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Determination of selenium in foods by pseudo-cyclic neutron activation and anti-coincidence gamma-ray spectrometry

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Abstract A pseudo-cyclic instrumental neutron activation analysis method in conjunction with anti-coincidence gamma-ray spectrometry (PC-INAA-AC) has been developed for the determination of ppb levels of Se. The method consists of repetitions of the irradiation-decay-counting cycles of a sample using the rapid transfer cyclic system at the Dalhousie University SLOWPOKE-2 reactor facility. The 162-keV γ-ray of ^{77m}Se ($t_{1/2} = 17.4$ s) has been found to be highly selective. The precision and detection limits are significantly improved and the total experimental time drastically reduced by this method. Detection limits are between 2 and 9 ng. The accuracy of the method has been evaluated by analyzing a number of nutritional reference materials. The PC-INAA-AC method has been applied for the routine determination of Se to the composites of 135 food samples with values ranging from as low as 1 ng g^{-1} for tea to 1,045 ng g^{-1} for organ meats on fresh weight basis.

Keywords Selenium · Foods · Pseudo-cyclic neutron activation · Anti-coincidence gamma-ray · Spectrometry

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Introduction

Although Se was reported to be biologically essential for animals in 1957, it was not until 1973 when glutathione peroxidase was proven to be a selenoenzyme. Dietary Se deficiency in China is reported to cause Keshan disease which is a type of cardiac muscle degeneration in both children and women of child bearing age. Although the aetiology of another disease in China called Kashin–Beck disease is said to be multifactorial, Se deficiency is said to be the underlying factor. Supplementation with Se can cure these afflictions. On the other hand, about two orders of magnitude higher levels of Se are known to cause toxic effects. Some reports have claimed that certain Se compounds can prevent carcinoma, slow the aging process, enhance sexual activities, etc. Obviously, there is an increasing interest in understanding the role of Se in human nutrition and metabolism [1]. The most widely used analytical techniques for Se determination in food, water, body tissues and fluids, etc. include either hydride generation or electrothermal atomization AAS, ICP-MS, and spectrofluorometry. The levels of selenium in many foods are too low for direct determination by most instrumental analytical techniques except neutron activation analysis (NAA).

Although Se has six stable isotopes which can produce seven radionuclides on thermal neutron activation, ^{75}Se ($t_{1/2} = 118.5$ d) and ^{77m}Se ($t_{1/2} = 17.4$ s) are most suitable for measurement by instrumental NAA (INAA). The 265-keV γ-ray of ^{75}Se could be interfered with by the 264-keV γ-ray of ^{182}Ta ($t_{1/2} = 114.4$ d). Additionally, the long-lived nuclide ^{75}Se requires lengthy irradiations at a high neutron flux, decay and counting periods lasting up to 2–3 weeks of total analysis time leading to an expensive procedure which may not be suitable for routine

analysis of Se in a large number of food samples. Alternatively, the short-lived ^{77m}Se nuclide can be routinely used at a significantly reduced cost and analysis time. Although the conventional INAA procedure involving an one-shot irradiation-decay-counting scheme can be employed, precision and detection limit can be significantly improved by using cyclic INAA (CINAA) where a sample is irradiated for a short time, rapidly transferred to a detector for counting for a short period, and the entire process is immediately repeated for an optimum number of cycles. If several minutes to days are allowed to elapse between repetitions of the irradiation-decay-counting cycles then the technique is called pseudo-cyclic INAA (PC-INAA). In the past, we have developed several PC-INAA methods using the rapid transfer cyclic system available at the Dalhousie University SLOWPOKE-2 reactor (DUSR) facility for Se in various matrices and optimized them for best sensitivity, detection limit, selectivity, rapidity, precision and accuracy [2–13]. Lately, we have found that food and diet samples with low amounts of Se in presence of high levels of Na and Cl cannot be that conveniently measured by PC-INAA coupled to conventional gamma-ray spectrometry. We have then developed PC-INAA methods in conjunction with anti-coincidence gamma-ray spectrometry (PC-INAA-AC) for these types of sample. One such method for food is reported here.

