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≪Original≫ Simultaneous Determination of Mercury, Bromine, Arsenic and Cadmium in Biological Materials by Neutron Activation Analysis*

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Abstract

A method for the simultaneous determination of mercury, bromine, arsenic and cadmium in biological samples is described. Following neutron activation and a simple distillation of volatile compounds, mercury and bromine were determined by gamma-ray spectrometry. Arsenic and cadmium were further separated by cation exchange separation and determined similarly. Determination limits for mercury, bromine, arsenic and cadmium were 0.001 μg, 0.003 μg, 0.001 μg and 0.02 μg, respectively. The method has been applied to the determination of mercury, bromine, arsenic and cadmium in rice and fish samples. Analysis of a standard kale powder yielded the values of 0.046 μg/g for mercury, 24.5 μg/g for bromine 0.17 μg/g for arsenic and 0.50 μg/g for cadmium.

요 약

생체 시료중에 함유된 수은, 브롬, 비소 및 카드뮴을 동시에 정량하였다. 시료를 원자로에서 중성자 조사 한다음 휘발성 물질인 수은과 브롬은 증류법을 사용하여 분리한다음 감마선 분광법으로 정량하였다. 그리고 비소와 카드뮴은 양이온 교환수지를 이용하여 계속분리하여 역시 감마선 분광법으로 정량하였다. 수은, 브롬, 비소 그리고 카드뮴의 정량한계는 각각 0.002μg, 0.003μg, 0.001μg 및 0.02μg이었다. 이 분석방법을 사용하여 쌀및 생선시료 중의 수은, 브롬, 비소 및 카드뮴을 정량하였다. 그리고 동방법을 사용하여 표준시료인 배추가루를 분석한 결과 수은이 0.046μg/gr, 브롬이 24.5μg/gr 비소가 0.17μg/gr이었고 카드뮴은 0.50μg/gr이었다.

1. Introduction

Wide-spread environmental contamination from various toxic compounds used in agriculture and certain industries makes it desirable to have a reliable picture of present and future backgrounds of toxic elements such as mercury, bromine, arsenic and cadmium in the biosphere. For that purpose neutron activation analysis has the advantage over conventional methods not only in its high sensitivities in the detection of these elements but also in the avoidance of contamination by reagents.

A number of methods have been described previously for the separation and the determination of mercury[1-4], bromine[5], arsenic[4-6] and cadmium[7-9] using activation techniques.

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However these methods are usually applicable to the determination of one or two of these elements and are not specific for the simultaneous analysis of these toxic elements.

This paper describes a method for the simultaneous determination of mercury, bromine, arsenic and cadmium in biological samples. Following the process of neutron activation, distillation and ion exchange separation, the elements mercury, bromine, arsenic and cadmium are identified and assayed by gamma-ray spectroscopy. The present method has been applied to the determination of mercury, bromine, arsenic and cadmium in rice and fish samples. A series of measurements of the elements in the standard kale powder of Bowen[10] has been made in order to provide a comparison between the present and other methods.

2. Experimental

1. Reagents and Apparatus

All the neutron irradiations were carried out by pneumatic transfer systems of TRIGA MARK II and III reactors. The neutron fluxes in the TRIGA MARK II and III reactors were 3×10^{12} and 1.5×10^{13} neutrons per cm^2 per sec., respectively.

After irradiation the decomposition of the organic samples was carried out in a closed system because of the volatility of some inorganic elements. The decomposition of samples as well as the distillation of mercury and bromine were therefore performed in the apparatus shown in Fig. 1. This apparatus was modified from the method reported by Smith[6]. The apparatus was consisted of round bottomed flask A with a long neck and a side tube C through which acid solutions were transferred. During digestion the distillates of mercury and bromine were collected into the bottle B in which 30 ml of 10 N sodium hydroxide solution were contained. In order to prevent the further vaporization of mercury, 1 ml of 6 N nitric acid solution was added in the bend of the trapping tube D.

A resin colum, Dowex, 50W ×8, 50-100 mesh, 15 cm long by 6 mm diameter, was used for the cation exchange separation. A fraction collector, Instrumentation Specialities Company, and a well type scintillation counter, Fujitsu E-14 Model, were used. A 400 channel gamma-ray pulse height analyzer, Hitachi RAH-403 Model. was connected with 3″×3″ NaI (Tl) crystal. The standard solution of mercury (5 μg of mercury per ml) was prepared by dissolving reagent grade mercuric chloride in distilled water in a volumetric flask. The additional standard solutions used here (bromine, arsenic, eadmium, sodium, potassium, copper and manganese) were made by dissolving ammonium bromide, arsenic oxide, cadmium metal, sodium carbonate, potassium nitrate, copper metal and manganese metal in appropriate solvents, respectively. The concentration of the elements were about 30μg per ml. All other chemicals used were reagent grades.

