DETERMINATION OF MERCURY AND SELENIUM IN BIOLOGICAL SAMPLES BY NEUTRON ACTIVATION ANALYSIS

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SUMMARY

Nuclear analytical techniques, specially neutron activation analysis (NAA) have been for a long time giving important contribution to trace element studies in the life sciences, due to their accuracy, precision, non-destructive and multielemental capabilities. In the present paper, NAA was applied to the determination of Hg and/or Se in several types of biological samples such as hair, nails, fish reference materials and also in selenium supplements. The hair samples analyzed were collected from a control group and mainly from Indian populational groups living in the Amazonic region, where very high Hg contents are being found, probably due to gold exploration activities and biomass burning. Hair of children submitted to odontological treatment with Hg amalgam were also analyzed. A comparison was made of NAA for Se using two different radioisotopes: the short-lived $^{77m}Se(t_{1/2} = 17.45s)$ and the long lived one $^{75}Se(t_{1/2} = 119.8d)$. Both methods were applied to determination of Se in samples of hair, nails and vitamin supplements.

Key words: neutron activation analysis; mercury; selenium; biological samples

INTRODUCTION

Studies on the role of trace elements in biological systems have grown considerably in the latest years, accompanying the development in analytical methods and increasing knowledge about the toxic effects or nutritional and metabolic roles of several elements.

The toxic effects of mercury have been known for a long time, initially related to occupational exposure in different kinds of activities, such as dentistry, mining, hat production, lamp and chloralkali industries and many others. Some consequences of mercury intoxication can be: damage to the liver, kidneys, brain and central nervous system.

In the 1950'S, the world's attention was drawn to the environmental tragedy of Minamata Bay in Japan, where methylmercury discharges by an acetaldehyde industry cost hundreds of lives and caused a severe illness in thousands of people, in what became lately known as the Minamata disease.[1] Although the signs and symptoms of Minamata disease can vary considerably, according to Takeuchi and Eto[1], the syndrome consists of: ataxia, dysarthria, constriction of the visual field, impaired hearing and sensory disturbance. Other neurological symptoms may also occur, like: tremor, muscle weakness, abnormal eye movement, disequilibrium, mental disorder and others.

In the 1970's, the Iraq tragedy, where bread prepared with mercury-treated seeds was consumed by the population caused again many deaths and illness.[2] Over 400 deaths and 6000 hospital admissions were attributed to mercury intoxication in this event.

In both cases hair, among other biological monitors like blood and urine, has shown to be very useful for monitoring human mercury exposure. Today, the WHO considers hair as a reliable monitor, specially for methylmercury.[2]

Gold mining activities have also for many years been responsible for environmental contamination with mercury, due to its use for gold extraction by amalgamation and its evaporation into open air by heating, after the extraction. In Brazil, several studies have revealed contamination by mercury in the Amazonic region, due to the gold rush that started in the 1980's.[3-6]

Another element that has been drawing much attention in trace element studies in biological systems is selenium, due to its essentiality to animals. Although the metabolic role of selenium is not totally understood, it is known as a component of many enzimes, like glutathione peroxidase, which is able to neutralize oxygen reactive species like H_2O_2 and O_2^- .

Some studies have suggested also that selenium can have a protective effect against the toxic action of mercury in biological systems. Drasch et al[7] have pointed out, after examining mercury and selenium concentrations in kidney cortex of 195

autopsies, and finding an 1:1 molar ratio of Hg and Se that, since in vitro mercury and selenium form relatively stable adducts, these results, suggest the formation of an 1:1 Hg-Se compound that may explain the mercury detoxification by selenium.

In the present paper, a nuclear analytical technique, instrumental neutron activation analysis (INAA), has been applied to the determination of mercury and/or selenium in biological samples, such as hair, nails, fish and also in selenium supplements. Special attention has been given to analysis of hair of Brazilian Indian populations living in the Amazonic region where, as already mentioned, gold exploration activities have been introducing mercury in the environment since the 1980's .