Experimental

Irradiations

All samples and standards were irradiated at a thermal neutron flux of 5×10^{11} cm^{-2} s^{-1} in an inner pneumatic irradiation site of the DUSR facility. The stability, homogeneity, and reproducibility of the DUSR neutron flux have previously been described [14–16]. The irradiation time (t_i), decay time (t_d), counting time (t_c), and the number of cycles (n) depended mainly on the sample matrix and the major elements present. For most of the food samples, these parameters were: t_i of 30 s, t_d of 10 to 50 s, t_c of 20 to 60 s, and n of up to 5.

Selenium comparator standards

Selenium comparator standards used in this work were made from the plasma emission spectroscopy standard solution with a certified purity of >99.999% supplied by SCP Canada Ltd. About 1 mL of the diluted standard solutions was pipetted into 1.2 mL (small) polyethylene vials, capped and heat-sealed. Eppendorf pipettes were carefully calibrated prior to use for dilutions and transfers. The comparator standards were of identical geometry and contained approximately similar amounts of selenium as the samples. The water used was first distilled in a quartz apparatus and then deionized using an ultrapure deionization column. This distilled deionized water (DDW) was used for making and diluting solutions and washing all apparatus. All materials and reagents used in this work were analyzed for "blanks" using experimental conditions similar to those of samples.

Reference materials

A number of reference materials (RMs) and Standard reference materials (SRMs) were obtained from the U.S. National Institute of Standards and Technology (NIST). These included Non-Fat Milk Powder (SRM 1549), Spinach (SRM 1570), Bovine Liver (SRM 1577b), Peach Leaves (SRM 1547), Whole Egg Powder (RM 8415), Corn Starch (RM 8432), and Corn Bran (RM 8433). The Horse Kidney (RM H8) was obtained from the International Atomic Energy Agency (IAEA). Between 100 and 700 mg depending on the minimum mass recommended, sample matrix, and dead time were used for evaluating the accuracy of the methods developed and for studying matrix interferences.

Conventional and anti-coincidence gamma-ray spectrometers

The principal detector used in both conventional and anticoincidence γ-ray spectrometry consisted of an E.G.&G Ortec HPGe p-type coaxial detector with a crystal diameter of 51.2 mm and a length of 65.2 mm. This detector had a peak-to-Compton ratio of 93:1, a relative efficiency of 25% with respect to a standard NaI(Tl) detector, and a resolution of 1.8 keV at the 1332-keV photopeak of ^{60}Co. The guard detector used in anti-coincidence γ-ray spectrometry consisted of a $10'' \times 10''$ NaI(Tl) annulus with five photomultiplier tubes (PMT) supplied by Harshaw and a $3'' \times 3''$ NaI(Tl) plug with one PMT supplied by Teledyne. The peak-to-Compton plateau ratio of this system was 582:1 at the 662-keV γ-ray of ^{137}Cs using the IEEE convention of the number of counts per channel in the Compton plateau (358–382 keV).

Samples

The food samples were provided by Health Canada. Market basket foods were collected at the retail level in the Toronto area, and prepared and composited at Kemptville Community College. About 135 composites along with the quality control samples were frozen and sent to our laboratory for analysis.

Results and discussion

The 162-keV γ-ray of ^{77m}Se ($t_{1/2} = 17.4$ s) is highly selective. In theory, it could be interfered with by the 162-keV γ-ray of ^{116m2}In ($t_{1/2} = 2.18$ s) and 161 keV γ-ray of ^{179m1}Hf ($t_{1/2} = 18.7$ s). Considering very low levels of In, if any at all, in foods and a decay time of 20 s used in the PC-INAA-AC method for Se measurements, it is highly unlikely that any interference from In would be encountered. Again, Hf is not commonly found in foods. Moreover, the more abundant 214 keV γ-ray of ^{179m1}Hf was not detected in any of the food samples analyzed in this work. Additionally, a detailed study on these interferences was conducted using t_i of 1 to 60 s, t_d of 0.6 to 30 s, t_c of 1 to 60 s. The half-life of ^{77m}Se through its 162 keV γ-ray varied between 17.3 and 17.5 s ruling out any interference. Moreover, the excellent agreement in Se content between our and certified values for RMs and SRMs (Table 1) proves the high selectivity of the 162-keV γ-ray of ^{77m}Se.

