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DETERMINATION OF PHOSPHORUS AND CALCIUM IN BIOLOGICAL SAMPLES BY ACTIVATION WITH 14 MEV NEUTRONS

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ABSTRACT

Instrumental procedures for phosphorus analysis in milk and bone, 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 in bone and phosphorus analysis in milk were performed by cyclic irradiation. Results obtained for the IAEA reference materials A3/74 were (15.6 ± 1.8)% for phosphorus and (31.8 ± 4.1)% for calcium in bone, and (0.9± 0.1)% by conventional irradiation and (1.0± 0.1)% by cyclic irradiation in milk reference material A-11. Detection limits for phosphorus and calcium were determined in diferent analyzed samples.

INTRODUCTION

The phosphorus analysis in biological samples is of special concern due to its vital role in biological process, specially in milk, that is ingered by people in the feed diet. Milk is one of major sources of phosphorus, moreover, 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 [1] is of special concern in recent years.

The aim of the present work was to establish an instrumental method for phosphorus and calcium analysis in milk and bone by activation using fast neutrons produced in a Van de Graaff accelerator. Phosphorus was determined via $^{31}P(n,\alpha)^{28}Al$ reaction, which emitts a gamma ray of 1778.9 keV and calcium was determined via $^{44}Ca(n,p)^{44}K$ reaction, which emitts a gamma ray of 1157 keV.

Considering that variations in the neutron flux occur frequently during the sample irradiation with fast neutrons [2], efforts were carried out in order to minimize errors in the counting rates. Therefore, a BF_3 detector was employed to follow the variations of neutron flux and all the counting rates were normalized. Besides, a cyclic irradiation procedure is also proposed, since a cumulative counting of the induced activity increases the sensibility and improves the detection limits.

EXPERIMENTAL

Ge Detector Calibration. A detector efficiency calibration at a short source to detector was performed using several nuclides emitting single gamma ray, according to KAWADE et al [3].

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 for 10 minutes and measuring the activity of ^{24}Na produced via $^{27}Al(n,\alpha)^{24}Na$.

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 neutron flux produced during the irradiation was calculated. This 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%[4].

Reference material. Milk (A-11) and Calcined Animal Bone (A3/74) from the International Atomic Energy Agency

Conventional Irradiation. (Phosphorus Analysis Procedure in milk and bone). The samples and reference material were pressed into pellets (7 mm diameter), weighed (mass varied from 30 to 300 mg) and transferred to polyethylene plastic envelops. These vials with cadmium shielding were irradiated for 5 minutes under a 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.

The neutron counting spectrum of the BF_3 detector obtained during the irradiation period was divided in small time intervals (10 seconds). By using the counting registered in each one of these intervals (C_l), and the value ϕ_m/C_m obtained in the BF_3 calibration, it was possible to calculate the neutron flux of each interval (ϕ_l), by the equation (1).

$$\phi_l = \frac{C_l}{C_m}\phi_m$$

where:

ϕ_l = neutron flux at the l interval.

C_l = medium counting at the l interval.

$\frac{\phi_m}{C_m} =$ 3.34 x 10^4 n cm^{-2} per counting of the BF_3 detector.

The concentration of phosphorus (F) was determined using the following relation:

$$F=\frac{C_{obs}\lambda M}{Nm\sigma f\varepsilon f_\gamma\left(1-e^{-\lambda t_i}\right)\left(1-e^{-\lambda t_c}\right)\sum_{1=1}^{n}\phi_1 e^{-\lambda t_{e_\ell}}} \qquad (2)$$

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;

$f\gamma$ = absolute transition probability by gamma decay;

t_i = irradiation time;

t_c = counting time, and

t_el = waiting time between the end of irradiation of the interval l and begining of the counting at the gamma system.

Cyclic irradiation. Calcium analysis in bone and phosphorus in milk sample was carried out by cyclic irradiation. 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.

The cycle irradiation was defined as:

t_i - irradiation time, 5 minutes;

t_e - waiting time, 2 minutes;

t_c - counting time, 5 minutes;

t_r - return time of the sample to the irradiation for the next cycle, 2 minutes.

T = the total time of the cycle irradiation, where;

$T = t_i + t_e + t_c + t_r.$

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

$$C_{obs_1}=k\sum_{\ell=1}^{n}\left(\phi_1\right)_\ell e^{-\lambda t_{e_\ell}}$$

were:

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

For the second irradiation cycle;

$$C_{obs_2} = k\left[\sum_{\ell=1}^{n}\left(\phi_2\right)_{\ell} e^{-\lambda t_{w\ell}} + \left(\sum_{\ell=1}^{n}\left(\phi_1\right)_{\ell} e^{-\lambda t_{w\ell}}\right) e^{-\lambda T}\right] \quad (5)$$

For the third irradiation cycle;

$$C_{obs_3} = k\left[\sum_{\ell=1}^{n}\left(\phi_3\right)_{\ell} e^{-\lambda t_{w\ell}} + \left(\sum_{\ell=1}^{n}\left(\phi_2\right)_{\ell} e^{-\lambda t_{w\ell}}\right) e^{-\lambda T} + \left(\sum_{\ell=1}^{n}\left(\phi_1\right)_{\ell} e^{-\lambda t_{w\ell}}\right) e^{-\lambda 2T}\right]$$

