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Determination of boron in a black mouse by prompt gamma activation analysis

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In the boron neutron capture therapy, an accurate determination of the boron content in a biological sample is very important. The boron content was investigated with a standard solution of boron which was administered intraperitoneally with a dose of 750 mg/kg body weight into mice induced cancer cells and tumors. The boron content for two types of a sample was compared to the boronophenylalanine for the tumor and the ethylamine derivatives for the induced cancer cell, which were also investigated for their accumulation rate in each organ such as blood, spleen, liver, kidney and brain. An analytical quality control was carried out by using certified reference materials such as Peach Leaves, Apple Leaves and Spinach Leaves. The relative error of the measured values was in good agreement within 2% to the certified values.

Introduction

Boron is present in animal tissue in low concentrations of about a few mg/kg and it is an essential micronutrient for humans. Though the essentiality of boron for animals has not yet been fully established,[1] there is growing evidence that it may have a metabolic role in human and animal nutrition systems.[2,3] Boron is also used as a source for the short-range alpha-particles in a cancer treatment using boron neutron capture therapy (BNCT).[4,5] In the biological research by a prompt gamma neutron activation analysis (PGAA), the boron contents in tumors, tissues, blood and cultured cells which were estimated from the calibration curves obtained by using standard samples including different boron concentrations were studied in previous works.[6,7]

The boron compound, N-Benzyl-2-(o-carboranyl)-ethylamine, and boronophenylalanine (BPA) have been used for the boron neutron capture therapy of cancer cells and tumors in experimental animals. When tissue cells accumulate these compounds and receive thermal neutrons, the tissue could be efficiently destroyed because the $^{10}B(n,\alpha)^{7}Li$ reaction releases a particle and a recoiling ^{7}Li ion with an average total kinetic energy of 2.34 MeV. These particles have the characteristics of high linear energy transfer (LET) radiations with large biological effects. However, their ranges are very limited, namely, they have a path length of approximately one cell in diameter (10 μm). Since this average path length is probably the size of a tumor cell of 10–14 μm, it is possible to selectively destroy a cancer without affecting the surrounding tissues. Generally, a single incident of a neutron capture is sufficient to destroy a cancer cell, and a concentration of 10–35 μg boron per gram for a tumor which is equivalent to 10^{8}–10^{9} atoms of boron per cell is able to destroy a tumor. This concentration range is due to the location of the boron compound at or inside the cells.[8] The boron contents of animal tissues are very important for BNCT and especially in the areas of cancer and tissue. Numerous investigations and clinical studies on BNCT are based on precise and accurate measurements of the boron concentrations in tissues and biological samples.[9] The aim of the present study is to confirm the accuracy of a boron analysis for biological samples by using the PGAA system at the HANARO research reactor, KAERI, and to examine the boron content in various organs with cancer cells by an administration of a boron compound by using a PGAA.[10,11]

Experimental

Sample preparation

To obtain an accumulation rate in each organ such as a skin cancer, blood, spleen, liver, kidney and the brain, a standard solution of boron was administered by i.p. injection with a dose of 750 mg/kg body weight for the C57BL/6 mice sample. The B16-F10 melanoma cells which were exponentially grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 100 U penicillin/ml, 100 μg streptomycin/ml and 10% fetal calf serum were inoculated ($1 \cdot 10^{5}$ cells) into the back of a 6-week-old male C57BL mice. About 12 days later, the tumors reached a suitable size for the experiments. The aqueous suspension of BPA and the boron derivatives were prepared at a concentration of 100 mg/ml. A dose of 750 mg/kg body weight of the boronophenylalanine (BPA) and the boron derivatives were administered to the mice intraperitoneally and the mice were sacrificed three hours later. These samples were frozen in a deep freezer overnight and then they were freeze-dried at –20 °C for six days. Dried samples were ground into powder and then placed into a polytetra-fluoroethylene (PTFE) vial with a lid and stored in a desiccator. For the preparation of a sample for irradiation, the powdered samples were kept in oven

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at 30 °C for two hours and then cooled to room temperature. To re-check the moisture content, the sample was weighed before and after drying. The moisture content of the sample was less than 2%. Each of the samples used was measured five times with two or three sample weights, respectively. The biological certified reference materials such as the Peach Leaves (NIST SRM 1547), Apple Leaves (NIST SRM 1515), Spinach Leaves (NIST SRM 1570a) and Tomato Leaves (NIST SRM 1573a) were used for the analytical control. The certified reference materials were pretreated by the recommended method of the certificates.

