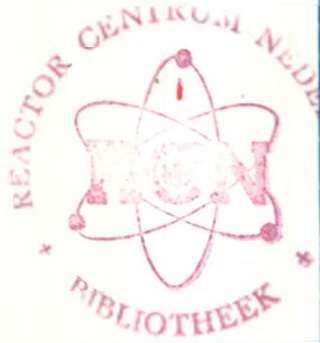

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REACTOR CENTRUM NEDERLAND

RCN REPORT

The Determination of Selenium in Blood.

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Summary:

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A procedure for the determination of selenium by neutron activation analysis in blood is given. The radionuclide used is ^{75}Se ($T_{\frac{1}{2}}$ = 120 d). Chemical separation is performed by precipitation of the element.

Keywords:

Activation analysis
Thermal neutrons
Carriers
Quantitative chemical analysis
Trace amounts
Precipitation
Biological Materials
Blood
Selenium
Selenium 75

CONTENTS

1. INTRODUCTION

The selenium-content of biological material seldomly exceeds 1 - 2 µg/g. If these concentrations have to be determined accurately in small biological samples, neutron activation analysis offers a reliable method. If naturally occurring selenium is irradiated with thermal neutrons, three radionuclides are formed with sufficiently high specific activities for analytical purposes.

	$T_{\frac{1}{2}}$	Main γ-ray energies
^{75}Se	120 days	136 keV, 265 keV, 280 keV, 401 keV, sum at 401
^{77m}Se	17.5 sec.	161 keV
^{81}Se	18.5 min.	280 keV ; 830 keV

The short-lived ^{77m}Se has been used for determining selenium in cases, where no chemical separation was required (1 - 4).
If the selenium has to be isolated from the matrix, ^{81}Se can be used (5). However, the abundances of the γ-rays of this radioisotope are very poor. Moreover, the simultaneous irradiation of large series is impossible.
The use of ^{75}Se offers the best possibilities from the radiochemical point of view (6 - 21). The main disadvantages are the long irradiation- and cooling-time. The resulting activity after irradiation for 24 hours in a thermal neutron flux of 5.10^{13} $cm^{-2}sec^{-1}$ is ≈ 600 d. sec^{-1} μg^{-1}.

Various chemical separation procedures are presented in literature:

a) Distillation as selenium tetrabromide (5, 22). Arsenic, mercury, bromine and iodine are distilled too. The selenium is precipitated as the element and the chemical yield is determined gravimetrically. The chemical purity can be improved by dissolving and repeating the precipitation.
b) Ion-exchange, followed by complexation and liquid-liquid-extraction (15, 23). Anion-exchange on Dowex-2 is used to eliminate zinc, mercury, and gold. Selenium is eluted with 6 N hydrochloric acid. It is then extracted in tetra as the DDC-complex at a pH of 4.2.

The selenium-content of blood is too low to permit a purely instrumental method of determination. The separation techniques mentioned above are too laborious for routine-purposes. Consequently, a rapid separation method was looked for, which should result in a high degree of radiochemical purity. The method presented here consists of precipitation as the element without any previous distillation.
The radionuclide used is ^{75}Se.

2. PRINCIPLE

After some initial experiments, the following procedure was adopted:
Samples are packed in quartz vials and irradiated for 24 hours in a thermal neutron flux of $\simeq 5.10^{13} - 10^{14}$ $cm^{-2}sec^{-1}$.
Standards are made by drying a standard solution (containing $\simeq$ 0.3 mg/ml) on "kale" powder.
After a cooling period of 6 - 8 days, the vials are opened. Each sample is transferred into a beaker and destructed with a mixture of conc. perchloric acid and nitric acid in the presence of selenium-carrier. Phosphate is added as a "hold-back" carrier. The resulting solution is slowly evaporated to a small volume. Evaporation to dryness should be avoided: This can result in explosions or losses of selenium. After the destruction has been completed, the mixture is diluted and heated. An excess amount of anhydrous sodium sulphite is added. Selenium is precipitated as the element, but dissolves in the sulphite solution. Elementary selenium is precipitated again by adding hydrochloric acid. The supernatant is decantated and the precipitate is washed with water and dried by heating. It is weighed out into polythene capsules, dissolved in HNO_3 and counted with a 3" x 3" NaI well-type crystal connected to a 400 channel analyzer. The observed specific count-rate in the 401 keV photopeak (combined with the sumpeak of 136 and 265 keV) is $\simeq 35.000$ c $m^{-1}(\mu g\ Se)^{-1}$. Gamma-ray spectrometry is necessary to detect possible residual impurities.
A coaxial Ge(Li) crystal is used to control the photopeak for interference by the 412 keV γ-ray of ^{198}Au ($T_{\frac{1}{2}}$ = 2.7 days).
Data obtained by measurements with a one-channel analyzer agree within 2% with the values found by γ-ray spectrometry.