A comparison was made also of INAA using two different radioisotopes: the short-lived ^{77m}Se ($t_{1/2}$ = 17.45s) and the long-lived one ^{75}Se ($t_{1/2}$ = 119.8d). Both methods were applied to the determination of selenium in samples of hair, nails and vitamin supplements.

The reference materials "Spiked Human Hair" IAEA-085, "Unspiked Human Hair" IAEA-086, "Dogfish Liver" DOLT-1, "Dogfish Muscle" DORM-1, BCR-CRM397, "Human Hair", GBW9101 "Chinese Human Hair" were also analyzed for Hg and or/Se for quality control of the analytical procedures.

EXPERIMENTAL

1. Sample Collection and Preparation

1.1. Hair Samples

The hair samples of the control group and of Indian tribes living in the Xingu Park, an Indian reservation located in the Amazonic region, were collected always from the occipital part of the head, with clean stainless steel scissors, according to the Protocol of the IAEA[8].

The hair was then cut in segments of about 0.5 mm and washed in a sequence of acetone-water-acetone, according to the same Protocol.

1.2. Nail Samples

The nail samples were collected from healthy individuals, most of them students, from all toes of both feet, also with clean stainless steel scissors.

They were cut in segments as small as possible and washed also according to the Protocol of the IAEA[8].

1.3. Selenium Vitamin Supplements

Firstly were randomly selected 10 pills and 10 capsules from flasks containing 100 units of vitamin supplements each, from two different brands.

After this selection, the pills and capsules were weighed one by one.

The pills were grinded and homogeneized in an agate mortar, previously cleaned with 10% nitric acid and distilled water.

For the capsules, this procedure was not necessary, since they were already in powder form.

Finally, each obtained powder was divided in two aliquots of about 200 mg, which were weighed in plastic envelopes, previously cleaned with dilute HNO_3.

2. Standard Preparation

2.1. Mercury standard

The mercury standard was prepared by dissolution of HgO (Aldrich Gold Label, 99.999%) in nitric acid and dilution with water, in order to obtain a stock solution with about 2mg/mL of Hg.

From this stock solution, a working standard was prepared, containing about 0.02 mg/mL of Hg, in hydrochloric acid. From this solution, 50 µL were pipetted on analytical filter paper, impregnated with thyoacetamide solution, to avoid losses of mercury during the irradiation.

2.2. Selenium standard

The selenium standard was prepared by dissolution of selenium metal powder (May & Baker, 99%) in HNO_3 concentrated. After appropriate dilution with water, a stock solution with about 2mg/mL of Se was obtained.

For the short irradiations of selenium, 100 µL of this solution were directly pipetted on analytical filter paper, corresponding to a mass of 200µg of Se.

For the long irradiations a working standard of selenium was prepared with a concentration of about 0.04 mg/mL of Se. From this solution, 50µL were pipetted on filter paper, corresponding to a mass of 2.0 µg of Se.

3. Irradiations and Radioactivity Measurements

3.1. Mercury analysis

About 200 mg of the prepared hair samples were irradiated in the IEA-R1 nuclear research reactor, for a period of 60 min, under a thermal neutron flux of about 10^{12} n.cm^{-2} s^{-1} .

The samples were irradiated simultaneously with the pipetted mercury standards, as already mentioned and with the reference materials, for quality control.

After a decay of about 48 hours, samples, standards and reference materials were measured in a gamma-ray spectrometer, constituted of a hyperpure Germanium detector, EURYSIS Model EGNC 25-190-R and associated electronics.

For mercury analysis, the gamma-radioactivity of ^{197}Hg, with a half-life of 64.1 hours and gamma-ray energy at 77keV, was measured.

