}{N m \sigma f \varepsilon f_\gamma \left(1 - e^{-\lambda t_i}\right)\left(1 - e^{-\lambda t_c}\right)\Phi} \quad (9)$$

RESULTS AND DISCUSSION

Table 1 shows the results of phosphorus and calcium analysis in bone samples.

Table 1 - Phosphorus and calcium percentage in bone samples

	Reference Bone (A3/74)	Animal Bone (A-104)
Experimental Value	(15.6±1.8)%P (n=13)	(16.0±1.8)%P (n=6)
Certified Value	(15.5±0.5)%P	(16.4±1.0)%P*
Experimental Value	(31.8±4.1)%Ca (n=4)	(25.9±3.6)%Ca (n=4)
Certified Value	(31.3±0.3)%Ca	-

*literature value

n= number of determinations

Results presented in Table 1 show that the phosphorus average percentage in the A3/74 reference material was (15.6 ± 1.8)% and for calcium was (31.8 ± 4.1)%. These results are in good agreement with the certified values, (15.5 ± 0.5)% and (31.3 ± 0.3)%, respectively. The average Ca/P ratio in this material was (2.04 ± 0.06)%.

The phosphorus average percentage in the A-104 animal bone sample was (16.0 ± 1.8)%. This result is comparable with the reported by *OLIVEIRA*[7].

Detection limits based on Currie's criterion[8] were determined for 100 mg of samples. The results were 0.24 mg for phosphorus and 14.3 mg for calcium.

Possible interfering elements were studied. For phosphorus, it could be Si by the reaction $^{28}Si(n,p)^{28}Al$ and for calcium, $^{45}Sc(n,2p)^{44}K$. Due to the low concentrations of silicium and scandium in bone, their interference can be considered insignificant.

The precision of the analysis method presented errors of about 12%, due to the errors associated

to the published values for nuclear data, mainly for cross-sections. The errors relative to the detector efficiency and counting rates were of 4%.

Calcium analysis was only possible by cyclic irradiation. The $^{44}Ca(n,p)^{44}K$ reaction leads to a small production of ^{44}K, because of the low cross section as well as the low isotopic abundance of ^{44}Ca. By cyclic irradiation, the counting rates at every counting period were summed to give a cumulative detector response. In the bone matrix, there is no activation of other elements to contribute to the background radiation of the photo peak of 1157 keV of ^{44}K. So, better detection limits can be obtained as well as the sensibility is improved.

CONCLUSION

Efforts made to minimize errors relative to neutron flux variation by means of the BF_3 calibration and normalization of the counting rates, give good results. The use of flux monitor in every irradiation was not necessary.

The instrumental method is rapid, simple, laborious chemical separation or a decay time are not required. It can be applied to routinely analysis of biological samples.

REFERENCES

1. Irigaray, J. L.; Capelani, J. C.; Chabard, J. L., 1979. Dosage par neutrons rapides des elementes P, Ca et N dans une biopse osseuse avant une analyse histologique. In: INTERNATIONAL SYMPOSIUM ON NUCLEAR ACTIVATION THECNIQUES IN LIFE SCIENCES, 22 - 26 May, 1978, Vienna. ***Proceedings***... Vienna: IAEA, 433 - 445.
2. Chindhade, V. K.; Gogte, V. D.; Joglekar, A. G.; Bhoraskar, V. N., 1982. Estimation of fluorine and phosphorus in bones using 14 MeV neutron activation analysis. ***Radiochem. Radioanal. Lett***., 52/3: 141-148.
3. Hoste, J.; Beek, J. O.; Gijbels, R.; Adams, F.; Winkel, P. V.; Soete, D., 1971. ***Instrumental and radiochemical activation analysis.*** London: CRC.
4. Givens, W. W.; Mills, W. R.; Caldwell, R., 1970. Cyclic activation analysis. ***Nucl. Instr. Methods***. 80: 95-102.
5. Kawade, K.; Ezuka, M.; Yamamoto, H.; Sugioka, K.; Katoh, T., 1981. Efficiency calibration of Ge(Li) detector at short source-to-detector distance. ***Nucl. Instr. Methods***. 190: 101-106.
6. Berretta, J. R.; Madi F°, T., 1994. BF_3 Detector calibration for neutron flux monitoring by a Van de Graaf accelerator. In: GENERAL CONGRESS OF NUCLEAR ENERGY, 28 August - 02 September. ***Proceedings***... Rio de Janeiro, Brazil, 2: 673 - 675.
7. Oliveira, R. M., 1994. ***Phosphorus determination in biological samples***, 1994. Master Thesis. Instituto de Pesquisas Energéticas e Nucleares. Brazil.
8. Currie, L. A., 1968. Limits for qualitative detection and quantitative determination. Aplication to radiochemistry. ***Anal. Chem***., 4: 586 - 593.