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行政院國家科學委員會專題研究計畫成果報告

超熱中子活化分析法應用於生物樣品的最佳化條件 Optimal condition for analyzing biosample via Epithermal neutron activation analysis

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一、中文摘要

為了降低由清華大學反應器($10^{12} \text{ n cm}^{-1}\text{s}^{-1}$)的中子束造成 ^{38}Cl , ^{24}Na 及 ^{42}K 對 γ 能譜上的干擾，建立一個應用於超熱中子活化分析(Epithermal Neutron Activation Analysis, ENAA)的最佳化條件，以 1 mm 厚鎘套(Cd)及 3.2 mm 厚的硼-聚乙烯(Borated polyethylene, BPE)為封套以尋求標準品鮮苔(lichen IAEA-336)及明日葉之生物樣本內的砷-76($T_{1/2} = 26.3 \text{ hr}$)，溴-80($T_{1/2} = 17.7 \text{ min}$)，錳-56($T_{1/2} = 2.58 \text{ hr}$)，碘-128($T_{1/2} = 24.9 \text{ min}$)、銻-122($T_{1/2} = 2.68 \text{ d}$)等元素建立確實又快速的同時多元素最佳化分析條件。結果證明 150 mg 的樣本中碘及溴以照射 10 分鐘、冷卻 10 分鐘、計測 5 分鐘，而於砷及銻是以 350 mg 的樣本照射 24 小時、冷卻 30 至 60 小時、計測 20 分鐘，得到的最小可測值 minimum detectable concentrations (MDC)的最佳化分析方法。

關鍵詞：最佳化條件，超熱中子活化分析，砷、溴、錳、碘、銻，最小可測值

Abstract

Optimal conditions for analysis of As, Br, Mn, I and Sb contents in lichen and *angelica keiskei* (AK) were investigated using Epithermal Neutron Activation Analysis (ENAA). The minimum detectable

concentrations (MDCs) of ^{76}As ($T_{1/2} = 26.3 \text{ hr}$), ^{80}Br ($T_{1/2} = 17.7 \text{ min}$), ^{56}Mn ($T_{1/2} = 2.58 \text{ hr}$), ^{128}I ($T_{1/2} = 24.9 \text{ min}$) and ^{122}Sb ($T_{1/2} = 2.68 \text{ d}$) were calculated at optimized conditions. This was accomplished by wrapped the irradiated target with 1 mm Cd cover and 3.2 mm Borated polyethylene (BPE) neutron filters which were adopted to screen original reactor fission neutrons and reduce of ^{38}Cl , ^{24}Na , and ^{42}K background activity. These results indicated that the MDCs of Br and Mn, I, measured under 10 min activation and 5 min counting followed by 10 min decay. MDCs of As, Sb were analyzed by 350 mg irradiated in 24 hr counting 20 min following by 30 to 60 hr decay was optimal for irradiation in a $10^{12} \text{ n cm}^{-1}\text{s}^{-1}$ thermal neutron flux.

Keywords: Optimal condition, ENAA, As, Br, Mn, I, and Sb, MDC

1. Introduction

Understanding the levels of toxic elements, such as As, Sb is the prime interest of toxicological studies and trace element. Iodine has, for many years, been known to be an essential nutrient and to participate in synthesizing thyroid hormones.¹ Many matrix elements, such as

Cl, K, and Na in *Angelica keiskei* (AK, Umbelliferae “Ashita-Ba” in Japanese) can be activated readily by thermal neutrons producing dramatic background activities of ^{38}Cl (1642.7 keV), ^{42}K (1524.7 keV) and ^{24}Na (1368.6 keV) which may interfere with direct counting of γ -activities of lower γ -ray energy isotopes like ^{76}As (559.2 keV), ^{80}Br (616.3 keV), ^{56}Mn (846.6 keV), ^{128}I (442.9 keV), ^{122}Sb (564.1 keV) and ^{153}Sm (103.2 keV) or increase Minimum Detectable Concentration (MDC).² For obtaining better analytical precision, enlarging sample weights, irradiating, lengthening decaying and counting durations are needed.³⁻⁵ Such long waiting time enlarges the MDCs of radionuclides with half-lives shorter than or comparable to those of interfering activities and generally reduces the analytical usefulness of INAA. Table 1 lists selected and interfering radionuclides in this study. The work deals with the analysis MDCs in lichen and some AK via a usual reactor, using 1 mm Cd cover and 3.2 mm Borated polyethylene (BPE) filter.