3.2. Selenium Analysis

3.2.1. Short Irradiation

About 200 mg of the samples (hair, nails or selenium supplements) were irradiated for a period of 20s, under a thermal neutron flux of 0.5 x 10^{12} n.cm^{-2}s^{-1} in the IEA-R1 nuclear research reactor. The pipetted selenium standard was irradiated simultaneously with the sample.

Immediately following irradiation, sample and standard were measured for 90s in a gamma-ray spectrometer, comprising a CANBERRA Model GX2020 hyperpure Germanium detector and associated electronics. For analysis, the Se 77m radioisotope ($t_{1/2}$ = 17.45s, γ = 161.9 keV was used).

Most of the Indian hair samples were analyzed using the fast-rabbit assembled at the interfaculty Reactor Institute (Delft, The Netherlands). Samples were irradiated in a thermal neutron flux of 1.5 x 10^{13} n.cm^{-2} s^{-1} for 17s and measured during 30s, after a waiting time of about 20s. Measurements were carried out using a coaxial 20% Ge detector (FWHM of 1.63 keV at 1332 keV of ^{60}Co) equipped with a loss free counting module.

3.2.2. Long Irradiation

For the long irradiation, about 200 mg of the samples, standards and reference materials were irradiated for a period of 8 hours, under a thermal neutron flux of 10^{12} n.cm^{-2} s^{-1} .

After a decay period of 15 days, samples, standards and reference materials were measured in an EURYSIS Model EGNC25-190R hyperpure Germanium detector and associated electronics.

The radioisotope used for analysis was Se.75 ($t_{1/2}$ = 119.8d ; γ = 265 keV).

Calculations

For data reduction of the gamma-ray spectra, the VISPECT2 software, developed by D. Piccot[9], from Saclay, France, was used.

The concentration calculations were made by the comparative method, using the expression:

$$Ca = \frac{Aamp}{ApMa} \, e^{[0.693(ta-tp)\,/\,t½]}$$

where

Aa and Ap are the counting rates obtained for sample and standard.
mp = mass of the element in the standard
M_A = mass of the sample
t = decay time
$t_{1/2}$ = half-life

RESULTS AND DISCUSSION

1. Analysis of Reference Materials

Table I presents the results obtained for Hg and/or Se in the reference materials, analyzed, i.e., IAEA-085, IAEA-086, DORM-1, BCR-CRM397 and GBW9101 DOLT-1, by instrumental neutron activation analysis.

It can be observed that the relative errors (e_r) and relative standard (s_r) deviations obtained, both for Hg and Se, were amost always less than 10%, which can be considered as good in these levels of concentration.

It has to be pointed out that in the case of Se in IAEA-085 and IAEA-086, the values given in the certificate are information values, not yet certified.

There was also a good agreement between the determinations of selenium in the reference materials using the long and short irradiations.

TABLE I. Results of the analysis of Hg and/or Se in reference materials by neutron activation analysis.

Reference Material	Hg ($\mu g.g^{-1}$)	Se ($\mu g.g^{-1}$) Long Irradiation	Se ($\mu g.g^{-1}$) Short Irradiation
IAEA-085	$\bar{x}$ = 24.7 s_r = 4.7% e_r = 6.5%	$\bar{x}$ = 1.06 s_r = 4.7% e_r = 0.9%	$\bar{x}$ = 1.05 s_r = 7.6% e_r = 1.9%
IAEA-086	$\bar{x}$ = 0.59 s_r = 5.1% e_r = 3.0%	$\bar{x}$ = 1.16 s_r = 8.6% e_r = 16%	$\bar{x}$ = 1.04 s_r = 15.4% e_r = 4%
DORM-1	—	$\bar{x}$ = 1.83 s_r = 4.4% e_r = 13%	$\bar{x}$ = 1.53 s_r = 11.1% e_r = 5.6%
DOLT-1	—	$\bar{x}$ = 7.24 s_r = 2.9% e_r = 1.4%	$\bar{x}$ = 7.68 s_r = 10.8% e_r = 4.6%
BCR-CRM397 Human Hair	$\bar{x}$ = 12.0 s_r = 7.2% e_r = 2.4%	—	—
GBW91-01 Chinese Human Hair	$\bar{x}$ = 2.20 s_r = 6.9% e_r = 1.8%	—	—

2. Analysis of biological samples

2.1. Analysis of mercury in hair samples

Table II presents the results obtained for analysis of mercury in hair samples of Brazilian populational groups living in the Amazonic region (NIMD Forum), by instrumental neutron activation analysis.

