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생체시료중 수은의 정량을 위한 중성자 방사화분석에 관한 연구

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A Study on the Neutron Activation Analysis for the Determination of Mercury in Biological Samples*

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요 약. 쌀, 생선 및 표준 기준 배추시료 속에 함유된 수은의 함량을 두 개의 독립적인 분석 방법 즉, 과거 본 연구실에서 개발한 방법과 Sjöstrand 방법에 의하여 분석하였다. 분석 결과는 시료의 종류에 따라서 서로 다른 매트릭스 효과를 보였다. 예를 들면 배추와 생선 시료는 같은 매트릭스 효과를 나타내었으나 쌀 시료는 이 두 종의 시료와 비교하여 다른 효과를 보였다.

ABSTRACT. Rice and fish samples as well as a standard reference kale sample have been analyzed for the mercury content using two independent methods, i.e., one developed previously in this laboratory and the other reported by Sjöstrand. The analytical results indicate differences in matrix effects depending on the type of sample, e.g., kale and fish samples show the same matrix effects, whereas rice samples show different effects compared to others.

INTRODUCTION

The determination of the trace amount of mercury in biological materials has become an important analytical task due to the wide-spread uses of various mercuric compounds in agriculture and industry. Neutron activation analysis is known to be highly accurate and sensitive for the determination of the element and it has been applied to biological samples by a number of authors. Most of them use post-irradiation separations in order to achieve sensitive determinations for mercury at ng level. These techniques involve digestion and distillation of mercury in a closed system,[1,2] precipitation,[3,4] solvent extraction,[5] ion exchange separation,[6] and isotope exchange[7] and others.[8]

Irradiation problems also need caution, because some parts of mercury are easily lost by volatilization or adsorption on the surface of ampoules. Except for a few works where both mercury standards and analytical samples were irradiated in polyethylene ampoules,[6,8] silica tubing was

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used for the irradiation.

A method was previously reported from this laboratory for the simultaneous determination of mercury, bromine, arsenic and cadmium in biological samples.[9] The method included the irradiation of samples in polyethylene vials, the digestion of the samples in a closed vessel, the distillation of mercury and bromine, and the separation of arsenic and cadmium by using a cation exchange column. In this work, the recovery of mercury during separations was found to be more than 95 %, which was confirmed by the ^{197}Hg tracer technique. However, when the method was applied to the reference kale powder, the content of mercury was found to be much lower by a factor of 3.6 ~5.2 than the best value of 0.167 ppm given by Bowen.[12] It was concluded that such differences might have been attributable to the loss of mercury during irradiation, because the analytical samples were irradiated in the polyethylene vials.

In order to rectify such as large difference between the two results, the reference kale sample has been analyzed after irradiation in a silica ampoule and cooling the ampoule in liquid nitrogen before opening. However, the results obtained were still lower by a factor of 2.3 than the value given by Bowen.

The present work has been attempted to investigate the cause for such differences. For this purpose, rice and fish samples have been analyzed as well as the reference kale, using two independent method, i.e., one by the method developed in this laboratory[9] and the other by Sjöstrand method.[1] The analytical results show different matrix effects which are dependent on the type of the samples as described below.

EXPERIMENTAL PROCEDURES for BIOLOGICAL SAMPLES

The Present Authors' Methods[9]. Samples of 0.5~1.0 g of the biological materials were weighed and put into polyethylene vials of 1.5 m*l* capacity or into silica ampoules and sealed. The silica ampoules were made from 4 mm I.D. silica tubing according to the method given by Bowen and Gibbons.[10] The samples were irradiated with a flux monitor for 1 hour using the pneumatic transfer system of either the TRIGA mark II or mark III reactors where the neutron fluxes were 3×10^{12} and 1.5×10^{13} neutrons per cm^2-sec respectively. The samples were left for 1 day to allow most of short-lived activities to decay. The irradiated samples in polyethylene vials were quantitatively transferred into the flask A of *Fig.* 1 using a few ml of concentrated nitric acid for washing. When silica ampoules were used, further treatment was adopted for the quantitative transfer into the flask A prior to the isolation of mercury as follows.