3. EXPERIMENTAL

3.1. Chemicals and equipment (all chemicals used are of "pro analisi" quality).

SeO_2
Na_3PO_4
Na_2SO_3 . 0 aq.
Conc.HCl
Conc.$HClO_4$
Conc.HNO_3
Distilled water
Liquid nitrogen
Quartz vials ; outer diameter 8 mm
100 ml beakers
1000 ml volumetric flask
filter paper
quartz paper filter
20 λ Eppendorf pipettes
A Dewar vessel
15 ml test tubes
A radiochemical chimney and a suction flask
A 3000 rpm centrifuge with 100 ml tubes
A stove
A 3" x 3" NaI well-type crystal, mounted in a "Philips" liquid sample changer and connected to an "Intertechnique" 400 channel analyzer.
An "IBM" electric type-writer is used for the recording of the spectra.

3.2. Procedure

1) 150-200 milligrams are packed in quartz vials, which are sealed off and irradiated for 24 hours in the poolside facility of the HFR at a thermal neutron flux of $\simeq 5.10^{13}$ $cm^{-2}sec^{-1}$ during one day. The samples are allowed to cool for 6 to 8 days.

2) The quartz vials are cooled by immersion in liquid nitrogen, put into plastic pockets and broken. Sample and quartz are transferred into a 100 ml beaker.

3) About 250 mg SeO_2 (weighed on a semi-microbalance) is added as a carrier.

Twenty mg Na_3PO_4 is added as a hold-back carrier.

4) About 5 - 8 ml. of a 1:1 mixture of conc. HNO_3 and $HClO_4$ is added and the resulting solution is heated on an open flame until the first white fumes of perchloric acid are formed. If the solution still displays a dark colour, 2 ml of conc. HNO_3 are added and the solution is evaporated to ≈ 2 ml.
The solution is now diluted with water to 15 ml.

5) The quartz is removed by filtering through a paper filter.
About 2 grams $Na_2SO_3 \cdot 0$ aq. is added to the solution. More Na_2SO_3 is slowly added until the effervescence subsides. This takes approximately 2 grams. Another 0.5 gram of Na_2SO_3 is added to ensure the complete dissolution of the fine fraction of the selenium.
A white precipitate of sodium-salts is present now, mixed with black granules of selenium. The supernatant is clear.

6) Now 5 ml conc. HCl is carefully added. A red-black precipitate of selenium is formed. The solution is heated and more HCl is added until the effervescence subsides. An excess of 2 ml HCl is added. The solution is diluted with water to 50 ml and heated. This causes the element to coagulate. The clear solution is decantated. The precipitate is washed with 25 ml water, crushed and washed twice. If the precipitate remains finely dispersed it is necessary to centrifugate.

7) The precipitate in the beaker or in the centrifuge tube is dried carefully in an open flame at moderate temperatures during a few minutes. Care should be taken that the element does not melt. It is then crushed again and dried in a stove at 150° C for one hour.

8) The precipitate is put into a polythene capsule and weighed. The material is transferred into a 15 ml test tube and the polythene capsule is weighed again to obtain the chemical yield.
This is usually 70 - 80 %.

9) The selenium is dissolved in 1 ml conc. HNO_3 and the solution is diluted to 3 ml.

10) The solution is counted with a 3" x 3" well-type NaI-crystal, connected to a 400 channel-analyzer. The counting-time is usually 30 - 60 minutes. The γ-ray spectrum is read out by an electric typewriter. The area of the 401 keV photopeak of ^{75}Se is determined.

In most cases integral counting with a one-channel spectrometer is possible.

