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A Simplified Method for the Determination of Arsenic by Means of Activation Analysis

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Dedicated to Professor *Ole Lamm* on his 60th birthday

A simple and rapid method for determination of arsenic in virtually all sorts of biological materials and also in other cases by means of activation analysis has been developed and evaluated. Arsenic is separated radiochemically by distillation and precipitation and the radioactivity of ^{76}As measured by γ-spectrometry or β^--counting. The sensitivity is $10^{-8}-10^{-9}$ g, depending on the measurement technique, when irradiation is performed at a flux of the order of 10^{12} neutrons cm^{-2} sec^{-1}. The procedure has been applied to the determination of the arsenic content of the remains of the 16th century King Erik XIV of Sweden and of other materials.

In this laboratory, methods of activation analysis have been studied for some seven years. The general method of activation analysis is to irradiate the sample (usually with reactor neutrons) and then measure the induced radioactivity either directly or after some sort of chemical separation of the desired element. Measurements of γ-activities can be made energy-selective using a scintillation detector and a pulse-height analyzer. Thus one often distinguishes between "spectrometric activation analysis" and "chemical activation analysis". For more detailed information on activation analysis the reader is referred to review articles by Westermark *et al.*[1] and by Atkins and Smales [2].

Since γ-spectrometric activation analysis is rapid and non-destructive it is naturally preferred when possible. However, disturbing γ-radiations from other nuclides present often make chemical separation necessary. It was found very convenient to make some simple and rapid separations, *e.g.* group separation, followed by γ-spectrometry. This combined method is fairly rapid, gives a good sensitivity in most cases and simultaneously yields an accurate identification of the nuclide in question.

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Earlier attempts by Westermark *et al.*[4,21] to determine arsenic in biological material by spectrometric activation analysis, showed that, due to disturbing γ-activities present, only an upper limit for the arsenic content of the sample can be given. Now a method of the "combined" type has been worked out.

Nuclear data of arsenic. The only naturally occurring isotope of As is stable ^{75}As, which when irradiated with thermal neutrons forms radioactive ^{76}As. According to Allen *et al.*[5] the activation cross section of the element for ^{76}As production is 4.2 barns. ^{76}As decays with a half-life of 26.5 h to stable ^{76}Se by the emission of β^-- and γ-radiation. The predominant maximum β^--energies are 2.97 (56 %) and 2.41 MeV (31 %) and the energy of the most abundant γ-ray is 0.56 MeV (45 %). In addition there are low intensity γ-rays such as 0.66 (6.3 %) and 1.21 MeV (5.3 %). The disintegration scheme can be found, *e.g.*, in a book by Dzhelepov and Peker [6]. The scheme indicates that some γ-rays are emitted in cascade and this fact might be used for selective measurements with a coincidence γ-ray spectrometer according to Ljunggren[7]. The intensities of the cascade emitted γ-rays are, however, weak ($\sim$8 %).

Neutron induced ^{76}As-activity can be formed by the following reactions:

$$^{75}\mathrm{As(n,\gamma)}^{76}\mathrm{As} \qquad \sigma = 4.2 \text{ b} \tag{1}$$

$$^{76}\mathrm{Se(n,p)}^{76}\mathrm{As} \qquad \sigma \approx 0.8 \text{ mb} \tag{2}$$

$$^{79}\mathrm{Br(n,\alpha)}^{76}\mathrm{As} \qquad \sigma = 10 \text{ mb} \tag{3}$$

$$\left.\begin{matrix} ^{74}\mathrm{Ge(n,\gamma)}^{75}\mathrm{Ge} \\ ^{75}\mathrm{As(n,\gamma)}^{76}\mathrm{As} \end{matrix}\right\} \xrightarrow[T_{1/2} = 82 \text{ min}]{\beta^-} {}^{75}\mathrm{As} \tag{4}$$

Reaction 1 predominates when arsenic is irradiated with thermal neutrons, while reactions 2 and 3 occur with fast neutrons. The activation cross section for reaction 2 is calculated from Inthoff [8] and for reaction 3 given by Blosser *et al.*[9] In the determination of arsenic in germanium reaction 4 can be a cause of error: Smales and Pate [10] have found an error corresponding to 0.08 ppm of arsenic for a 75 h irradiation of pure germanium dioxide at 2×10^{12} neutrons cm^{-2} sec^{-1}.

