Jointly published by
Elsevier Science S. A., Lausanne and
Akadémiai Kiadó, Budapest

J.Radioanal.Nucl.Chem.,Letters
213'(3) 185-192 (1996)

PHOSPHORUS DETERMINATION IN MILK AND BONE SAMPLES BY NEUTRON ACTIVATION ANALYSIS

Ieda I.L. Cunha, Rosangela M. de Oliveira

Divisão de Radioquímica,
Instituto de Pesquisas Energéticas e Nucleares - IPEN-CNEN/SP, Caixa Postal 11049 Pinheiros, São Paulo - Brasil

Received 19 March 1996
Accepted 2 April 1996

The determination of phosphorus in milk and bone samples by both radiochemical and instrumental neutron activation analysis is described. The radiochemical method consists of thermal neutron irradiation of samples and standards, sample dissolution, phosphorus precipitation as ammonium phosphomolybdate, use of zinc holdback carrier and counting of the phosphorus-32 ß-activity. The instrumental method involves thermal neutron irradiation of samples and standards, waiting for a decay time and ß-counting. The methods were applied to commercial samples and reference materials.

INTRODUCTION

Phosphorus analysis in biological samples, especially in milk and bone is important because of its vital role in various biological processes. Milk is one of the majors sources of phosphorus, beside meat, fish and eggs

0236-5731/96/US $ 12.0

and it is ingested by people in the feed diet. Bone serves as an important storage area for phosphorus and other essential elements. The role of these elements in the development of bone diseases and in the monitoring of changes in their concentrations after various treatments are of great concern.

Neutron activation analysis is a sensitive method for phosphorus determination. This element can be determined by fast neutron reaction $^{31}P(n,\alpha)^{28}Al$. Since the same radionuclide is produced from aluminium via $^{27}Al(n,\gamma)^{28}Al$, both thermal and epithermal neutron activation are required. This method is dependent on the ratio of the fast to thermal neutron flux density[1] and the phosphorus to aluminium ratio[2]. Another analysis method is based on the counting of phosphorus-32 by means of the reaction $^{31}P(n,\gamma)^{32}P$.

Phosphorus-32 is a pure ß-emitter but the measurement of ß-radiation is not selective so, a decay time for the interferences to negligible levels is necessary or they need to be separated from the matrix before activity measurement. Besides, bremsstrahlung produced by phosphorus-32 sometimes becomes a strong interference in the analysis of some radionuclides and its removal by a radiochemical method is required.

There are several papers in the literature about radiochemical and instrumental methods for phosphorus analysis[3-8]. Most of the radiochemical methods are based on the precipitation of ammonium phosphomolybdate (AMP), while the instrumental methods were developed in order to avoid laborious separations, to determine several elements in the same sample and to obtain quick results in a short time.

The aim of the present work was to establish routine methods for phosphorus analysis, that could be applied

in any biological sample, independently of the aluminium to phosphorus ratio or the reactor flux density. Data obtained by both radiochemical and instrumental methods for milk and bone are presented and compared between themselves. The accuracy of the methods was checked with reference materials.

EXPERIMENTAL

Preparation of sample

Phosphorus analyses were performed by using samples of commercial milk powder (500 mg) and animal bone samples (20 mg, codes A-104, A-702) from calves. Samples were dried at 70 °C overnight, weighed into polyethylene bags and heat-sealed.

Standards of Powder Milk (A-11, 0.91% P), Calcined Animal Bone (A 3/4, 15.4% P) and ammonium phosphate (5 to 15 mg in phosphorus) were prepared in the same way.

Irradiation

The polyethylene bags were inserted into rabbits and irradiated under a thermal neutron flux of the 10^{12} $n.cm^{-2}.s^{-1}$, for 30 min, using a pneumatic transfer system at the IPEN reactor.

Radiochemical method

After the irradiation, milk samples were dissolved in a mixture of conc. perchloric acid (1 ml) - concentrated nitric acid (5 ml) under heating, in the presence of 5 mg of phosphorus carrier and 10 mg of zinc carrier. Ammonium molybdate (1.0 g) was dissolved in 25 ml of

2.5M nitric acid and this solution was added to the milk sample. The solution was heated at 70 °C for 2 h. The precipitate was filtered, dried and transferred to a planchet.

The IAEA standard analysis was carried out similarly to the milk samples.

In the case of bone, this matrix was dissolved by using concentrated perchloric acid (1 ml) in the presence of phosphorus and zinc carriers. The other steps were the same as employed for milk analysis.

Counting

The phosphorus precipitate was counted in a Geiger-Müller detector. Corrections for decay time and dead time were performed. To verify the purity of the phosphorus-32 precipitate, its half-life was determined by counting the precipitate during some weeks, and the half-life obtained was compared with the value of 14.3 d. The precipitate purity was also checked by γ-counting employing a Ge detector. It was verified that zinc is precipitated with ammonium phosphomolybdate. So, this interference was avoided using a zinc holdback carrier.

Instrumental method

After irradiation, the bone samples and standards in their plastic bags were placed in aluminium planchets.

In the case of milk, this procedure was not possible due to the large amount of sample irradiated, about 500 mg. The counting of a ß-emitter is sensitive to the counting geometry, the homogeneity, thickness and count rate being important. So, milk samples were dissolved in water, diluted to 10 ml and weighed. Aliquots of 1 ml were

TABLE 1

Phosphorus analysis in the reference milk sample (A-11)

Decay time, d	P (mg) certified value	P (mg) found at this work
1	0.455	0.504
16	0.455	0.449
21	0.455	0.452

pipetted onto aluminium planchets and evaporated to dryness under an infrared lamp.