3.3. Carrier and standards

Pure solid SeO_2 is used as a carrier. The gravimetric separation procedure requires at least 100 mg Se. As the average chemical yield is 70 - 80 %, addition of at least 200 mg SeO_2 is necessary.

Three types of standards can be used:

a) Kale powder. According to Bowen the most probable Se-content is 145 ± 15 ng/g [26]. Aliquots of about 100 mg of kale powder are weighed out in quartz vials. These are closed and irradiated together with the samples. The kale powder is processed in the same way as the samples.

b) Small amounts of SeO_2 on aluminium foils. These standards are made from a solution containing ≈ 420 mg SeO_2 (≈ 300 mg Se) per liter. Twenty λ amounts of this solution are pipetted on aluminium-foils of 20 x 40 mm^2. After drying at room temperature, the foils are carefully wrapped and rolled to thin cylinders. These are packed in quartz vials and irradiated together with the samples. The standards are dissolved in conc. HNO_3 and counted in the same geometry as the samples.

c) Twenty λ amounts of the SeO_2-solution are pipetted on 100 mg aliquots of the kale powder in quartz vials. The liquid is evaporated at 60° C in a stove. The quartz vials are closed and irradiated together with the samples.

The influence of flux variations is corrected for by the following procedure:

1) Small iron rings (≈ 45 mg) are clamped around the quartz vials at the centre of the sample.

2) The monitors are counted integrally with a 1½" x 2" NaI detector and a single channel spectrometer. This yields the relative integrated fluxes.

3) The observed count-rates in the 401 keV photopeak of ^{75}Se are corrected for the differences in the integrated flux.

4. RESULTS

4.1. Standards

The γ-ray spectrum of a standard, measured with a 3" x 3" well-type NaI crystal is given in figure 1. The ratio of the peak-areas is as 136 keV : 265 keV : 401 keV = (0.14 ± 0.01) : (0.16 ± 0.01) : 1. The average specific count-rate in the 401 keV photopeak is $\simeq 3.10^4$ $c.m^{-1}$ $(\mu g\ Se)^{-1}$ after a cooling-period of one month. If the sample is placed on top of the detector, the area of the 401 keV photopeak decreases to 4.7 % of its original value. This decrease of the 401 keV photopeak-area is a useful control on interferences by other radionuclides.

4.2. Blood samples

The average data obtained in three runs for the blood samples from uraenic patients before and after dialysis, and for a group of normal persons are given in table I. The average uncertainty in the concentrations is 5 ng/g. Kale powder served as the standard; its Se-content was put at 150 ng/g. The data available in literature on the Se-content of human blood are scanty and inconsistent. Bowen finds 0.26 - 0.37 μg/ml |5| but Morrison reports 0.02 μg/ml |25|. Mazière quotes ≃ 85 ng/ml for one sample and ≃ 200 ng/ml for another |15|.

4.3. Kale powder *

Results for kale powder obtained in five runs point to an average value of (137 ± 6) ng/g. The Se-precipitate was occasionally contaminated with ^{181}Hf ($T_{\frac{1}{2}}$ = 45 d). The 401 KeV photopeak is not interfered by this radionuclide. By putting the sample on top of the detector, the area of the peak decreases to (4.7 ± 0.1)% of the original value. The observed concentrati agrees reasonably with the average quoted in literature |22,24,26|.

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* This material was prepared and issued by Dr. H.J.M. Bowen in 1966, as a standard for intercomparison purposes.