When arsenic is irradiated with fast neutrons reaction 5 occurs:

$$^{75}\mathrm{As(n,2n)}\ ^{74}\mathrm{As} \quad \sigma \approx \begin{cases} 50 \text{ mb}\ [11] \\ 545 \text{ mb}\ [27] \end{cases} \tag{5}$$

^{74}As decays with a half-life of 18 days emitting β^+-, β^-- and γ-radiation. The energy of the most abundant γ-ray is 0.596 MeV (62 %) [5]. In addition, the emitted positrons give rise to 0.51 MeV annihilation γ-rays.

In the determination of arsenic by activation analysis the reaction ^{75}As(n,γ) ^{76}As is the preferred one. The procedure is to irradiate the sample together with an arsenic standard in the thermal neutron flux of a reactor for about one half-life (26.5 h) and then separate the arsenic activity which is measured. Comparison of the activities of sample and standard gives the amount of arsenic present in the sample.

Radiochemical separation of arsenic. Activation analysis for arsenic has been reported by Smales and Pate [10], Lenihan and Smith [12], Lenihan [13] and Smith [14]. A compilation of the radiochemistry of arsenic is given by Beard [15].

In addition references to arsenic determinations can be found in bibliographies on activation analysis by Gibbons *et al.*[16],[17] and Bock-Werthmann and Schulze[18].

The radiochemical procedures described in these earlier arsenic determinations seem to involve too many steps. We have therefore developed a simplified method with only three steps preceding γ-spectrometry:

1. Dissolution with $HNO_3-H_2SO_4$
2. Distillation, as $AsCl_3$ and $AsBr_3$, with HCl—HBr
3. Precipitation, as the element, with $NH_4H_2PO_2$.

EXPERIMENTAL

Preparation of sample and standard. The sample, weighing 0.1—0.5 g, was sealed in a quartz ampoule. (Sealing technique see Westermark and Sjöstrand [3].) The sample (s) was then irradiated for about 20—30 h together with a suitable arsenic standard at a flux of $1-2 \times 10^{12}$ neutrons cm^{-2} sec^{-1} in the Swedish reactor R 1. The standard was prepared by sealing a known volume (0.1 ml) of a water solution of As_2O_3 in a quartz ampoule to give a known amount of 1—2 μg arsenic. After irradiation, the ampoules were left for a few hours to let shortlived activities decay.

Separation of arsenic. After careful cleaning, the ampoule was crushed in a plastic tube and transferred to a flask for decomposition of the organic material in 10 ml of a

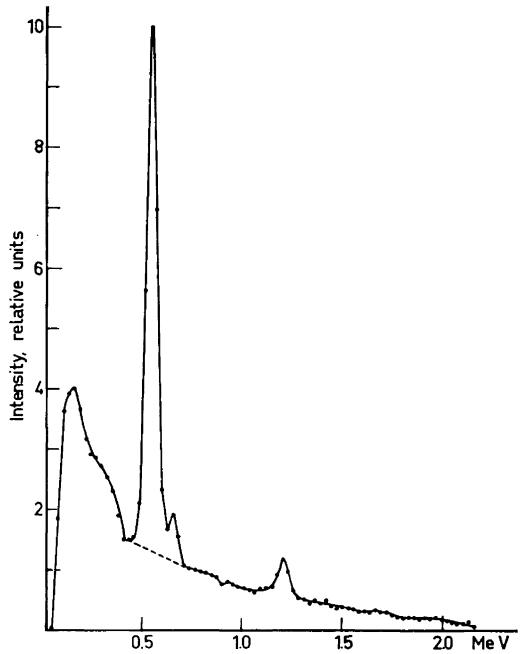

Fig. 1. ^{76}As γ-spectrum obtained from one of the samples (black smooth velvet from the 16th to the 17th century). Relative intensity is plotted against energy in MeV. The area enclosed by the curve at about 0.6 MeV and the dotted "back-ground" line was taken as a measure of the arsenic content of the sample. The γ-peaks at 0.66 MeV and 1.21 MeV are clearly visible in addition to the main peak at 0.56 MeV.

mixture of 3:1 fuming nitric acid and concentrated sulfuric acid. A known amount of about 25 mg As_2O_3 was added as a carrier. The flask was heated until no more nitrous gases or excess nitric acid were evolved; otherwise the hydrobromic acid added subsequently would be oxidized to free bromine. The flask was left to cool, then 15 ml hydrochloric acid was added dropwise. The sample was then transferred to the distillation flask and 10 ml 48 % hydrobromic acid was added. The flask was connected to the distillation apparatus and the distillation was continued until the volume in the distillation flask had been reduced by about one half. The temperature of the fumes was allowed to rise to 120—125°C. A stream of nitrogen was used as a carrier for the arsenic halides.