2. Experimental procedure

2.1 Material

Lichen (IAEA-336) was chosen as an analytical standard and carefully weighed in clean PE before being placed in an electron desiccator.⁶ Elements in AK are considered to promote human health and have been extensively studied by the Taiwan Agricultural Research Institute (TARI). Raw AK, including about 5 kg of roots, leaves and stems, was taken simultaneously from the five-planted farms to ensure diversity in Taiwan. These samples were endorsed by TARI. The collected samples were first washed in distilled water and then very carefully homogenized using Potytron Homogenizes (Model PT-11, Inyon Switzerland) with high quality stainless steel blades, before analysis by a method published elsewhere.⁴ These samples were subsequently freeze-dried using Labconco for two days at -40°C , under 13 mm-Hg. Lichen, and AK were divided into four

groups by weight 50 ± 10 mg, 150 ± 10 mg, 250 ± 10 mg, and 350 ± 10 mg. A weighed sample was packed into a 3×3 cm² polyethylene (PE) bag and immediately doubly sealed in another PE bag for irradiation in a pneumatic tube (PT) and vertical tube (VT) of Thing Hua Open-pool Reactor (THOR).⁴ An empty PE bag of identical size, also double-sealed, was used as a blank correction. Each sample and standard was prepared in triplet to minimize the statistical uncertainty, as described elsewhere.^{4,6} Each irradiated sample was paired with a 10 mg Ni-foil monitor used to record neutron flux fluctuation in PT.⁴⁻⁶

2.2 Ratios of R_{Cd} and R_{BPE}

To suppress the reactor neutrons from Open-pool Reactor of Tsing Hua University (THOR), effectively, all the irradiated samples were wrapped in 1 mm Cd and 3.2 mm thickness BPE. A quantified index was defined and expressed in Tab. 1 as R_{Cd} , and R_{BPE} . These ratios are acquired peak of radionuclide measurement with and without Cd or BPE during identical irradiation. These results indicated that the gold of R_{BPE} was 17.9 ± 1.4 , which is consistent with published data.⁷⁻⁹ It can be observed that the As, Br, Mn, I, Sb and Sm are 3.63, 3.32, 21.5, 2.63, 6.29 and 1.94 higher than those of the interfering nuclides, respectively. R_{BPE} are in agreement with the reference values as shown in Tab. 1.^{2,9-11}

2.3 MDA and MDC

$$\text{MDA}_{(\text{ENAA})} = \frac{K \cdot (2.71 + 4.65\sqrt{B})}{\bar{\Phi} \cdot \bar{\sigma} \cdot m \cdot \text{Eff}_{(\text{E}\gamma)} \cdot I_{\gamma(\text{E}\gamma)} \cdot S \cdot D \cdot C} \quad (1)$$

Where B represents the background of the photopeak; $\bar{\Phi}$ is the average epithermal neutron flux; $\bar{\sigma}$ is the mean cross section of the nuclide with an epithermal neutron; m is the sample weight, and $\text{Eff}_{(\text{E}\gamma)}$ is the absolute detector efficiency at the photopeak with intensity $I_{\gamma(\text{E}\gamma)}$; $I_{\gamma(\text{E}\gamma)}$ is the intensity of the γ -ray. Here, $S = 1 - \exp(-\lambda t_i)$, $D = \exp(-\lambda t_d)$, and $C = (1 - \exp(-\lambda t_c)) / \lambda$, are the saturation factor, decaying factor, and counting factor,

respectively. K represents an overall calibration factor that relates to the chemical processes, if there is any.

$$MDC_{(sample)} = \frac{MDA_{(sample)}}{MDA_{(std)}} C_N K_f \quad (2)$$

Where C_N is the lichen contents of As, Br, Mn, I, Sb and Sm were simultaneously by measuring the counting rate C_N is given in $ng\ g^{-1}$. K_f is the correction factor of epithermal neutron flux determined by gold-foil.^{4,12} Figure 1 and 2 plot these MDCs by ENAA using thermal neutron filters.