It can be immediately observed that, for all the 13 Indian groups analyzed and for the populations of three localities in the State of Amapá, in the Amazonic Region, the mercury concentrations (arithmetic means $\bar{x}$, median and geometric means $\bar{x}_g$) are much higher than for the controls of a Brazilian population non exposed to mercury contamination. An application of the ANOVA test confirmed this hypothesis.

This fact can be attributed to the very frequent fish consumption of these populations, since fish is traditionally the main protein source of Brazilian Indian populations.

The World Health Organization[2] considers that the population in general is not subject to risk of contamination by mercury (mainly the more toxic form, methylmercury). On the other hand, certain populational groups with high fish consumption can attain blood mercury levels of about 200 µg/L, which corresponds to 50 mg Kg^{-1} in hair, associated with a low risk (5%) of neurological damage in adults. In the case of the populational groups studied in the present work, only one individual had mercury concentration higher than 50 mg kg^{-1}, in the Indian Group 6 .

TABLE II. Summary of the results obtained for total mercury contents in the hair of the controls, of the Xingu Indian Park and of three localities in the Amazonic region ($\mu g.g^{-1}$), by neutron activation analysis.[5]

Populational group	$\bar{x}$	S	Median	$\bar{x}_g$	Range
CONTROLS	1.1	0.6	1.0	0.9	0.3-2.9
INDIAN GROUP 1	18.5	5.9	18.0	17.1	6.9-34.3
INDIAN GROUP 2	12.0	4.0	10.7	11.4	6.5-21.6
INDIAN GROUP 3	8.7	3.0	8.2	8.2	4.5-18.5
INDIAN GROUP 4	13.2	3.8	13.0	12.7	4.8-25.3
INDIAN GROUP 5	10.6	3.9	11.5	9.4	1.7-15.1
INDIAN GROUP 6	20.6	10.0	18.8	19.0	8.1-57.3
INDIAN GROUP 7	16.5	5.5	15.8	15.5	2.5-30.2
INDIAN GROUP 8	17.2	6.0	16.2	16.3	2.1-31.7
INDIAN GROUP 9	21.8	6.1	20.8	21.0	12.4-34.2
INDIAN GROUP 10	8.1	9.0	2.8	4.7	1.5-33.1
INDIAN GROUP 11	18.2	7.8	16.2	16.7	5.5-41.8
INDIAN GROUP 12	12.2	3.1	12.5	11.8	6.6-18.8
INDIAN GROUP 13	3.6	2.4	2.6	3.1	1.2-11.1
SERRA DO NAVIO	3.73	3.63	2.11	2.44	0.21-20.58
VILA NOVA	5.42	2.27	5.32	5.02	2.61-8.62
TARTARUGALZINHO	11.34	9.80	6.60	7.34	1.19-28.62

2.2. Analysis of selenium in hair samples

Table III presents the results obtained for selenium contents in the hair of the Brazilian populational groups studied, by instrumental neutron activation analysis[10].

It has to be pointed out that the anomalous results obtained for selenium, in the case of the control population and of the Billings group (people who live by a Dam that could be contaminated by mercury) are probably due to the use of hair products that contain selenium, like anti-dandruff shampoo.