The 162-keV γ-ray of ^{77m}Se is not coincident with other γ-rays, and should be suitable for anti-coincidence counting. At low count rates (e.g. dead time <6%), the peak efficiency reduction factor (PERF) of the 162-keV peak was measured as 0.98 ± 0.04. Therefore, the counts in this peak area will hardly be reduced by anti-coincidence counting while some of the background activities arising from the Compton scattered events can be suppressed. A partial γ-ray spectrum of NIST Bovine Liver (SRM 1577b) presented in Fig. 1 shows that the background was suppressed by a factor of 3.5. The background suppression factor was found to vary between 3 and 5 for the RMs and SRMs listed in Table 1.

Several experimental parameters that could affect anti-coincidence counting efficiency were optimized for measuring Se levels by the PC-INAA-AC method.[17, 18] These included: (i) relative position of the NaI(Tl) annulus with respect to the HPGe detector; (ii) distance of the sample from the HPGe detector surface; (iii) highest sensitivity; (iv) lowest relative standard deviation (RSD) of

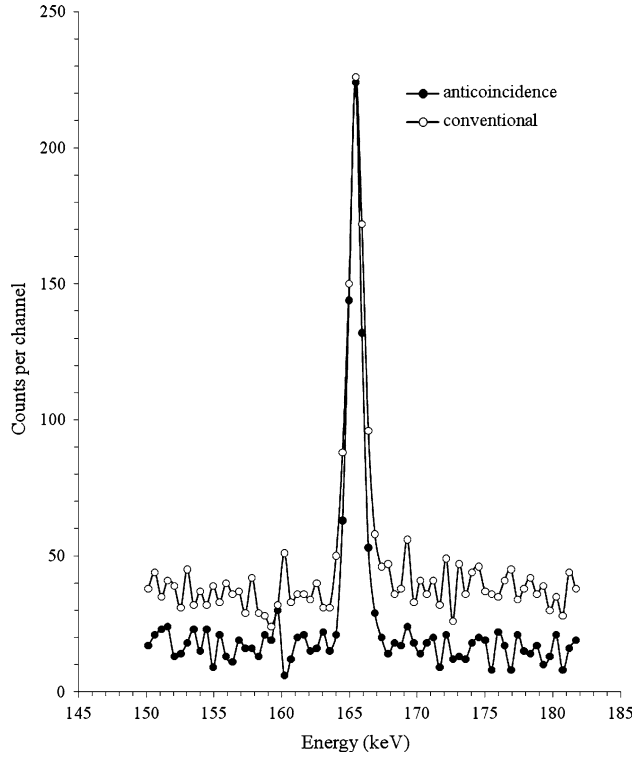

Fig. 1 Gamma-ray spectra near the 162-keV photopeak of ^{77m}Se in Bovine Liver (NIST SRM 1577b) using conventional and anti-coincidence spectrometry

counts; (v) decay time, and (vi) counting time. Using IAEA Horse Kidney (RM H8) as a typical example the values of the above parameters obtained were: (i) 9.5–12.5 cm; (ii) 0.1 cm; (iii) 1,800 counts/μg; (iv) RSD of 5.16%; (v) 10 s; and (vi) 40 s.

We have developed a theoretical model called Analytical Figure of Merit (AFOM) which can be conveniently used for the optimization of number of cycles in PC-INAA-AC [17]. In order to evaluate the applicability of AFOM to the determination of Se, the Whole Egg Powder (NIST RM 8415) was analyzed using $t_i = 30$ s, $t_d = 10$ s and $t_c = 40$ s, and counted in both anti-coincidence and conventional counting modes. The delay time ($t_{d'}$) between

Table 1 Levels (ng g^{-1}) and detection limits (ng) of selenium in biological RMs and SRMs by PC-INAA-AC

NIST RMs & SRMs	This work	Certified, [literature]	Detection limit (ng)	
			Conventional	Anti-coincidence
RM 8432	<5	–	4.3	2.3
SRM 1570	41 ± 9	40 ± 14	13	2.9
RM 8433	44 ± 4	45 ± 8	7.4	3.5
SRM 1549	115 ± 9	110 ± 10	9.4	7.3
SRM 1547	145 ± 13	120 ± 9	11	5.2
SRM 1577b	710 ± 11	730 ± 60	13	9.1
RM 8415	1370 ± 25	1390 ± 170	16	7.4