Analysis of boron

The equipment and measurement system of a PGAA are described in a previous paper.[12] The boron concentrations in the standard and the real samples were determined by the prompt gamma neutron activation analysis system at the HANARO research reactor, KAERI, Korea. The neutron beam flux and Cd-ratio for gold at the sample position are $8.4\pm0.0\cdot10^7$ n·cm^{-2}·s^{-1} and about 300, respectively. Flux uniformity was within 4% for the central area of 1×1 cm^2 of the total beam cross section of 2×2 cm^2. The PTFE vial of a cylindrical shape was used and the vial size was made to be less than the beam dimensions and then the irradiation was performed in air. The sample frame was inclined 45° with respect to the diffracted beam direction. All of the samples were measured for 3,600 second per sample at the same sample position. The boron peak was overlapped with the prompt gamma-rays from ^{24}Na with 472 keV. The results are either known from the literature or can be determined using the standards experimentally. These intensity ratios can be used to subtract the appropriate counts from the combined boron plus the sodium peak in the 468–487 keV region.[13,14] The correction of the B concentration was obtained from the total peak area of the region of interest subtracted by the interference peak area corresponding to the standard Na concentration under the same measurement condition.

The combined uncertainties were in the range of 3.86 to 4.0% for the boron analysis of the relevant materials. The main sources of the uncertainties are due to statistical errors (0.20–0.34%), the detection efficiency (2.8%), the background subtraction of 472 keV Na (2.4–2.65%), and error sources including some other corrections, as shown in Table 1.

Results and discussion

Firstly, for the analytical control of the experimental procedure, the boron concentrations of the NIST SRM samples were determined from the measured count rate of the boron peak, the sample mass and the boron sensitivity. The results are summarized in Table 2 together with the certified values. The relative errors of the measured values are 1.0–2.0% for a high boron concentration such as the Apple Leaves, and they also showed about 3.5% for the 5 mg/kg concentration such as Typical Diet and above 6% for below the 3 mg/kg concentration such as Corn Bran when compared with the previous measurement. The analysis of the boron content by using the present PGAA facility is in good agreement with the certified value of the NIST SRM above 10 mg/kg, but below a 5 mg/kg concentration, the measured values are higher than the certified values. Figure 1 shows the *Z*-score from a comparison between the experimental and the certified values in terms of the ratio of the experiment to certified values.

The boron concentrations in the tumor and other tissues after a BPA and a boron sulfur hydride (BSH) administration were measured as shown in Fig. 2. By this measurement, the value of the kidney in the BAP, BSH and a combination of the BPA and the BSH was a few points higher than the other tissues. The boron content in the tissues after a BPA and derivatives administration were measured as shown in Table 3. For the BPA, for the boron content within each tissue, the tumor was the highest, however the stomach and kidney also had a high value. In the case of a cancer cell, the boron distribution after the administration of the boron derivatives is also shown in Table 3. The skin cancer value shows a higher count than the other tissues and also the kidney and stomach show a similar pattern with the BPA for the tumor. The total dose rate is about 13.2% with 99 mg/kg for the boron derivatives of 750 mg/kg. The boron content of each tissue by the boron derivatives shows higher counts than the BPA after an administration of the same amount (750 mg/kg).

In the boron concentration for the BAP and boron derivatives, the value of the tumor and skin cancers were higher than in other tissues. The data reported here shows that all the experimental organs contained boron contents, suggesting that the BPA and the boron derivatives might not be dominant accumulators in the tumor and cancer cells. Further, to be higher, for an accumulation of the boron concentrations in the cancer cell rather than in the brain and liver, new more powerful boron compounds have to be created and also more basic BNCT studies are needed. In the present measurement, most of the measured values were shown to be within 6% standard deviation in a comparison to the obtained value for each prepared sample for the same tissue, as shown in Fig. 3.

