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TRACE ELEMENTS IN HUMANS

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

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ABSTRACT

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, instrumental neutron activation analysis (INAA) 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 were found (means from 3.6 to 21.8 $\mu g\, g^{-1}$), probably due to gold exploration activities and biomass burning. A comparison was made of INAA for Se using two different radioisotopes: the short-lived ^{77m}Se ($t_{1/2} = 17.45$ s) and the long-lived one ^{75}Se ($t_{1/2} = 119.8$ d). Both methods were applied to determination of Se in samples of hair, nails and vitamin supplements.

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Key Words: Instrumental neutron activation analysis; Mercury; Selenium; Biological samples

Abbreviations: IAEA, International Atomic Energy Agency; INAA, instrumental neutron activation analysis; NAA, neutron activation analysis; WHO, World Health Organization

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 chlor-alkali industries and many others. Some consequences of mercury intoxication can be: damage to the liver, kidneys, brain and central nervous system.

In the 1950s, 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 1970s, 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 1980s.[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 enzymes, 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 1980s.

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

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," GBW09101 "Chinese Human Hair" were also analyzed for Hg and/or Se for quality control of the analytical procedures.

EXPERIMENTAL

Sample Collection and Preparation

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.

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]

Selenium Vitamin Supplement

Firstly were randomly selected 10 pills from one flask containing 100 units of vitamin supplements. After this selection, the pills were weighed one by one. The pills were grinded and homogeneized in an agate mortar, previously cleaned with 10% nitric acid and distilled water. 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.

Standard Preparation

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 $2\,mg\,mL^{-1}$ of Hg. From this stock solution, a working standard was prepared, containing about $0.02\,mg\,mL^{-1}$ 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.

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 $2\,mg\,mL^{-1}$ 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 200 µg of Se.

For the long irradiations a working standard of selenium was prepared with a concentration of about $0.04\,mg\,mL^{-1}$ of Se. From this solution, 50 µL were pipetted on filter paper, corresponding to a mass of 2.0 µg of Se.

Irradiations and Radioactivity Measurements

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 h, samples, standards and reference materials were measured in a γ-ray spectrometer, constituted of a hyperpure Germanium detector, EURYSIS Model EGNC 25-190-R and associated electronics.

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

Selenium Analysis

Short Irradiation

About 200 mg of the samples (hair, nails or selenium supplement) were irradiated for a period of 20 s, under a thermal neutron flux of 0.5×10^{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 90 s in a γ-ray spectrometer, comprising a CANBERRA Model GX2020 hyperpure Germanium detector and associated electronics. For analysis, the ^{77m}Se radioisotope ($t_{1/2} = 17.45$ s, $\gamma = 161.9$ 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 1.5×10^{13} n cm^{-2} s^{-1} for 17 s and measured during 30 s, after a waiting time of about 20 s. 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.

Long Irradiation

For the long irradiation, about 200 mg of the samples, standards and reference materials were irradiated for a period of 8 h, 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 counting times for samples and standards were of 50.000 s.

The radioisotope used for analysis was ^{75}Se ($t_{1/2} = 119.8$ d; $\gamma =$ 264.7 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:

$$C_a = \frac{A_a m_p}{A_p M_a} e\,[0.693(t_a - t_p)/t_{1/2}]$$

where:

A_a and A_p are the counting rates obtained for sample and standard.

C_a = concentration, in $\mu g\,g^{-1}$
m_p = mass of the element in the standard, in μg
M_a = mass of the sample, in g
t_a = decay time for the sample, in s
t_p = decay time for the standard, in s
$t_{1/2}$ = half-life of the radioisotope measured, in s

The formula supposes the same counting times for sample and standard.

RESULTS AND DISCUSSION

Analysis of Reference Materials

Table 1 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 GBW09101 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 almost 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.

Analysis of Biological Samples

Analysis of Mercury in Hair Samples

Table 2 presents the results obtained for analysis of mercury in hair samples of Brazilian populational groups living in the Amazonic region (NIMD Forum '99), 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

Table 1. Results of the Analysis of Hg and/or Se in Reference Materials by Neutron Activation Analysis

