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DETERMINATION OF CALCIUM, PHOSPHORUS AND FLUORINE IN BONE BY INSTRUMENTAL FAST NEUTRON ACTIVATION ANALYSIS

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The determination of fluorine, calcium and phosphorus in bone by instrumental fast neutron activation analysis is described. Results for the IAEA standard material "Animal Bone" A3–74' are: Ca = 313 ± 10 mg/g, P = 155 ± 5 mg/g and F = 613 ± 20 μg/g. The accuracy for all three elements is ⩽ 4%, precision being about 3%. The limit of determination for F is 120 μg, for Ca 30 mg and for P 1 mg in a sample of 500 mg.

Introduction

As part of a system of activation analysis techniques for the determination of essential trace elements in human body organs and fluids, it was necessary to develop a method for the determination of fluorine. Attention was paid to bone as it is the target tissue for fluorine.[1] From many papers describing activation analysis of fluorine in bone[1–10] fast neutron activation analysis appears to be a suitable technique, while calcium and phosphorus contents can be measured simultaneously.[11–15]

Principle

Fluorine gives three nuclear reactions during irradiation with fast neutrons.[16] Two of them, $^{19}F(n, p)^{19}O$ and $^{19}F(n, \alpha)^{16}N$ suffer from serious oxygen interference.[17] Consequently, the third possibility, $^{19}F(n, 2n)^{18}F$ is chosen as a base for F determination. The radionuclide formed emits 511 keV annihilation radiation, and has a half-life of 109.7 min. There is interference from other positron emitters, mainly ^{13}N and ^{30}P, with half-lives of 9.96 min and 2.50 min, respectively.

Fig. 1 and Table 2 show the decay curve of the 511 keV photopeak of a bone sample. The contribution of ^{13}N is negligible after a waiting time of 100 min. Calcium is determined by measuring the 1157 keV γ-ray of ^{44}K, formed via

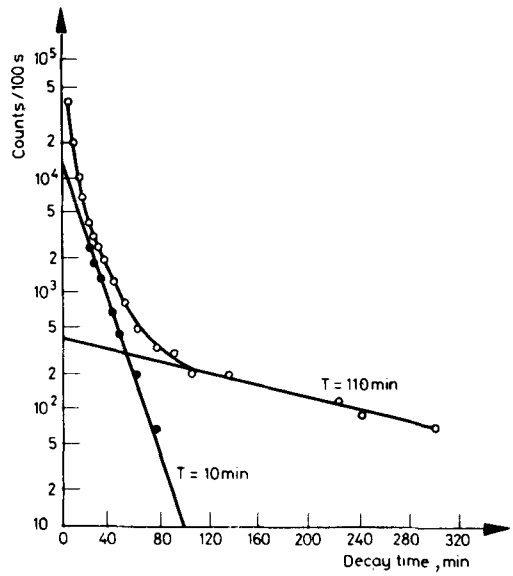

Fig. 1. The decay-curve of the 511 keV photopeak of a bone sample

$^{44}Ca(n, p)^{44}K$, with a half-life of 22.15 min. The determination of P is based on the measurement of ^{28}Al, formed via $^{31}P(n, \alpha)^{28}Al$, with a γ-ray energy of 1779 keV and a half-life of 2.24 min. Possible interfering element in the determination of P could be Si, via $^{28}Si(n, p)^{28}Al$, but its low content in bone compared to P (Si: 17 μg/g, P: ±150 mg/g,)[20] makes interference negligible.

Experimental

Standards and samples

Standards for the detemirnation of F are mixtures of p-fluorobenzoic acid and cellulose, with varying F contents. For Ca and P determination, aliquots from a mixture of calcium fluoride and calcium dihydrogen phosphate are used as standards. The samples are 500 mg portions of the IAEA standard material "Animal Bone" A3–74.

Irradiation and measuring equipment

After weighing and packing, samples and standards are transferred to the irradiation facility, a SAMES neutron generator, by a pneumatic system. Flux corrections for successive irradiations are made by means of a fission chamber. After irradiation,

Table 1
The observed and calculated values for an actual determination.
Calculation is done by non-linear curve fitting,
assuming contributions of ^{18}F, ^{13}N and ^{30}P

Waiting time, min	Observed value, counts/100 s	Calculated value, counts/100 s
4	44 217	44 268
8	20 016	19 802
12	10 132	10 364
16	6 499	6 324
20	4 212	4 324
24	3 084	3 170
28	2 367	2 418
32	1 883	1 798
40	1 165	1 158
50	780	725
60	495	501
75	338	331
90	299	266
105	198	230
120	207	206
135	206	187
220	115	127
240	86	98
300	70	71

samples and standards are counted in a 3″ X 3″ NaI(Tl) well-type detector connected to a Nuclear Data 512 multichannel analyzer for F and P determination, and on a coaxial 45 cm^3 Ge(Li) detector connected to a Nuclear Data 4096 multichannel analyzer for Ca determination. Counting data are stored on magnetic tape and evaluated by computer, assuming a straight base-line. Properties of the neutron generator and a detailed discussion on flux corrections are described elsewhere.[22]

Irradiation and measuring scheme

As a result of preliminary experiments to find optimum irradiation, waiting, and counting times, the scheme in Table 2 is used for the simultaneous determination of F, P and Ca.