TABLE III. Summary of the results obtained for selenium contents in the hair of the Brazilian populational groups studied ($\mu g.g^{-1}$) [10]

Populational group	$\bar{x}$	s	Median	$\bar{x}_g$	Range
CONTROLS	0.43	0.04	0.43	0.43	0.34 - 84.3
BILLINGS	0.38	0.12	0.36	0.36	0.17 - 26.8
INDIAN GROUP 1	0.47	0.01	0.43	0.45	<0.28 - 0.86
INDIAN GROUP 2	0.352	0.033	0.352	0.351	0.328 - 0.375
INDIAN GROUP 3	0.28	0.04	0.28	0.3	0.25 - 0.32
INDIAN GROUP 4	0.563	0.401	0.429	0.498	0.402 - 1.63
INDIAN GROUP 5	0.314	-----	-----	-----	0.314
INDIAN GROUP 6	0.372	0.028	0.372	0.371	0.34 - 0.41
INDIAN GROUP 7	0.329	-----	-----	-----	0.329
INDIAN GROUP 8	0.343	0.032	0.351	0.691	0.28 - 0.39

In Table IV, the results are presented for Hg and Se in $nmolg^{-1}$, for the sake of comparison with results obtained by other authors.[10]

TABLE IV. Results of the selenium and mercury ratios found in the hair of the Brazilian populational groups studied.[10]

Populational Group	$\bar{x}$ Hg (n mol/g)	$\bar{x}$ Se (n mol/g)	Se/Hg	Hg/Se
CONTROL	5.49	5.45	0.993	1.007
BILLINGS	4.39	4.82	1.098	0.911
INDIAN GROUP 1	92.3	5.96	0.0646	15.49
INDIAN GROUP 2	59.85	4.46	0.0745	13.42
INDIAN GROUP 3	43.40	3.55	0.0818	12.23
INDIAN GROUP 4	65.84	7.14	0.108	9.221
INDIAN GROUP 5	52.87	3.98	0.0753	13.28
INDIAN GROUP 6	102.74	4.71	0.0458	21.81
INDIAN GROUP 7	82.29	4.17	0.0507	19.73
INDIAN GROUP 8	85.79	4.35	0.0507	19.72

These results agree with those of DRASCH et al[7] who determined Hg and and Se concentrations in kidney cortex samples of 195 autopsies. The authors point out that, since in vitro Hg and Se form relatively stable adducts, these results suggest the formation of an 1:1 Hg-Se compound that may explain the Hg detoxification by Se.

In an animal study, on the other hand, female monkeys were exposed to methylmercury[11] for up to 18 months and the concentrations of Hg and Se were determined in their brains. The results obtained indicated an association between concentrations of mercury in both occipital pole and thalamus in the methylmercury exposed animals and the conclusion was that an important role for selenium in the retention of mercury in brain could be indicated.

Table V presents the comparison of analysis of selenium using the radioisotopes ^{77m}Se and ^{75}Se.

TABLE V. Analysis of selenium in hair of the Indian populational groups – comparison between analysis with ^{77m}Se and ^{75}Se.

Sample Code Number	Se ($\mu g.g^{-1}$) using ^{77m}Se	Se ($\mu g.g^{-1}$) using ^{75}Se
5192	0.33 ± 0.04	0.38 ± 0.01
1926	0.32 ± 0.04	0.34 ± 0.01
1941	0.32 ± 0.04	0.30 ± 0.01
1946	--	0.31 ± 0.01
1979	--	0.34 ± 0.01
133	0.50 ± 0.04	0.47 ± 0.02
176	0.42 ± 0.03	0.39 ± 0.01
514	0.44 ± 0.04	0.39 ± 0.01
607	1.63 ± 0.07	1.44 ± 0.01
611	0.42 ± 0.03	0.43 ± 0.01

It can be observed that there was a good agreement between the averages obtained using ^{77m}Se and ^{75}Se radioisotopes.

On the other hand, the standard deviations obtained using ^{77m}Se were higher.