The condensate was collected in 10 ml water in an ice-cooled receiver flask and hydrochloric acid was added to it until the solution contained about 8 N HCl. Arsenic was then precipitated as the element by adding about 2 g of ammonium hypophosphite. After the ammonium hypophosphite had dissolved completely the solution was heated on a water-bath for 45 min. The precipitate formed was collected on a fine pore porcelain filter.

Recovery determination. The recovery of arsenic was determined by drying and weighing the porcelain filter before and after the filtration. The filters were dried to constant weight at 105°C. Comparison with the added amount of arsenic carrier gave the chemical yield which was regularly 80—100 %.

Standard treatment. Since the quartz in the ampoules contains small amounts of arsenic it seemed necessary to remove the standard solution from the ampoule before measuring its activity. It is also possible to correct for the As-content in the quartz by irradiating an empty ampoule (of the same quartz) together with the samples. In this case the standard activity should be measured by γ-spectrometry without breaking the ampoule.

In our procedure we have chosen to break the ampoule and let the standard undergo exactly the same procedures as the sample.

ACTIVITY MEASUREMENTS

After radiochemical separation of the arsenic the γ-spectra of samples and standards were measured using a 3″ × 3″ NaI(Tl)-scintillation detector coupled to either a multi-channel pulse height analyzer, of the Hutchinson-Scarrott type, or a single-channel analyzer. The latter is quite adequate when the radioactivity is not too weak. In Fig. 1 the γ-spectrum obtained from one of the samples is shown up to 2 MeV. The shape of the spectra from this and all other samples were identical with those obtained from the arsenic standards and the energy of the observed photo-peaks matched the γ-energies of ^{76}As within $\pm$ 15 keV. Half-life measurements yielded values between 25.0 and 27.5 h, which is in good agreement with the value 26.5 h given by Allen *et al.*[5]

Inspection of the $T_{1/2}$-E_γ-chart for radionuclides formed by neutron capture, published by Westermark and Sjöstrand [4], shows ^{76}As to be the only possible nuclide with the observed values of γ-energies and half-life.

As can be seen from Fig. 1 the emitted γ-rays of 0.56 MeV energy give rise to the largest peak and the area of this peak, plus that of the small 0.66 MeV peak, was used to determine the arsenic content of the samples.

Parallel to the spectrometric measurements, the β^--activity of the samples and standards was measured by counting with a G—M tube coupled to a scaler. These determinations gave half-lives of 25.5 to 26.5 h and values of arsenic content which were in excellent agreement with those obtained by γ-spectrometry, as can be seen in Table 1.

Table 1. Amount of arsenic in μg found by using different methods for measuring the ^{76}As-activity.

Sample	γ-spectrometry with single-channel analyzer 3″ × 3″ solid crystal	γ-spectrometry with multi-channel analyzer 3″ × 3″ solid crystal	Measurement of total γ-radiation 3″ × 3″ solid crystal	Measurement of total β^--radiation G—M tube	Average	Standard deviation in %
A	2.88	2.84	2.84	2.86	2.86	± 1
B	0.315	0.298	0.305	0.318	0.309	± 3
C	0.145	0.130	0.132	0.138	0.136	± 6
D	1.08	1.03	1.02	1.08	1.05	± 3

Table 2. Sensitivity limits of As-determination for different methods of activity measurement after irradiation in a flux of $\sim 10^{12}$ neutrons · cm^{-2} sec^{-1}.

Measurement method	γ-spectrometry			Total activity measurement		
	Multi-channel 3″ × 3″ crystal	Single-channel 3″ × 3″ crystal	Multi-channel well-crystal 1¾″ to 3″ diam.	3″ × 3″ crystal	well-crystal 1¾″ to 3″ diam.	G—M tube
Sensitivity limit in grams	10^{-8}	$\sim 10^{-7}$	$1-2 \times 10^{-9}$	5×10^{-9}	$5-10 \times 10^{-10}$	$5-10 \times 10^{-10}$

ARSENIC DETERMINATIONS

The method described has been applied to the determination of the arsenic content of the remains of the late King Erik XIV of Sweden. It has for a long time been a controversial problem in Swedish history whether King Erik was poisoned to death in 1577 by his brother King Johan III or not. Determination of the arsenic in King Erik's remains was of interest for this reason.

The samples were provided by Professor C. H. Hjortsjö at the University of Lund. Table 3 shows the arsenic concentrations in parts per million found in samples from the remains of King Erik and some samples used for comparison. The samples were taken for analysis as they were without previous drying or ashing.