2. Standardization

When samples are irradiated the flux fluctuations in the irradiation sites will cause some errors. Moreover, when the irradiation of a sample takes place with many standards the voluminous targets will cause inhomogeneous irradiation, giving rise to different reaction rates inthe sample and standards. In order to eliminate these difficulties, iron wire was used as a single comparator, which homogeneously contained sufficient manganese to produce a suitable ^{56}Mn activity as described in the previous report[11]. For the use of iron wire as the comparator, the activities from the standard samples were compared, as described

below, with the gamma-ray activities under the 0.84 MeV. peak of ^{56}Mn from the wires which were each irradiated along with samples.

It was reported by previous authors that the standard solution of mercury should be sealed in a silica tube because other capsules such as those of polyethylene adsorb mercury[1]. Moreover, after irradiation the tube should be treated[4] in order to trap the mercury vapour that might be formed during the irradiation. In order to eliminate these operations for the mercury standard, experiments were conducted as follows.

One ml of the standard solution of mercury was pipetted into a polyethylene vial of 1.5 ml capacity. The vial was sealed with a flame. Ten mg of the iron wire was attached on the outside wall of the vial as a flux monitor. The vial was irradiated for 1 hr and cooled for 1 day. The vial was counted at fixed geometry without opening it. From the counting rate thus obtained, the equivalent counting rate in other geometry, *i. e.*, the solution of standard mercury in 50ml flask as described below, was estimated using the relative counting efficiencies which have been previously determined in both geometries. For the measurement of these relative counting efficiencies, the tracer ^{197} Hg was used.

Other standard solutions of 1.2ml were each pipetted, transferred into polyethylene vials, and sealed as described above for the mercury standard. Ten mg of the iron wire was attached to the outside wall of each vial. The vial was irradiated for 1 hr and cooled for 1 day. The vials were opened and 1 ml of each solution was transferred into a volumetric flask of 50 ml capacity and diluted to volume. Gamma-ray spectrometry was applied for the measurement of the gamma-ray activity. The gamma-ray energies and half lives of the nuclides used in this work are shown in Table 1.

Each irradiated iron wire was attached to the center of an aluminium plate and the area under the 0.84 MeV. peak of ^{56}Mn was measured with the analyzer. The activity of each standard element was normalized with the activity of the manganese monitor. The normalized activites in counts per min per μg of standard elements are shown in Table 2. For this data the standards irradiated were cooled for 20 hours.

3. The decomposition of Samples and the Distillation of Mercury and Bromine

One g of the irradiated sample was heated with 0.7 ml of concentrated sulfuric acid and 3 ml of concentrated nitric acid in flask A of Fig. 1. During heating additional quantities of nitric acid were added until the organic tissues were completely digested. The total volume of concentrated nitric acid added was about 7 ml. The excess nitric acid was then expelled from the flask by further heating. The time needed for the digestion was about one hour. During digestion the mercury and bromine were distilled and collected as described above under section 2.1. Recoveries of both elements were determined by tracer work, *i.e.*, by repeating these procedures with the radioactive

Table 1. The gamma-ray energies selected

Isotope	Half-life	Selected energy	Isotope	Half-life	Selected energy
^{197}Hg	65hr.	0.77and 0.068 MeV.	^{42}K	12.4hr.	1.52 MeV.
^{82}Br	35.5hr.	0.78 MeV.	^{64}Cu	12.9hr.	0.511 MeV.
^{72}As	26.5hr.	0.56 MeV.	^{56}Mn	2.58hr.	0.845 MeV.
^{24}Na	15.0hr.	1.37 MeV.	^{115}Cd	53.5hr.	0.34 MeV. of ^{115m}In

Table 2. Counts of each element per min. per μg

Element	Counts of element	Element	Counts of element
Hg	2,548±161	K	22.6±3.7
Br	1,557±67	Cu	1,456±16
As	3,147±10	Mn	905±43
Na	874±33	Cd	229±15

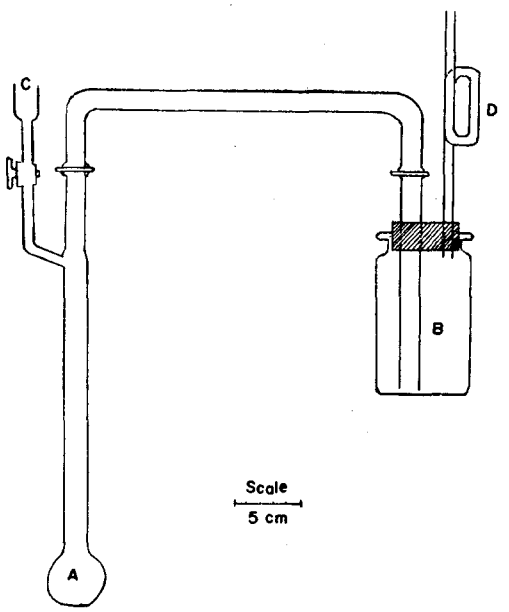

Fig. 1. Apparatus for the destruction of biological samples.

tracers of ^{197}Hg and ^{82}Br along with samples of non-irradiated rice. The recovery of bromine was 100% and that of mercury was more than. 95%.