Table VI presents the results of analysis of selenium mineral supplement, also using the short-lived and the long-lived radioisotopes.

In this case the averages obtained using the two radioisotopes were different in about 6% and they were both lower than the nominal value of 200 µg declared by the producer. The individual standard deviations were higher when the short-lived radioisotope was used.

TABLE VI. Analysis of selenium in a mineral supplement (Vitamin World) by neutron activation analysis, using the radioisotopes ^{77m}Se and ^{75}Se.

Sample Aliquot	Mass of Se/capsule in µg Se 77m	Relative Standard Deviation (%)	Mass of Se/capsule in µg Se 75	Relative Standard Deviation (%)
C1	184.6 ± 5.2	2.8	171.1 ± 1.0	0.6
C2	198.6 ± 6.4	3.2	174.4 ± 1.0	0.6
C3	198.7 ± 5.8	2.9	175.8 ± 1.0	0.6
C4	205.1 ± 6.4	3.1	176.5 ± 1.1	0.6
C5	180.4 ± 5.4	3.0	179.0 ± 1.1	0.6
C6	197.6 ± 6.5	3.3	180.4 ± 1.3	0.7
C7	187.1 ± 6.4	3.4	172.3 ± 1.2	0.7
C8	182.2 ± 5.5	3.0	170.6 ± 1.2	0.7
C9	193.6 ± 5.8	3.0	198.8 ± 1.4	0.7
C10	176.5 ± 5.5	3.1	188.9 ± 1.3	0.7
	n = 10		n = 10	
	$\bar{x}$ = 190.4		$\bar{x}$ = 178.8	
	s = 9.5		s = 8.7	
	S_{rel} = 5.0%		S_{rel} = 4.9%	
	µg Se/capsule = 200 µg (nominal)			

In table VII are presented the results obtained for selenium in nail samples of Brazilian subjects.

TABLE VII. Analysis of selenium in nail samples by neutron activation analysis, using the radioisotopes ^{77m}Se and ^{75}Se.

Sample Code Number	Se (µg.g^{-1}) using ^{77m}Se	Relative Standard Deviation (%)	Se (µg.g^{-1}) using ^{75}Se	Relative Standard Deviation (%)
U1	0.59 ± 0.05	8.5	0.50 ± 0.04	8.0
U2	0.48 ± 0.01	2.1	0.51 ± 0.04	7.8
U3	0.403 ± 0.001	0.2	0.45 ± 0.01	2.2
U4	0.42 ± 0.05	11.9	0.44 ± 0.01	2.3
U5	0.45 ± 0.01	2.2	0.44 ± 0.01	2.3
U6	0.66 ± 0.01	1.5	0.61 ± 0.03	4.9

In this case there was a good agreement between the averages obtained using ^{77m}Se and ^{75}Se (0.66 and 0.61 $\mu g.g^{-1}$, respectively). Similar results for selenium concentrations in toenails of Brazilian subjects were obtained by Aguiar[12], also using the radioisotope ^{75}Se for the analysis by neutron activation.

CONCLUSIONS

Neutron activation analysis proved to be a reliable method for analysis of mercury and selenium in different kinds of biological samples, using both the short-lived isotope, ^{77m}Se and the long-lived one, ^{75}Se for selenium and the radioisotope ^{197}Hg for mercury analysis.

Very high mercury concentrations were found in hair of Brazilian Indian populational groups living in the Amazonic region, probably due to their very frequent fish consumption.

The Hg/Se ratios found for the hair samples of control population and for the Indians showed similar trends as those found by other authors for kidney cortex of human autopsies and for brain tissue of female monkeys exposed to mercury.

The results obtained for selenium in human nails of Brazilian subjects using both radioisotopes of selenium were similar to the ones found by another Brazilian author.

It is import also to point out that these are contributions to the knowledge of selenium and mercury status of Brazilian populational groups, for whom these data are scarce.

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