The irradiated silica ampoules were thoroughly cleaned to remove the possible surface contamination and dipped in liquid nitrogen to prevent possible loss of mercury by volatilization. Each

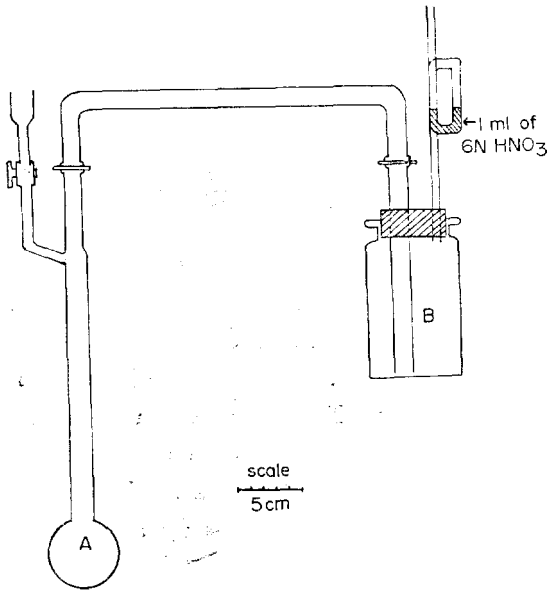

Fig. 1. Apparatus for decomposition of organic materials and for distillation of mercury.

of the ampoules was inserted into a piece of plastic tube which was previously sealed at one end. The tube was then filled with 3 m*l* of an oxidation mixture which consisted of 90 % concentrated nitric acid and 10 % concentrated sulfuric acid in order to cover the ampoule completely. The plastic tube was sealed at the other end with a Hoffman clamp. A pressure was applied to crush the ampoule. The sample was then immediately mixed with the oxidation mixture. The gases were absorbed by shaking the tube. The plastic tube was then opened. The irradiated sample and broken pieces of the ampoule were quantitatively transferred into the flask A of *Fig.* 1 using a few m*l* of concentrated nitric acid for washing.

The sample was heated with a total volume of 0.7 m*l* of concentrated sulfuric acid in flask A of *Fig.* 1. During heating, additional quantities of nitric acid were added until organic tissues were completely digested. The total volume of concentrated nitric acid was 7~8 m*l*. The excess nitric acid was then expelled from the flask by further heating. The time needed for the digestion was about one hour. During heating, mercury was distilled and absorbed into the Bottle B which contained 30 m*l* of 10 *N* sodium hydroxide solution. The solution in bottle B was transferred into a 500 m*l* beaker. Three m*l* of mercury carrier solution (4 mg of mercury per m*l*) were added and the solution was adjusted to pH 8~9 with 6 *N* hydrochloric acid. The precipitation of mercuric sulfide was then carried out by adding 3 m*l* of thioacetamide (T. A.) solution (5 mg of T. A. per m*l*).[3] The precipitates were allowed to settle for 2 hours and centrifuged. The precipitates were dissolved in a saturated sodium sulfide solution, transferred into a 50 m*l* volumetric flask and finally counted with 3″×3″ NaI(Tl) crystal which was coupled with a 400 channel analyzer. The mercury content was calculated by comparing the peak area of 68+77 keV gamma-rays with the normalized counts of ^{197}Hg per min-μg which were independently obtained under the same experimental conditions and given in the previous paper.[9]

Modified Sjöstrand's Method.[1] An accurate amount of the sample was sealed in a silica ampoule before irradiation. After cooling for 1 day the ampoule was cleaned to remove surface contamination. The ampoule was broken in a plastic tube as described above. The sample and the broken pieces of the ampoule were transferred into the flask of *Fig.* 2. One m*l* volume of mercury carrier solution containing 20 mg of mercuric chloride was added. A 15 m*l* volume of