It was also confirmed by similar tracer work with ^{76}As that the arsenic quantitatively remained in the reaction flask A. This is in good agreement with the results of Smith[6].

4. Further Separation and gamma-ray Measurements

The solution of 10 N sodium hydroxide in bottle B, which contained mercury and bromine, was transferred to a 50 ml volumetric flask and diluted with distilled water to volume. Gamma-ray spectrometry was performed for the activity of the bromine using a 400 channel analyzer which was connected to the 3″×3″ NaI (TI) crystal. After counting the bromine, the solution in the flask was quantitatively transferred to a 500 ml beaker. Three ml of the mercury carrier solution (4 mg of mercury per ml)were added and the solution was adjusted to pH 8-9 with 6N hydrochloric acid. The precipitation of mercuric sulfide was then carried out by adding 3 ml of thioacetamide (T. A.) solution (5mg of T. A. per ml). The precipitates were allowed to settle for 2 hours and centrifuged. The precipitates were dissolved in a saturated sodium sulfide solution, transferred to a 50 ml volumetric flask and finally counted with the analyzer. The digested sample solution in flask A was transferred to a 50 ml volumetric flask and diluted to volume with distilled water. Twenty five ml of the solution were loaded on a cation exchange resin column and eluted with 50 ml 0.1 N HCl for the quantitative separation of the arsenic, as shown on the elution curve of Fig. 2.

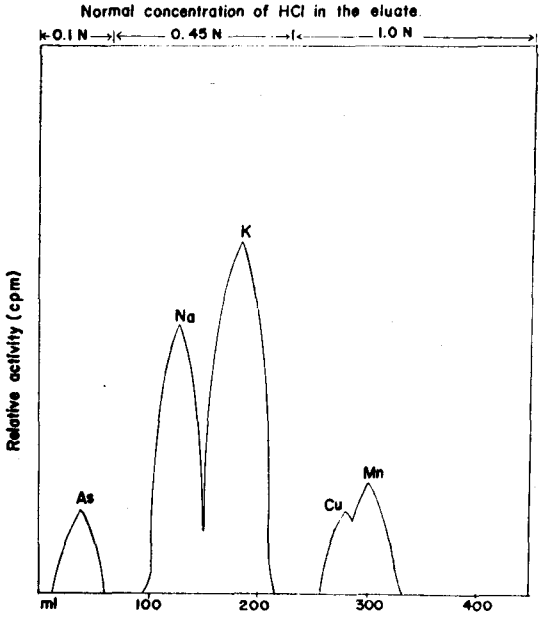

Fig. 2. Elution curve of trace elements in a rice sample

Table 3. The contents of elements analyzed in the rice ane fish (p.p.m.)

Name	The Places	Hg	Br	As	Na	K	Cu	Mn	Cd
Rice	Suwon, Kyungki	0.14±0.02	0.30±0.01	0.08±0.01	14±0.8	640±7	3.5±0.3	19±0.3	0.02±0.002
//	Pohang, Kyungbuk	0.023±0.006	0.41±0.05	0.12±0.01	26±2	690±5	2.7±0.1	12±0.6	0.075±0.004
//	Mockpo, Junnam	0.015±0.002	0.59±0.03	0.43±0.02	31±0.3	630±5	1.6±0.08	11±0.9	0.10±0.007
Pomfret	Inchun, Kyungki	0.049±0.006	27±0.9	7.2±0.3	5230±110	3820±540	4.4±1.0	7.9±0.8	0.69±0.02
Snakehead	Kwangchun Chungnam	1.8±0.3	7.9±0.57	0.22±0.002	2630±78	8320±540	5.5±0.25	2.9±0.3	0.77±0.02
Lobster	Daechun, Chungnam	0.053±0.003	33±0.6	9.0±0.4	330±3	2000±310	16±0.2	2.3±0.3	2.1±0.09

The flow rate was about 1 ml/min. The eluate was collected in fractions of 60 drops by means of the fraction collector. The activity of each fraction was measured for 1 min. with a well type scintillation counter and was plotted against the volume of the eluate as shown in Fig. 2.

The column was further eluted with 150ml 0.45 N HCI for the quantitative separations and the measurements of Na and K. The copper and manganese were then separated by eluting the column with 100 ml 1 N HCI. Each eluate was collected into a 50ml volumetric flask after reducing the volume by heating. Mn and Cu could easily be counted with the 1 N HCI eluate, which would also contain alkaline earths[13]. Rare earths could be further eluted with 3 N HCI solution[13,14], but this was omitted in this work.