2.4 Optimized conditions selected

Some factors are responsible for the limitations, the analytical conditions must be considered. First, the samples were counted only when the dead times of multichannel analyzer (MCA) system were within 20% for the peak integrated in high purity germanium (HPGe).^{4,5} Second, the irradiated samples also had to be cooled for a suitable time for avoiding too high Compton scattering plateau on which the γ -ray are situated. This Compton scattering plateau is mainly generated by 1368.6 keV of ^{24}Na ($T_{1/2} = 15.06$ hr), 1524.7 keV of ^{42}K ($T_{1/2} = 12.35$ hr), 1642.7 keV of ^{38}Cl and 1779 keV of ^{28}Al ($T_{1/2} = 2.24$ min). That is, decaying time must be enough to permit the decay of interfering nuclides to accept limits and to prevent Compton continuum overlapping the low energy of γ -spectra as listed at Tab. 1 and Fig. 3(d) and 4(e). Third, the irradiating weight of evaluated sample must be as lower as possible in the limited radiation container for the huge irradiated samples in routine works. Thus, the MDC analysis of As, Br, Mn, I, Sb and Sm of lichen was displayed in Fig 2(a), to 2(c). Table 1 presents the characteristics of selected nuclides, including the I_{reso}/σ_{ther} , half-lives, γ -ray energy and Cd, boron ratio.^{2,10-12} Several analytical schemes were adopted in Tab. 2.

Table 2. Irradiation and analytical periods for elemental concentration analysis in lichen (IAEA-336) and *Angelica keiskei*

Sample weight	Irradiating time	Decaying time	Group
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(mg)			
<i>Short irradiation</i> ^{a,b}			
50	2 min	2 min	G22
150	5 min	5 min	G55
250	2 min	20 min	G220
350	7 min	10 min	G710
350	10 min	5 min	G105
350	10 min	20 min	G1020
350	10 min	60 min	G1360
<i>Middle irradiation</i> ^{c,d}			
350	1 hr	24 hr	M350.24
<i>Long irradiation</i> ^{c,d}			
50	24 hr	20 hr	L50.20
150	24 hr	20 hr	L150.20
250	24 hr	20 hr	L250.20
350	24 hr	30 hr	L350.30
350	24 hr	60 hr	L350.60
350	24 hr	80 hr	L350.80
350	24 hr	520 hr	L350.520

^a Irradiation was conducted at the pneumatic tube with epithermal neutron flux $1.8 \times 10^{11} n\ cm^{-2}s^{-1}$

^b Sample to detector in the γ -ray HPGe was 8 cm

^c Irradiation was conducted at the vertical tube with epithermal neutron flux $1.1 \times 10^{11} n\ cm^{-2}s^{-1}$

^d Sample to detector in the γ -ray HPGe was 2 cm

2.5 Loss of elements during irradiation

Determining whether any loss of elements occurs during and after irradiation is very important, because of the high temperature in the Cd filter.^{6,13} The possible loss of elements during irradiation was confirmed by examining the activity of the elements. 150 mg of lichen in doubly sealed PE bags was irradiated when wrapped in a 1 mm Cd filter under counting period of 5 min after a decaying period of 10 min. Elemental concentrations, Br, Cl, I, Mn, and Na, were measured for irradiation periods of 2 to 13 min, as displayed in Fig. 5. Any loss of any one of these elements by evaporation would be manifest by a lack of proportionality between the activity and the irradiation period. The figure shows that, under the conditions employed in these examples, no leakage of elements occurs during ENAA when the samples are enclosed in the doubly sealed PE bags and wrapped in a Cd filter.⁵

3. Results and discussion

3.1 Short irradiation

The experimental results confirm that ENAA simultaneously determined ^{76}As , ^{80}Br , ^{128}I , ^{122}Sb and ^{153}Sm and with reasonable sensitivity. Figures 1(a) and 1(b) show the MDC analyses of ^{80}Br and ^{128}I . G22, the analytical groups clearly showed significant discrepancies, even when no iodine was detected. G22 yields the poorest results. Moreover, the MDC values also improve as the irradiation time increases. Table 3 shows that a significant discrepancy of Br concentration assessed from both the analytical group G22 and G55 owing to shorter time of irradiation than other group.