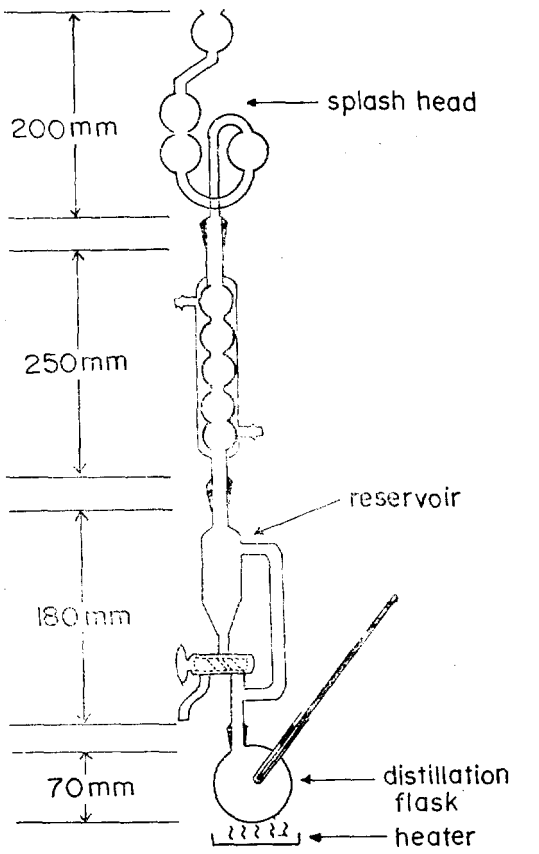

Fig. 2. Apparatus for controlled decomposition of organic materials, also used as distillation apparatus.

an oxidation mixture of 90 % concentrated nitric acid and 10 % concentrated sulfuric acid were added. The flask was connected to a Bethge apparatus as shown in *Fig.* 2. A few m*l* volume of water was poured into the splash head. The tap was closed and the flask was heated until the nitric acid was distilled into the reservoir. The temperature was allowed to rise to over 300 °C. The distillate was run back into the flask when cold. Heating cycles were repeated until the solution was completely clear and colorless. After cooling, a mixture of 5 m*l* of 70 % perchloric acid and 0.5 g of glycine dissolved in 5 m*l* of water was added to the dissolved sample. Heat was applied to the flask. When the solution was boiling under reflux the tap was closed and the mercury fraction was collected in the reservoir. The distillation was interrupted when the temperature exceeded 250 °C. The distillate was tapped into a 600 m*l* glass beaker. The mercury in the distillate was further separated as follows.

The solution was adjusted to pH 8~9 with 6 *N* ammonium hydroxide solution. The precipitation of mercuric sulfide was carried out by adding T. A. solution as described above. The precipitates were settled and centrifuged. The precipitates were washed with a few m*l* of distilled water and centrifuged. The precipitates were dissolved in a saturated sodium sulfide solution, transferred to a 50 m*l* volumetric flask and counted with the analyzer as described above. The content of mercury was calculated as described above.

RESULTS and DISCUSSION

The mercury content in the reference kale sample which was obtained in the previous report[9] by using the TRIGA mark II and mark III reactors is shown in the second and the third columns of *Table* 1 respectively. In these works, polyethylene vials were employed for the irradiation of the samples in the reactor. These two values are much lower by a factor of 3.6~5.2 compared to the best value of 0.167 ppm reported by Bowen. It was thought that such discrepancies might be attributable to the absorption and vaporization of mercury during irradiation because

Table 1. Comparison of mercury contents(ppm) obtained from reference kale.

Discription of method	Irradiation in TRIGA II reactor using polyethylene vial and disitllation by the previous method	Irradiation in TRIGA III reactor using polyethylene vial and distillation by the previous method	Irradiation in TRIGA III reactor using silica ampoule and distillation by previous method	Irradiation in TRIGA III reactor using silica ampoule and distillation by Sjöstrand method
Results	0.049	0.023	0.070	0.143
	0.063	0.036	0.073	0.149
	0.038	0.020	0.082	0.142
	0.040	0.041	0.059	0.154
	0.050	0.027	0.076	0.149
	0.041	0.029	0.077	0.157
	0.048	0.043	0.070	0.152
	0.042	0.037	0.076	0.149
Average($\pm 1\sigma$)	0.046±0.008	0.032±0.009	0.073±0.007	0.149±0.005
Discrepancy factor between the value by Bowen and the results obtained	3.6	5.2	2.3	1.1

the samples were irradiated in polyethylene vials.

The values in the second column are more reproducible than those in the third column. The difference in the values and their reproducibilities might results from the higher temperature effects in TRIGA mark III which is operated at ~2 MW compared to the TRIGA mark II operated at 250 KW.

In order to confirm the possible cause for the loss of mercury, a silica ampoule was employed for the irradiation instead of a polyethylene vial. After irradiation, the silica ampoule containing the sample was cooled in liquid nitrogen before opening as described above under the Experimental Procedure. The results are given in the fourth column of *Table* 1. The average value, 0.073± 0.007ppm, obtained by this procedure, was still found to be much lower by a factor of 2.3 than the best value of 0.167 ppm given by Bowen. From these results it was concluded that the volatilization and absorption of mercury on the polyethylene vials during irradiation could not fully explain the total loss of the element in the previous procedures.