The arsenic concentrations found in King Erik's remains are high compared with normal values for tissue given by Monier-Williams [19], Underwood [20] and Lenihan [12,13]. However, the high arsenic concentration in the remains of King Erik does not alone answer the poisoning question. A book with an account of all investigations, including activation analysis for arsenic and mercury and a discussion on the poisoning evidence (carried out after the opening of King Erik's tomb in 1958) is to be published in the autumn 1962 [21].

Table 3. Arsenic content of analyzed samples, in parts per million of As.

Sample	Character of sample	Arsenic content, ppm. (Standard deviation $\leq \pm$ 6 %)
1	Soft part from loin region	21.9
11	"Embalming paste"	1.1
12	Probably left lung	93.4
18 A	Scalp and hair	9.0
19	Possibly dry organ parts from the coffin-bottom under the stomach	8.4
22	Throat muscles	6.1
24	Possibly dry organ part from the coffin-bottom	4.2
32	Wood from the old coffin (two different samples)	9.7 12.9
Not numbered	Wood from the bottom of the new coffin	0.15
33	Part of rib-cartilage	7.3
38	Part of the seventh rib	2.6
Nail I	Root of nail	~18
Nail II	Center of nail	17.2
Nail III	Top of nail	18.8
Not numbered	Root of head hair	8.3
»	Top of head hair	2.6
»	Velvet from King Erik XIV's dress	33.8
»	Velvet from King Gustav Vasa's grave-clothes	2.8
»	Velvet lining from King Johan III's cap	5.7
»	Black smooth velvet from the 16th to the 17th century	8.2

In addition a work by Forshufvud *et al.*[22] might be mentioned here, namely determination of the arsenic content of a sample of hair stated to be probably taken from Napoleon I.

EVALUATION OF THE METHOD

A weak point in the analytical procedure is the decomposition and dissolution of the sample, a weakness which is shared with all other analytical procedures involving chemical operations. Investigations of trace element losses have been reported by Pijck *et al.*[23] and Gorsuch [24,25] and according to them losses of arsenic during wet oxidation is not serious. Wet oxidation has been found sufficient unless arsenic is present in samples with heterocyclic compounds as in, *e.g.*, tobacco leaves.

The chemical yield of the procedure is as high as 80 to 100 %, provided that the distillation is not stopped too early.

The sensitivity of the method is in the order of 10^{-8} g when irradiation in a flux of $\sim 10^{12}$ neutrons cm^{-2} sec^{-1} and γ-spectrometry with a solid 3″ × 3″ NaI(Tl)-crystal, 5 cm from the sample is performed. With β^--counting, using, *e.g.*, a G—M tube, the sensitivity can be increased by a factor of 10—15, depending on the counting geometry. When using β^--counting, accurate decay measurements are, of course, important.

Total γ-activity measurements with a $3'' \times 3''$ solid NaI(Tl)-crystal should increase the sensitivity by a factor of ~ 2 compared to spectrometric measurements.

By the use of a well-type NaI(Tl)-scintillation crystal and γ-spectrometry the sensitivity will be increased by a factor of 5—10, depending on the size of the crystal, compared to the sensitivity obtained with the use of a solid $3'' \times 3''$-crystal at a distance of 5 cm from the sample. The estimated sensitivities for the different methods of measurement are summarized in Table 2.

The sensitivity limit for the determination of arsenic by activation analysis is, according to Leddicotte *et al.*[26], 10^{-3} ppm at a flux of 5×10^{11} neutrons cm^{-2} sec^{-1}.

Production of ^{76}As from the reactions $^{76}Se(n, p)^{76}As$ and $^{79}Br(n,\alpha)^{76}As$ has been considered. For activation in the Swedish reactor R 1, which has a flux of fast neutrons approximately one tenth of the thermal flux, the ratio of ^{76}As formed from selenium, to ^{76}As formed from arsenic has been calculated to be about 2×10^{-6}. The analogous ratio for the ^{79}Br-reaction is about 10^{-4}.

The bromine concentration of biological matter, including human and animal tissue, is according to Underwood[20], of the order of 1—10 ppm. Thus the ^{79}Br-reaction must be considered when determining arsenic concentrations of 10^{-2}—10^{-3} ppm or lower.

Errors due to neutron flux depression in samples and standards are small when no material with exceedingly high neutron cross section is present in quantity. In our case all these errors were negligible. The standard deviation of the determined arsenic concentrations did not exceed $\pm$ 6 %. Some similar samples have been analysed for arsenic by both chemical and activation analysis[21] and the agreement was fairly good.

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