3.2 ^{80}Br and ^{128}I

Based on the MDC of Br analysis, significant differences ($p < 0.05$) were observed in roots, stems, and leaves of AK under G22, G55, and G1360 conditions from that under G105 conditions. Bromine concentrations of the stems of AK taken from Puli (central Taiwan) showed a significant difference ($p < 0.05$) under both G22 ($88 \pm 6 \mu\text{g g}^{-1}$) and G55 ($88 \pm 5 \mu\text{g g}^{-1}$) conditions compared to the G105 ($47 \pm 2 \mu\text{g g}^{-1}$) condition. This difference may be the result of high concentrations of Cl and Na and Mn in stems of AK. The iodine concentration in the leaves was $0.38 \pm 0.08 \mu\text{g g}^{-1}$, significantly higher than in other parts of AK. From these results, the identification of ^{28}Mg by INAA at 843.8 keV can be difficult, because of strong interference from the 846.6 keV ^{56}Mn .^{4,12} A longer irradiating period time (15 min) in Cd filter cause difficulty at a large γ -spectral dead time (over 20%) making the identification of the spectral photopeak impossible. An irradiated Cd filter has a very strong residual radioactivity and the special protection afforded by a hot cell is needed. From Tab. 3, iodine cannot be detected under G22, G55, G220, or G710 conditions in the roots, stems, or leaves of AK.

3.3 ^{27}Mg and ^{56}Mn

As shown in Tab. 3, with the exception of G22, G55, G220, and G710, the differences among Mg measurements (843.8 keV) may be attributed to inaccurate determination caused by interference of the 846.6 keV ^{56}Mn .

The relatively high concentration of ^{56}Mn in the AK still reduces the statistics quality of ^{27}Mg using ENAA. Under G105, G1020 and G1360 conditions, both twin peaks are resolvable using Sampo90 software. Moreover, in the activation scheme of ^{56}Mn nuclides, only 2 min of irradiation may cause a high γ -ray peak at 846.6 keV, as shown on Fig.3(b). Figure 3(c) shows that the corresponding Compton scattering plateau overlaps the γ -ray peaks generated from 442.9 keV ^{128}I , 616.6 keV ^{80}Br , and 843.8 keV ^{27}Mg . This overlapping phenomenon may increase the MDC and also minimize the analytical precision of the identification of nuclides. In using the ENAA techniques, the activation scheme must thus be constrained in several minute orders to avoiding overheating the Cd filter and unwanted interference from ^{56}Mn .

3.4 Long irradiation

Following 30 hrs of decay, BPE cover could not measure the 564.1 keV of ^{122}Sb ($T_{1/2} = 2.70$ day) under L150.20 condition. On the other hand, irradiated heavy-samples within 20 hr decay (L350.20) encounter dead time 30% making spectra photopeak identification impossible. In Figure 2, the MDCs of As, Sb, and Sm can be decided after 24 hr irradiation. The M350.24 condition always has the worst MDC values, due primarily to shorten irradiating durations (1 hr) among eight conditions. From seven conditions in the long irradiation, it was clear that the analytical conditions (L50.30) of 50 ± 10 mg irradiation have significant discrepancies even there were no As, Sb, Sm can be investigated. The analytical concentrations of As, Sb, and Sm irradiated with 350 ± 10 mg following 80 hr decay (L350.80) has lower MDC than those in 250 ± 10 mg (L250.30) analysis. Table 3 shows a significant discrepancy of Sb concentration assessed from both the M350.24 and L50.20, due primarily to shorten time of irradiation among other conditions. Because of less sample weight and shortening duration of irradiating, both of M350.24 and G50.20 always appear with worse MDCs among each condition even there were no As, Sb, and Sm can be

investigated. In addition, the MDC values also improve with an increase of sample weight. However, in L350.30, L350.60, L350.80 that had 350 ± 10 mg weight with a cooling time longer than 30 hrs, the MDCs only improve mildly. However, lengthening the decaying time (30 to 80 hr) can improve the MDCs only mildly regardless of the variation in decaying time, as shown in Fig. 2(a) to (c). Therefore, the best optimal conditions are L350.30, L350.60, and L350.80. The results indicates the optimized conditions of elemental contents of medicinal herbs using ENAA wrapped by 3.2 mm BPE can be decided from sample weight, irradiating, and cooling durations.

Figure 4(a) shows the γ -spectrum of an empty double-sealed PE bag under L350.30 condition. It is clear that the interference from irradiated blank, double-sealed PE, is negligible.⁵ Contents of As, Sb, and Sm in the PE blank were all below the MDCs.