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

$$C_{obs_t} = k\left\{\sum_{\ell=1}^{n}\left(\phi_1\right)_{\ell} e^{-\lambda t_{w\ell}} + \left[\sum_{\ell=1}^{n}\left(\phi_2\right)_{\ell} e^{-\lambda t_{w\ell}} + \left(\sum_{\ell=1}^{n}\left(\phi_1\right)_{\ell} e^{-\lambda t_{w\ell}}\right) e^{-\lambda T}\right] + \right.$$

$$\left. + \left[\sum_{\ell=1}^{n}\left(\phi_3\right)_{\ell} e^{-\lambda t_{w\ell}} + \left(\sum_{\ell=1}^{n}\left(\phi_2\right)_{\ell} e^{-\lambda t_{w\ell}}\right) e^{-\lambda T} + \left(\sum_{\ell=1}^{n}\left(\phi_1\right)_{\ell} e^{-\lambda t_{w\ell}}\right) e^{-\lambda 2T}\right] + \ldots\right\}$$

From 7,

$$C_{obs_t} = k\ \Phi$$

where:

$$\Phi = \left\{\sum_{\ell=1}^{n}\left(\phi_1\right)_{\ell} e^{-\lambda t_{w\ell}} + \left[\sum_{\ell=1}^{n}\left(\phi_2\right)_{\ell} e^{-\lambda t_{w\ell}} + \left(\sum_{\ell=1}^{n}\left(\phi_1\right)_{\ell} e^{-\lambda t_{w\ell}}\right) e^{-\lambda T}\right] + \right.$$

$$\left. + \left[\sum_{\ell=1}^{n}\left(\phi_3\right)_{\ell} e^{-\lambda t_{w\ell}} + \left(\sum_{\ell=1}^{n}\left(\phi_2\right)_{\ell} e^{-\lambda t_{w\ell}}\right) e^{-\lambda T} + \left(\sum_{\ell=1}^{n}\left(\phi_1\right)_{\ell} e^{-\lambda t_{w\ell}}\right) e^{-\lambda 2T}\right] + \ldots\right\}$$

Therefore, we obtain the concentration of phosphorus F, evaluating the following relation.

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

Blank Analysis. Samples of blank consisted of polyethylene plastics were irradiated and counted at the same conditions for phosphorus and calcium analysis. The values found by means of equation 2 and equation 10 was discounted from the level of phosphorus and calcium determined at the different irradiated samples.

RESULTS

Table 1 shows the results of phosphorus analysis by conventional irradiation.

TABLE 1. Phosphorus Percentage in Milk

Comercial Milk				A-11 Milk	
mass (g)	% P	mass (g)	% P	mass (g)	% P
0.17893	0.7 ± 0.1	0.15788	0.7 ± 0.1	0.12210	0.9 ± 0.1
0.07952	0.7 ± 0.1	0.03441	0.8 ± 0.1	0.12210	0.9 ± 0.1
0.07163	0.8 ± 0.1	0.05686	0.8 ± 0.1	0.12210	1.0 ± 0.2
0.11330	0.7 ± 0.1	0.10378	0.6 ± 0.1	0.12210	0.9 ± 0.1
Mean value	0.7 ± 0.1			Mean value	0.9 ± 0.1
				Certified value	0.910 ± 0.102

By using a cyclic irradiation, the milk sample was irradiated by 5 cycles, being obtained the medium value of (1.0 ± 0.1)% of phosphorus in the milk A-11.

Table 2 shows the results of phosphorus and calcium analysis in bone samples using cyclic and convencional irradiation.

TABLE 2 - Phosphorus and calcium percentage in bone

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

*literature value

n= number of determinations

DISCUSSION

Phosphorus analysis in milk (A-11) by conventional and cyclic irradiation presented the medium values of (0.9 ± 0.1)% and (1.0 ± 0.1)%, respectively, these values are in good agreement to the certified value, (0.910 ± 0.102)%. The concentration of phosphorus in commercial milk is lower, P = (0.7 ± 0.1)%.

Results presented in Table 2 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 phosphorus average percentage in the A-104 animal bone sample was (16.0 ± 1.8)%. This result is comparable with the reported by *OLIVEIRA* [5].

Results obtained in the cyclic irradiation showed that there is an improvement in the counting statistics as well as in the detection limits. The detection limits for phosphorus analysis calculated by the Currie's criterion [6], were of 0.12 mg for conventional analysis and 0.04 mg for cyclic analysis per 100 mg of sample irradiated, for calcium analysis the results was 14.3 mg.

The results of the analysis presented errors of about 12%, due to the errors associated to the published values for data nuclear, mainly for cross-sections. In this work, the errors of detector efficiency and counting rates were of 4%.

The phosphorus determination by reactor leads to the formation of other gamma or beta emitter radionuclides. So, the analysis methods require a radiochemical separation of phosphorus, as ammonium phosphomolybdate or a decay time of the interferences, specially.

These interferences can be avoided by activation with 14 MeV neutrons produced by a Van de Graaff accelerator. The very low cross sections for 14 MeV neutrons for the majority of the elements and the neutrons sharp energy spectrum allow us to obtain the gamma spectrum in the phosphorus analysis, free of interference on the photopeak of ^{28}Al.

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.

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 the samples studied, their interference can be considered insignificant.

The normalization method of the neutron flux here employed gives an advantage that fluctuation of the neutron flux during the irradiation does not effect the results. These use of flux monitor in every irradiation is not required. It can be applied to routinely analysis of phosphorus in biological samples.

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