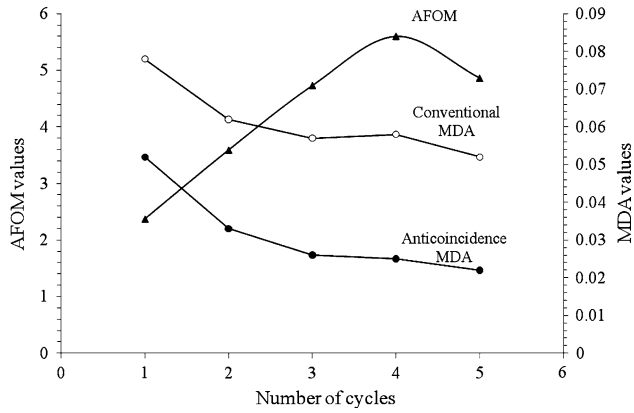

Fig. 2 Variation of minimum detectable activity (MDA) for conventional as well as anti- coincidence counting modes and analytical figure of merit (AFOM) with the number of cycles for the 162-keV photopeak of ^{77m}Se in Whole Egg Powder (NIST RM 8415)

two cycles was 24 h. We defined minimum detectable activity (MDA) for anti-coincidence and conventional counting modes, MDA_{anti} and MDA_{conv}, respectively, at each cycle. The variation of MDA and AFOM values with the number of cycles are graphically presented in Fig. 2. For the 162-keV peak of ^{77m}Se, both MDA_{anti} and MDA_{conv} decrease with increasing number of cycles. The rate of decrease is greater for MDA_{anti} compared to MDA_{conv}. The rate of improvement in MDA_{anti} appears to slow down beyond the fourth cycle due to the build-up of background activities from nuclides such as ^{38}Cl and ^{56}Mn present in the irradiated samples. As a result, the difference between the MDA_{anti} and MDA_{conv} values becomes smaller with increasing number of cycles. Consequently, the AFOM term starts with a low value in the first cycle then increases with increasing number of cycles, and passes through a maximum at the fourth cycle before starting to decrease. Hence, the optimal number of cycles (n) should be four for the Whole Egg Powder RM. In summary, the experimental conditions selected for Se analysis using the short-lived nuclide ^{77m}Se were: $t_i = 30$ s, $t_d = 10$ s and $t_c = 40$ s, $n = 4$ cycles, NaI(Tl) annulus position of 9.5–12.5 cm from the HPGe detector, and a sample-to-HPGe detector distance of 0.1 cm.

Because of both nutritional and toxicological significance of Se, a large number of food and diet samples are being analyzed for this element in our and other laboratories. It is imperative that the Se levels be measured under an extensive quality assurance program. Both internal and external quality assessments of Se measurements in foods and diets by PC-INAA-AC using conventional and anti-coincidence gamma-ray spectrometry have been studied in detail and described elsewhere [17, 18].

The accuracy of the method was evaluated by analyzing a number of reference materials. The average of five measurements for 7 NIST RMs and SRMs are presented in Table 1. Except in one case, the RSD varied between ±1.8 and 9.1%. It is evident that our values agree very well with the certified values since their ratios are within ±5%, without the propagated uncertainties, for all except in the case of SRM 1547.

The detection limits for the same 7 NIST RMs and SRMs obtained using both conventional and anti-coincidence counting modes are also shown in Table 1. In all cases, the anti-coincidence detection limits are superior. The degree of improvement in detection limits varies between 1.3 times for Non-Fat Milk Powder and 4.5 times for Spinach SRMs. It has been pointed out earlier that the 162-keV γ-ray of ^{77m}Se is not coincident with any other γ-rays and thus there should not be any decrease in its PERF. Anti-coincidence counting, on the other hand, can suppress some of the background activities arising from the Compton scattered events (Fig. 1) which in turn gives an improvement of detection limit for the 162-keV γ-ray of ^{77m}Se.