Radiochemical purities of mercury, bromine, arsenic, copper, manganese, sodium and potassium for the analysis of rice and fish samples were determined by checking half lives and gamma-ray spectra.

It was ascertained by tracer work that cadmium was quantitatively eluted in the 0.45 N HCI eluate in Fig. 2, as predicted from the data of the distribution coefficients[15]. In order to determine cadmium in biological samples the following experimental procedure was adopted. Twenty mg of Cd carrier were added to the 0.45 N HCI eluate and the Cd was precipitated as CdS by passing H_2S gas. The Cadmium sulfide was reprecipitated remove Na and K. The precipitates were to dissolved in 1ml 6 N HCI and diluted to 15 ml with dist. water 1.5 ml of 3N NH_4Cl was added and pH was adjusted to 7 by the dropwise addition of 6N NH_4OH. 7 ml of 1.5 N $(NH_4)_2$ HPO_4 solution were added to the solution. Cadmium precipitates were collected on weighed filter paper discs, dried, weighed as $CdNH_4$ $PO_4 \cdot H_2O$, and counted with multichannel anal yzer after about 16 hours to allow the ^{115m}In daughter to grow into equilibrium[16].

3. Results and Discussion.

The procedures described above were applied

Table 4. Analytical results of the standard kale powder (p.p.m.)

Element	Results of this work (±1 standard deviatign)	Previous results[10]	Best mean (1972)[18]
Hg	0.046±0.0078	0.150	0.167
Br	24.5±2.2	24.3	26.1
As	0.17±0.002	0.127	0.141
Na	2300±113	2488	2506
K	15319±1030	18890	24615
Cu	5.05±0.19	4.81	4.99
Mn	11.7±0.38	14.7	14.73
Cd	0.50±0.05	0.89[9] 0.384[7]	0.74

Table 5. The content of mercury in kale powder (p. p. m.)

	1	2	3	4	5	6	7	8	Average	Std. Dev. (%)
Triga mark II	0.049	0.063	0.038	0.040	0.050	0.041	0.048	0.042	0.046	17
Triga mark III	0.023	0.036	0.020	0.041	0.027	0.029	0.043	0.037	0.032	27

Table 6. Counting times and limits for the determination

Element	Counting time (min.)	Determination limit(μg)
Hg	30	0.001
Br	30	0.003
As	30	0.001
Na	10	0.01
K	10	0.5
Cu	15	0.005
Mn	15	0.007
Cd	30	0.02

to the simultaneous determination of mercury, bromine, arsenic and cadmium in rice and fish samples. The contents of each element in the samples were calculated by comparing the activity of each element with the corresponding values given in Table 2. Typical results are summarized in Table 3. The contents of sodium, potassium, copper and manganese are given in the Table because these elements were also analyzed simultaneously.

The standard sample of dried kale powder was included in order to provide a running check of the method. The results of the elements in the dried kale are shown in Table 4. The values for the bromine and arsenic are in agreement with the values reported by Bowen[10], but the concentration of mercury is much lower than the values of 0.15 μg/g determined by other works using activation analysis. A possible explanation of the discrepancies between the values determined by neutron activation analysis may lie in the vaporization of mercury during irradiation. In contrast to procedures adopted in this work, others irradiated the samples in silica ampoules and placed these in liquid air before opening in order to condense any mercury vapour that might be formed during irradiation. The analytical results, obtained with the TRIGA MARK II reactor which was operated at 250 KW, were reproducible as shown in Table 5. The results, obtained with the TRIGA MARK III which was operated at 2 MW, showed less reproducibility and this discrepancy may result from the vaporization of mercury during irradiation, because of higher temperature in TRIGA MARK III compared with TRIGA MARK II.

It is, however, concluded that mercury contents in biological samples can be determined by this method with a good reproducibility, which is comparable to other methods when the sample is irradiated in TRIGA MARK II. However, when the sample is irradiated in TRIGA MARK III the present method gives worse reproducibility.

In the determination of cadmium, nearly the same weight of the standard as the expected weight of the element in the samples was irradiated in order to eliminate the high self-shielding effect of the cadmium[9]. The analytical results for cadmium in the standard kale samples are comparable with the values reported by previous workers as shown in Table 4. The contents of sodium, copper and manganese which were determined simultaneously from the standard kale samples are in good agreement with the values reported as shown in Table 4. The potassium content however showed lower results by factor of 1.2 than the values reported previously by the same

methods as shown in the same Table.

The quantitative determination limits of the elements have been calculated by Curie's method and are given in Table 6, which shows the weights giving 100 counts[17]. The sensitivities are sufficient to permit the determination of these elements when irradiating for one hour, except cadmium which needs further irradiation to improve the sensitivity

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