Table 2
Irradiation and measuring scheme

	Duration, min	Cumulative time, min	Radionuclide measured
Irradiation	15 m	0– 15	
1st decay period	3 m	15– 18	
1st measurement	200 s	18– 21	^{28}Al
2nd decay period	10 m	15– 25	
2nd measurement	2000 s	25– 55	^{44}K
3rd decay period	100 m	15–115	
3rd measurement	2000 s	115–145	^{18}F

Results

Specific count rates

The number of counts under the 511 keV photopeak of ^{18}F is determined in five different mixtures of p-fluorobenzoic acid and cellulose. These mixtures yield an average specific count rate of 6.8 counts/μg F, with a standard deviation of 2.5%. With this value, all F's are calculated. Known and calculated amounts are in good agreement (Table 3). Measurements on mixtures of CaF_2 and $Ca(H_2PO_4)_2 \cdot H_2O$ yield specific count rates of 1.9 counts/μg P with a standard deviation of 1.8% and 6.2 counts/mg Ca, with a standard deviation of 2.1%. The standard deviations in specific count rates are included in the standard deviations of the elemental contents.

Accuracy and precision

Accuracy and precision of the method are determined by multiple analysis of samples with known element contents. Fluorine has been analyzed in a mixture of calcium fluoride and calcium dihydrogen phosphate, with a theoretical F-content of 20.22%. Result of a triplicate analysis was (19.40 ± 0.55)%.

Calcium and phosphorus have been analyzed in hydroxyapatite. One gram theoretically contains 399 mg Ca and 185 mg P. The results obtained for Ca are 394 ± 9 mg/g and 189 ± 4 mg/g for P. The average Ca/P ratio is 2.08 ± 0.06.

The determination of F, Ca and P in the IAEA standard material "Animal Bone", A3–74

Fluorine, Ca and P have been analyzed in IAEA standard material "Animal Bone". Detailed results are shown in Table 4. The average fluorine content is

Table 3
Fluorine analysis in mixtures of p-fluorobenzoic acid and cellulose with known fluorine contents

Sample	Known amount of F, μg	Calculated amount of F,* μg
A	283	260
B	1041	1037
C	1268	1362
D	2317	2328
E	3154	3174

*Calculated with a specific F count rate = 6.8 counts/μg.
Regression line: F(calc.) = 1.01 F(known) + 7, $r^2 = 0.999$.

Table 4
Determination of F, Ca and P in the IAEA standard material "Animal Bone" A3-74 (as received)

Sample, No.	F, mg/g	P, mg/g	Ca, mg/g	Ca/P
1	607	152	300	1.97
2	628	169	329	1.95
3	610	154	332	2.16
4	563	148	305	2.06
5	658	153	301	1.97
Average	613	155	313	2.02
S.D.*	±20	±5	±10	0.07

*Standard deviations of specific count rates are included.

613 ± 20 μg/g, the average Ca-content 313 ± 10 mg/g, and the average P-content 155 ± 5 mg/g. The average Ca/P ratio in the material amounts to 2.02 ± 0.07.

The limit of determination

The limit of determination is calculated from Currie's criterion L_D,[18] applied to γ-spectra,[19] and the specific count rates given in section "Specific count rates" of this paper. Limits of determination for a 500 mg sample are 120 μg F, 30 mg Ca and 1 mg P.

Table 5
Calculation of the P and N-content of bone from the decay curve of the 511 keV photopeak by way of the absolute comparator method

		F	N	P
C	Count rate (Counts/100 s at tw = 0)	419 ± 156*	14795 ± 315*	121 684 ± 785*
σ	Cross-section, mb[21]	60.6	5.7	11
a	Abundance, %	100	99.63	100
M	Atomic weights, g/mol	19.00	14.01	30.97
a'	Decay abundance, %[8]	97	100	99.5
λ	Decay constant, s^{-1}	$1.05 \cdot 10^{-4}$	$1.16 \cdot 10^{-3}$	$4.62 \cdot 10^{-3}$
t_b	Irradiation time, s	900	900	900

*Deviations are 68% confidence limits on linear hypothesis.

Calculation of the P and N content from the 511 keV photopeak decay curve

From the decay curve of the 511 keV photopeak (Fig. 1), the count rates of the contributing radionuclides (^{18}F, ^{30}P and ^{13}N) at the end of the irradiation can be calculated by curve fitting with a non-linear regression program. The relation between elemental content and count rate can be calculated from the activation formula. From the ratio of count rates of two contributing nuclides, the ratio of elemental contents can be calculated.

Table 5 summarizes the calculation of the N and P content from the 511 keV photopeak decay curve (Fig. 1), using F as an absolute comparator. Results for the standard material "Animal Bone", calculated with a known F content of 613 ± 20 µg/g, are a P-content of 143 ± 6 mg/g and a N-content of 2.3 ± 0.1%.

With a known fluorine content of 613 ± 20 µg/g and the nuclear data listed in Table 5, results for the IAEA standard material "Animal Bone" are 143 ± 6 mg P/g and 23 ± 1 mg N/g. Following the suggestion of HOLMBERG[13] of deducing the Ca content from the known Ca/P ratio (2.02 ± 0.07) and the P-content, a Ca-content of 289 ± 14 mg/g can also be given.

Conclusion and discussion

Activation analysis of bone samples with fast neutrons is a suitable technique for the simultaneous determination of F, Ca and P. Though sensitivity is moderate, limits of determination are sufficient for elemental analysis in samples of 300–500 mg weight.

The Ca/P ratio of the IAEA standard material "Animal Bone" A3–74 is in good agreement with that of pure hydroxyapatite, while the P and Ca contents both are 80% of those in apatite.

Measurement of the decay of the 511 keV photopeak of bone samples offers the opportunity to calculate N and P contents if the F content is known. If the suggestion of HOLMBERG et al.[13] to calculate Ca contents from known Ca/P ratios and P contents is followed, it is possible to determine the F, N, P and Ca content from the 511 keV photopeak decay curve only.

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