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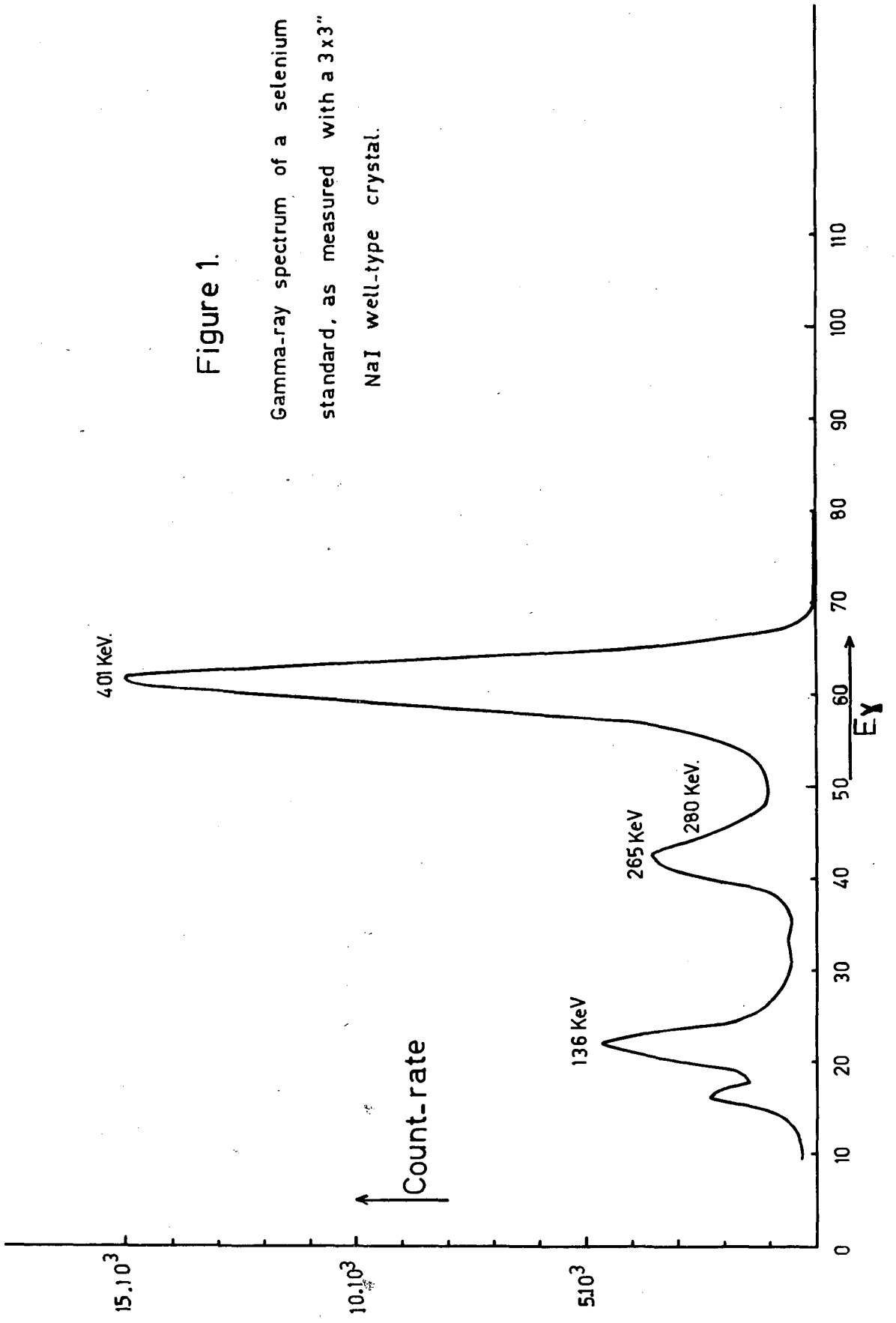
TABLE I

Sample Number	Weight of blood	Weight of freeze-dried sample	Ratio dry/wet	Average Se content in ng/g	
Control group					
1	10.258 g	1.600 g	0.219	103	
2	9.972	1.290	0.215	83	
3	10.004	1.326	0.217	125	91 ± 10
4	9.881	1.201	0.200	93	
5	9.758	1.186	0.206	70	
Uraemic patients before dialysis					
6	9.416	0.760	0.143	87	
7	9.695	1.038	0.193	93	
8	9.423	0.740	0.140	78	81 ± 4
9	9.378	0.700	0.145	73	
10	9.400	0.748	0.139	85	
11	9.485	0.825	0.155	70	
Uraemic patients after dialysis					
12	9.493	0.840	0.153	83	
13	9.242	0.666	0.121	58	
14	9.847	1.169	0.206	75	70 ± 6
15	9.525	0.947	0.182	63	

The average specific gravity of wet blood is 1.06 ± 0.01 g ml^{-1}.

Figure 1.

Gamma-ray spectrum of a selenium standard, as measured with a 3x3" NaI well-type crystal.



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