The PC-INAA-AC method was applied to the determination of Se in about 135 food composites. A detailed presentation of these data and their nutritional interpretation are beyond the scope of this paper because of the page limitation. Selenium levels, along with their standard deviations obtained from counting statistics, of randomly selected samples of 14 Health Canada food groups are shown in Table 2. The Se content has been found to range from as low as 1 ng g^{-1} for tea to 1045 ng g^{-1} for organ meats on fresh weight basis, and is similar to that of the

Table 2 Selenium content of selected food composites by PC-INAA-AC

Composite code (Food type)	Content (ng g^{-1}, fresh wt.)
A-01 (Milk, Whole)	15 ± 1
B-10 (Organ Meats, Liver and Kidney)	1045 ± 50
C-01 (Eggs)	251 ± 15
D-01 (Fish, Marine, Fresh or Frozen)	392 ± 20
E-04 (Soup, Dehydrated)	15 ± 2
F-02 (Bread, Whole Wheat)	392 ± 15
G-13 (Onion)	11 ± 1
H-05 (Blueberries)	2 ± 0.4
I-01 (Cooking fats and Salad oils)	12 ± 1
J-04 (Honey)	4 ± 0.3
K-05 (Tea)	1 ± 0.2
L-02 (Desserts)	24 ± 2
M-04 (Frozen entrees, Oven)	88 ± 7
N-01 (Pizza)	184 ± 12

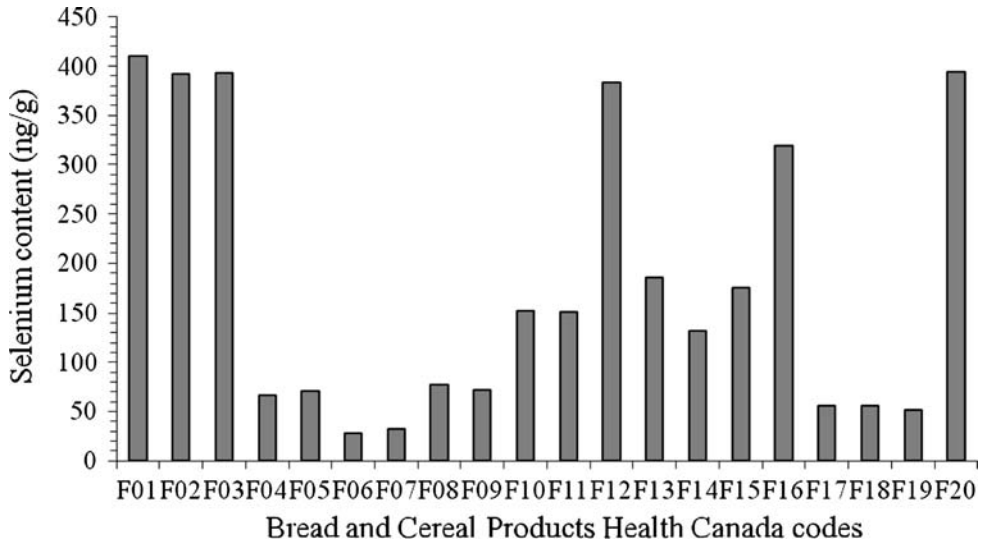

Fig. 3 Selenium content (fresh weight basis) of Bread and Cereal Products food group in Canadian composites.Legend for codes: *F01* Bread, white; *F02* Bread, whole wheat; *F03* Bread, rye; *F04* Cake; *F05* Cereals, cooked wheat; *F06* Cereals, corn; *F07* Cereals, oatmeal; *F08* Cereals, wheat & bran; *F09* Cookies; *F10* Crackers; *F11* Danish and donuts; *F12* Flour, wheat; *F13* Muffins; *F14* Pancakes; *F15* Pasta, mixed dishes; *F16* Pasta, plain; *F17* Pie, apple; *F18* Pie, other; *F19* Rice; and *F20* Rolls & English muffins

USA [19]. Even within the same food group, for example the Health Canada "F" group of bread and cereal products, the Se content varied widely as shown in Fig. 3. For this reason one needs the analytical method to be linear over several orders of magnitude. The PC-INAA-AC method developed in this work meets this requirement.

Conclusions

It can therefore be concluded that the anti-coincidence counting technique in combination with pseudo-cyclic neutron activation provides a more reliable measurement in terms of improved precision and detection limits for short-lived nuclides compared to both one-shot and pseudo-cyclic INAA coupled to conventional counting. A lower detection limit is always obtained in anti-coincidence counting for the same number of cycles compared to conventional counting. The maximum value for AFOM is generally obtained in the third or fourth cycle. This factor can be used for optimizing the number of cycles in PC-INAA in anti-coincidence counting mode. The PC-INAA-AC method can be applied to routine analysis of foods for Se without the need of dissolving the samples and tedious chemical treatments.

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