3.5 The ^{76}As and ^{122}Sb

The dramatic activity coming from ^{82}Br in Chinese Medicine herbs still reduces the statistical quality of ^{76}As and ^{122}Sb . In general, ENAA technique only improves the MDCs of ^{76}As and ^{122}Sb marginally, since during long irradiations (24 hours), ^{82}Br , ^{76}As and ^{122}Sb isotopes displayed comparable I_{reso}/σ_{ther} ratios and half-lives, the 554-559-564 trine peaks. However, the two peaks were sufficiently resolved to permit reasonable quantitative determination of As and Sb. However, Figure 4(d) demonstrated that these peaks were sufficiently resolved to permit reasonable quantitative determination of As, and Sb.

3.6 Elemental concentrations in AK

Figure 4 and 6 show the concentrations of six elements in freeze-dried AK collected from five farms. The average elemental concentrations, under optimal ENAA conditions (G105), are also shown. In general, elemental concentrations vary from 10^3 to 10^{-1} $\mu\text{g g}^{-1}$. The concentrations of iodine range from 0.18 to 0.38 $\mu\text{g g}^{-1}$; those of bromine range from 2.1 to 41 $\mu\text{g g}^{-1}$ in the roots of AK from the five main farms. The maximum concentration of Mg is 1470 $\mu\text{g g}^{-1}$

in AK from Lalashan (high mountain), but is negligible in the roots, stems, and leaves taken from Chuchi (southern Taiwan). The variations of elemental content among farms are mainly attributed to the differences in soil mineral composition, fertilizers, and climatological conditions.

Conclusions

In general, ENAA is favorable for those elements with high I_{reso}/σ_{ther} values. In short irradiation, seven analytical groups were selected. The MDCs of Br and I, measured with a 10 min activation period and a 5 min counting time after 10 min of decaying (G105) with a 350 mg sample wrapped in a 1 mm Cd filter, are optimal conditions for rapid and reliable routine works. The MDCs of Br and I are reduced to 0.33 and 0.11 $\mu\text{g g}^{-1}$, using G105. This work has demonstrated that the ENAA techniques might be successfully applied to Br and I in AK. Enclosed in doubly sealed PE bags, elemental leakage occurs during 2-13 min of irradiation is negligible. The concentrations of these elements have been found to range from 10^4 to 10^{-1} $\mu\text{g g}^{-1}$ in AK. In long irradiation, eight conditions were selected for optimal analysis herein. The MDCs of As, Sb, and Sm, determined with 24 hr irradiated duration and a 20 min counting time after 30 to 60 hr decaying with 350 mg. The entire period of analysis of the AK may be reduced to 30 min for routine analysis. Samples wrapped in 3.2 mm BPE are optimal condition for analysis the As, Sb, and Sm simultaneously.

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- Figure 3(d). γ -spectrum of lichen measured under L350.30 condition
- Figure 3(e). γ -spectrum of lichen measured under L350.520 condition
- Figure 4(a). γ -spectrum of an empty double-sealed PE bag measured with G105 condition.
- Figure 4(b). γ -spectrum of lichen measured with G22 condition.
- Figure 4(c). γ -spectrum of lichen measured with G105 condition.
- Figure 4(d). γ -spectrum of lichen measured with G1360 condition.
- Figure 5. Elemental concentrations of 350 mg lichen for irradiation periods of 2 to 13 min for Br, Cl, I, Mn, and Na.
- Figure 6. Elemental concentrations distributed in the roots, stems, leaves of *Angelica keiske* AK taken from: ■ Chuchi; ○ Lalashan; ▲ Minder ; ▽ Puli ; ◆ Taian farms.
- Figure 7. (a), (b) Elemental concentrations of capsules and tea bags in the five planted areas.

Figure Captions

Figure 1. The analysis of minimum detectable concentration (MDC) for ^{80}Br and ^{128}I in lichen of various analytical groups with ENAA: solid for ^{80}Br and open for ^{128}I , Irradiating time (min) : ■ 2; ● 5; ▲ 10; ▼ 13.

Figure 2. The analysis of minimum detectable concentration (MDC) for ^{76}As , ^{122}Sb , and ^{153}Sm in lichen of various analytical conditions with ENAA: (a) ^{76}As , (b) ^{122}Sb , and (c) ^{153}Sm , Irradiated weight (mg) : ■ 50; ○ 150; ▲ 250; ▽ 350.

Figure 3(a). γ -spectrum of an empty double-sealed PE bag measured under L350.30 condition.

Figure 3(b). γ -spectrum of lichen measured under M350.24 condition

Figure 3(c). γ -spectrum of lichen measured under L50.30 condition