In order to understand the cause of such discrepancies among the values in *Table* 1, the previous procedures were rechecked as follows. For this purpose, only the inactive mercury solution, 5 μg of mercury per 1 m*l* of distilled water, was added into the polyethylene vial of 1.5 m*l* capacity without the reference kale sample. After irradiation for 1 hour, it was found that about 50 % of mercury was so strongly absorbed on the inner surface of the vial that the mercury could not be recovered by the repeated washing with concentrated nitric acid. The non-absorbed mercury remaining in the solution was digested and distilled in similar manner as described above under the section of the Present Authors' Method, and its recovery was found to be 50~ 60%. In total, the overall recovery of mercury appeared to be about 30 % throughout this procedure. This low recovery of mercury may explain, at least partly, the differences among the analytical results given in the second, third and fourth columns in *Table* 1. In contrast with this, however, when the previous procedures for the digestion and distillation were adopted with the mixture of the tracer of ^{197}Hg and the non-irradiated samples of rice, it was found that the recovery was more than 95 %.[9] The reason why such a large differences existed between the present and previous recoveries was not fully investigated. It was, however, concluded that the previous procedure was not adequate for the digestion and distillation of mercury. A modified Sjöstrand method which is described above was, therefore, adopted for the determination of mercury. Eight analyses for the mercury were carried out and the results are summarized in the last column of *Table* 1. Further improvement in the values obtained by this method is evidenced by the agreement between two values, i.e., one obtained by this method and the other given by Bowen.

The loss of mercury in the fish samples was corrected by multiplying the analytical results by the factors given in *Table* 1, which were deduced from the ratio of the mercury content given by Bowen to the values by previous procedures. A good agreement was obtained as shown in *Table* 2. In contrast with the fish samples, the correction was found unnecessary for the rice samples because a fairly good agreement was obtained as shown in the last two columns in *Table* 2. It was, therefore, thought that such differences of the mercury content among the samples might be attributable to the hardness of the sample surface. Throughout the present work, the rice grains were irradiated without pulverization as described by Tölg.[11] Following his suggestion, dried fish were broken into a large pieces for irradiation. The standard reference kale powder was delivered from IAEA in a pulverized state and irradiated directly as received. The surface structures of both

Table 2. Mercury contents of rice and fish show definite matrix effects.

Sample No.	Species	Sampling site	Contents(ppm)		
			Irradiation in TRIGA mark II reactor usingpolytehylene vial and distillation by the previous method		Irradiation in TRIGA mark III reactor using silica ampoule and distillation by Sjöstrand method
			Results obtained	Results corrected by the factor of 3.6	
R-1	Rice	Inchun, Kyungki	0.042		0.037±0.002
R-4	Rice	Chunan, Chungnam	0.038		0.040±0.002
R-7	Rice	Yoju, Kyungki	0.027		0.027±0.001
R-8	Rice	Ychun, Kyungki	0.030		0.027±0.001
R-9	Rice	Kwangju, Kyungki	0.011		0.011±0.001
R-10	Rice	Ulsan A, Kyungnam	0.012		0.012±0.002
F-18	Snakehead	Daechun,Chungnam	0.42	1.5	1.7±0.3
F-20	Carp	Euiam, Kwangwon	0.075	0.27	0.23±0.01
F-21	Carp	Soyang, Kwangwon	0.058	0.21	0.17±0.008
F-22	Crucian	Chungpyung, Kyungki	0.058	0.21	0.18±0.02
F-24	Carp	Kwangnaru, Seoul	0.30	1.1	0.83±0.05
F-25	Carp	Paldang, Kyungki	0.26	0.94	0.77±0.04

the kale and the fish samples are similar in softness. On the other hand, rice grains are hard and, therefore, it is reasonable to expect that mercury will find difficulty in escaping from rice grain.

It was concluded from the above consideration and the results of *Table* 2 that the previous procedures are adequate for the determination of mercury in rice sample as the Sjöstrand method, but not suitable for the analyses of such soft samples as fish and kale powder. The correction factors, which were obtained by the analysis of the reference kale and which were presented in *Table* 1, could be applicable to fish sample only.

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