For ammonium phosphate to be used as standard, this salt was dissolved in water in the presence of unirradiated powder milk (500 mg) in order to maintain the same geometry conditions.

Counting

Samples were counted by employing a Geiger-Müller detector for different decay times.

RESULTS AND DISCUSSION

Table 1 shows the influence of decay time in the instrumental analysis of phosphorus in reference milk sample.

By means of γ-spectrometry, the presence of bromium-82, sodium-24, antimony-122 radionuclides was verified. For a decay time of 1 d, the influence of these radionuclides is so great that the accuracy of the analysis method was only 10.7%. For 16 d of decay the accuracy improved to 1.3% and for 21 d it was 0.7%. Then, it was

TABLE 2

Percentage of phosphorus in milk and animal bones

	Commercial milk	Bone (A-104)	Bone (A-702)
Radiochemical method	(0.86±0.09)% n=10	(16.5±1.9)% n=10	(13.5±1.0)% n=10
Instrumental method	(0.89±0.06)% n=8	(16.4±1.0)% n=12	(15.9±0.9)% n=12

n = number of determinations.

established that, after a decay time of 15 d, samples and standards can be counted, since the short-lived interfering radionuclides drop to negligible levels.

Milk samples were analyzed applying a decay time of 15 d.

For bone samples, the interference of sodium-24 and potassium-42 radionuclides was verified by γ-spectrometry. A decay time of 10 d was enough to allow the decay of these radionuclides.

The results of the phosphorus analyses are presented in Table 2.

The results of phosphorus analysis in commercial milk, employing both radiochemical (0.86±0.09%) and instrumental methods (0.89±0.06%) are in good agreement. Although the radiochemical method is laborious, it can be applied without waiting for any decay. The instrumental method is very simple but requires a time of at least 15 d for decay of the radioactivity of short-lived radionuclides to an insignificant level.

Results obtained for phosphorus analysis in bones employing thermal neutron activation analysis with and without radiochemical separation are also in good agree-

ment. By the radiochemical method, animal bones gave 16.5±1.9% of phosphorus in the A-104 bone and 13.5±1.0% for the A-702 bone. By instrumental method the results were 16.4±1.0% for A-104 bone and 15.9±0.9% for the A-702 bone.

In all experiments, the radiochemical purity was checked through the phosphorus-32 half-life. The values obtained varied from 14.1 to 14.4 d and they are close to the value published in the literature (14.3 d).

Results for IAEA milk standard indicate the good accuracy of the method established for phosphorus.

The precision for phosphorus determination is in the range of 5 to 10%, because a very simple Geiger-Müller detector (low counting efficiency, high background level) was employed for ß-counting. This can be improved by using a convenient detector or by increasing the irradiation time, since better counting statistics will be obtained.

The detection limits determined for bone samples employing radiochemical and instrumental methods were 26 µg and 14 µg of phosphorus, respectively. In the case of milk, the detection limit was 14 µg for both analysis methods.

The interference introduced by the reactions $^{32}S(n,p)^{32}P$, $^{35}Cl(n,\alpha)^{32}P$ and $^{30}Si(n,\gamma)^{31}Si \xrightarrow{\beta} {}^{31}P$ was estimated by irradiating salts of phosphorus, chloride, sulfate and silicon in quantities similar to these elements in milk. Samples were counted under the same conditions for phosphorus-32. It was noticed that this radionuclide produced 31 times more activity than silicon, 20 times more than chlorine and 14 times more than sulfur. Reactions (n,γ) are more favorable than (n,p) or (n,α), because the IPEN reactor has a neutron spectrum mainly formed by thermal neutrons.

CONCLUSION

The instrumental method of phosphorus analysis in milk and bones involves only a simultaneous irradiation of samples and standards and counting without chemical processes, but it requires a decay time of 15 and 10 d, respectively. Although laborious, the radiochemical method can be employed without any decay time. The anarial, being of interest in disease diagnosis in live animals.

The radiochemical method can also be used for isolating phosphorus from biological samples before the determination of other elements when phosphorus becomes an interference, the ß-activity of phosphorus-32 masks the γ-spectra of neutron-activated biological samples. The removal of phosphorus employing ammonium molybdate is efficient, selective and provides a recovery of phosphorus higher than 95%.

REFERENCES

1. W. Gatschke, D. Gawlik, J. Radioanal. Chem., 56 (1980) 203.

2. Z.B. Alfassi, N. Lavi, Analyst, 109 (1984) 959.

3. N. Lavi, F. Lux, Z.B. Alfassi, J. Radioanal. Nucl. Chem., 129 (1989) 93.

4. F. De Corte, A. Speecke, J. Hoste, J. Radioanal. Chem., 8 (1971) 277.

5. A. Gaudry, B. Maziere, D. Comar, J. Radioanal. Nucl. Chem., 29 (1976) 77.

6. R.G. Weginwar, D.L. Samudralvar, A.N. Garg, J. Radioanal. Nucl. Chem., 133 (1989) 317.

7. S. Bajo, A. Wittenbach, Talanta, 35 (1988) 741.

8. E. Damsgaard, K. Heydorn, J. Radioanal. Nucl. Chem., 113 (1987) 267.