Table 1. Standard uncertainties estimated in the boron analysis by a PGAA

Uncertainties due to	Uncertainty, %
Statistical error	0.20–0.34
Detection efficiency	2.80
Mass (sample weight)	0.05
Detection sensitivity	0.50
Background subtraction from blank	2.40–2.65
472 keV, Na influence	1.00
Total uncertainty:	3.86–4.00

Table 2. Comparison of the measured and certified values for the NIST SRM samples

Sample (NIST SRM)	Measured, mg/kg	Certified, mg/kg	RE, %
Peach leaves (1547)	29.3 ± 1.0	29.0 ± 2.0	1.0
Apple leaves (1515)	27.3 ± 0.8	27.0 ± 2.0	1.1
Spinach leaves (1570a)	37.9 ± 1.5	37.6 ± 1.0	0.8
Tomato leaves (1573a)	33.9 ± 1.2	33.3 ± 0.7	1.8

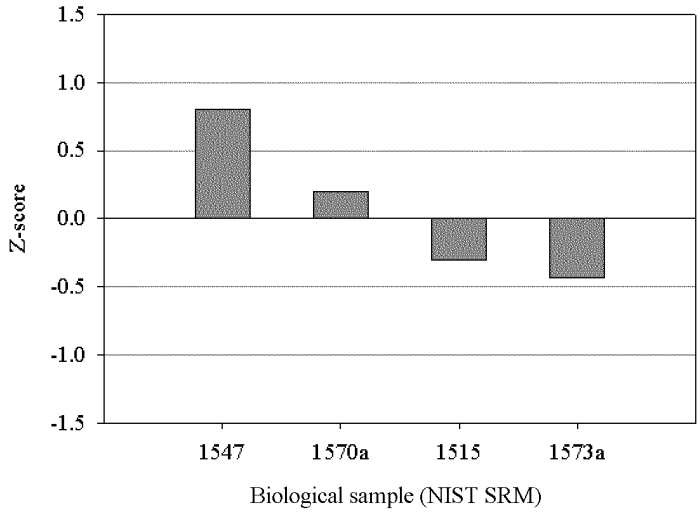

Fig. 1. Comparison of the analytical results for the biological NIST SRM samples

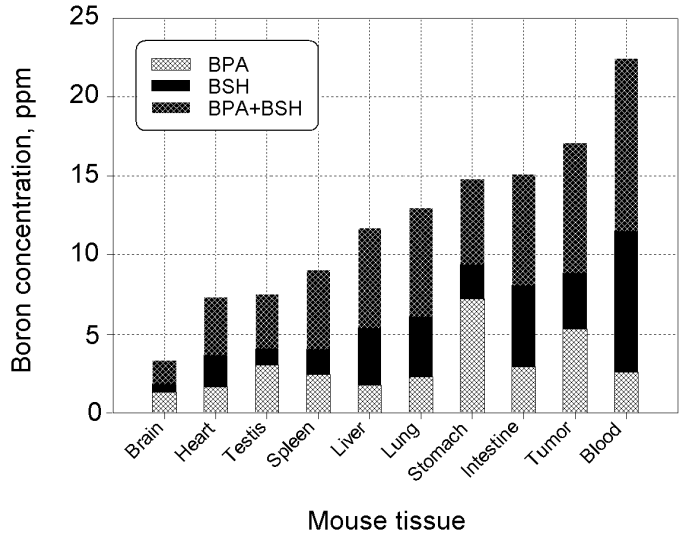

Fig. 2. Comparison of the boron concentration in the BPA, BSH and a combination of the BPA and the BSH

Table 3. Boron concentration (in mg/kg) of various tissues after an administration of the BPA 750 mg/kg/b.wt. and boron derivatives 750 mg/kg/b.wt., (mean ± SD)

Tissue	BPA (tumor)	Boron derivatives (cancer cell)
Blood	3.6 ± 0.2	6.6 ± 0.6
Lung	4.3 ± 0.3	8.1 ± 0.4
Heart	2.6 ± 0.2	n.d.
Liver	3.8 ± 0.2	15.7 ± 1.0
Spleen	2.5 ± 0.3	2.7 ± 0.4
Kidney	6.3 ± 0.5	11.6 ± 0.8
Testis	3.1 ± 0.3	3.4 ± 0.3
Stomach	7.3 ± 0.6	12.2 ± 1.0
Brain	2.3 ± 0.2	11.8 ± 0.4
Intestine	2.9 ± 0.3	3.1 ± 0.4
Tumor	9.3 ± 0.4	n.d.
Coronary	n.d.	6.1 ± 0.4
Skin cancer	n.d.	17.9 ± 0.6

n.d.: Not determined.

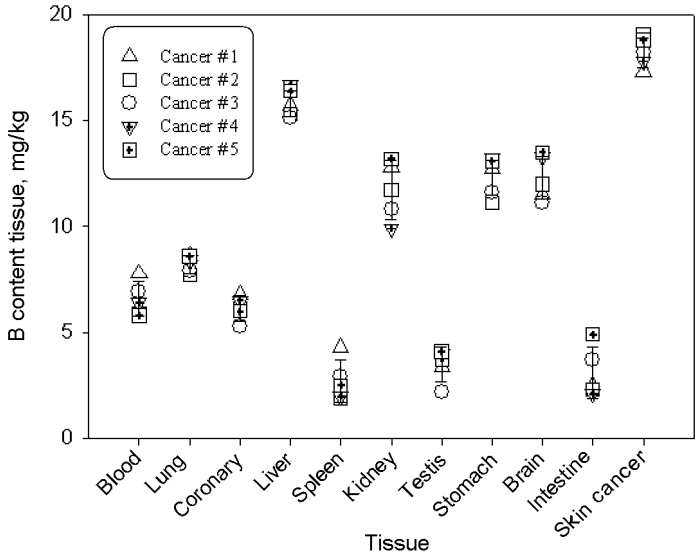

Fig. 3. Comparison of the boron concentration with the cancer cells

Conclusions

The degree of the spectral interference peaks as a matrix effect for a sample was established for an accurate peak analysis, and then an analysis of the boron by a PGAA was investigated by using certified reference materials (NIST SRM). Under a stable beam flux which had a fluctuation below 2%, the measured values for the SRM are in good agreement with the certified value.

One of the important tissues for the basic and clinical studies on the BNCT is a precise and accurate measurement of the boron concentrations in biological samples. Analysis of the boron concentrations which had accumulated in tumor and cancer cells were experimentally performed and they were compared with the boron concentration in the BPA and boron derivatives for two types to create good boron compounds from an accumulation aspect.

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References

1. F. H. NIELSEN, Biol. Trace Elem. Res., 27 (1990) 599.
2. F. H. NIELSEN, Nutr. Today, 27 (1992) 6.
3. D. L. ANDERSON, W. C. CUNNINGHAM, T. R. LINDSTOM, J. Food Comp. Anal., 7 (1994) 59.

4. C. P. RAAIJMAKERS, M. W. KONIJNENBERG, L. DEWIT, D. HARITZ, R. HUISKAMP, K. PHILIPP, A. SIEFERT, F. STECHER-RASMUSSEN, B. J. MIJNHEER, Acta Oncologica, 34 (1995) 517.
5. D. W. NIGG, Intern. J. Radiation Oncol. Biol. Phys., 28 (1994) 1121.
6. Y. S. CHUNG, H. J. CHO, J. H. MOON, S. H. KIM, Y. J. KIM, Anal. Sci. Technol., 16 (2003) 391.
7. C. H. LEE et al., Medical Research in BNCT, KAERI/CM-666/2002.
8. T. U. PROBST, Fresenius J. Anal. Chem., 364 (1999) 391.
9. B. J. JUN et al., Development of Cancer Therapy Facility of HANARO and Medical Research in BNCT, Research Report, KAERI/RR-2364/2002.
10. K. J. CHUN et al., Korean Assoc. Radiat. Prot., in press.
11. K. J. CHUN et al., Korean Assoc. Radiat. Prot., in press.
12. S. H. BYUN, G. M. SUN, H. D. CHOI, Nucl. Instr. Meth., A487 (2002) 521.
13. H. J. CHO, Y. S. CHUNG, Y. J. KIM, Nucl. Instr. Meth., B229 (2005) 499.
14. HYUN-JE CHO, YONG-SAM CHUNG, YOUNG-JIN KIM, J. Radioanal. Nucl. Chem., 264 (2005) 701.