Reference material	Concentration Hg ($\mu g\,g^{-1}$)	Concentration Hg ($\mu g\,g^{-1}$)	Concentration Se ($\mu g\,g^{-1}$)	Se ($\mu g\,g^{-1}$) Long Irradiation	Se ($\mu g\,g^{-1}$) Short Irradiation
IAEA-085 "Human Hair"	23.2[a] (22.4–24.0)	$\bar{x} = 24.7$ $s_r = 4.7\%$ $e_r = 6.5\%$	1.07 (0.96–1.17)[b]	$\bar{x} = 1.06$ $s_r = 4.7\%$ $e_r = 0.9\%$	$\bar{x} = 1.05$ $s_r = 7.6\%$ $e_r = 1.9\%$
IAEA-086 "Human Hair"	0.573[a] (0.534–0.612)	$\bar{x} = 0.59$ $s_r = 5.1\%$ $e_r = 3.0\%$	1.0 (0.80–1.2)[b]	$\bar{x} = 1.16$ $s_r = 8.6\%$ $e_r = 16\%$	$\bar{x} = 1.04$ $s_r = 15.4\%$ $e_r = 4\%$
DORM-1 "Dogfish Muscle"	—	—	1.62 ± 0.12[c]	$\bar{x} = 1.66$ $s_r = 3.0\%$ $e_r = 2.5\%$	$\bar{x} = 1.58$ $s_r = 6.3\%$ $e_r = 2.5\%$
DOLT-1 "Dogfish Liver"	—	—	7.34 ± 0.42[c]	$\bar{x} = 7.24$ $s_r = 2.9\%$ $e_r = 1.4\%$	$\bar{x} = 7.68$ $s_r = 10.8\%$ $e_r = 4.6\%$
BCR-CRM397 "Humam Hair"	12.3 ± 0.5[c]	$\bar{x} = 12.0$ $s_r = 7.2\%$ $e_r = 2.4\%$	—	—	—
GBW09101 "Human Hair"	2.16 ± 0.21[c]	$\bar{x} = 2.20$ $s_r = 6.9\%$ $e_r = 1.8\%$	—	—	—

e_r = relative standard deviation (% deviation from the certified value).
The number of determinations was 6 for Hg and 6 for Se.
[a]Recommended values.
[b]Information values.
[c]Certified values.

Amazonic region, the mercury concentrations (arithmetic means $\bar{x}$, 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. Fish, specially predatory species, are known to concentrate mercury present in water.[3]

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 $200\,\mu g\,L^{-1}$, 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 2. 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 by Neutron Activation Analysis[5]

Populational Group	$\bar{x}$	s	N	$\bar{x}_g$	Range
Controls	1.1	0.6	38	0.9	0.3–2.9
Indian Group 1	18.5	5.9	27	17.1	6.9–34.3
Indian Group 2	12.0	4.0	18	11.4	6.5–21.6
Indian Group 3	8.7	3.0	35	8.2	4.5–18.5
Indian Group 4	13.2	3.8	46	12.7	4.8–25.3
Indian Group 5	10.6	3.9	11	9.4	1.7–15.1
Indian Group 6	20.6	10.0	20	19.0	8.1–57.3
Indian Group 7	16.5	5.5	39	15.5	2.5–30.2
Indian Group 8	17.2	6.0	41	16.3	2.1–31.7
Indian Group 9	21.8	6.1	11	21.0	12.4–34.2
Indian Group 10	8.1	9.0	27	4.7	1.5–33.1
Indian Group 11	18.2	7.8	51	16.7	5.5–41.8
Indian Group 12	12.2	3.1	18	11.8	6.6–18.8
Indian Group 13	3.6	2.4	20	3.1	1.2–11.1
Serra do Navio	3.73	3.63	51	2.44	0.21–20.58
Vila nova	5.42	2.27	5	5.02	2.61–8.62
Tartarugalzinho	11.34	9.80	10	7.34	1.19–28.62

[5]Vasconcellos, M.B.A., et al. (1999).

Analysis of Selenium in Hair Samples

Table 3 presents the results obtained for selenium contents in the hair of the Brazilian populational groups studied, by instrumental neutron activation analysis.[10]

In Table 4, the results are presented for Hg and Se in n mol g^{-1}, for the sake of comparison with results obtained by other authors.[10] These results agree with those of Drasch et al.[7] who determined Hg 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 5 presents the comparison of analysis of selenium using the radioisotopes ^{77m}Se and ^{75}Se. 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 3. 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	0.34–0.50
Billings	0.38	0.12	0.36	0.36	0.17–0.64
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

[10]Vasconcellos, M.B.A., et al. (2000).

Table 4. Results of the Selenium and Mercury Ratios Found in the Hair of the Brazilian Populational Groups Studied[10]

Populational Group	$\bar{x}$ Hg ($n\,mol\,g^{-1}$)	$\bar{x}$ Se ($n\,mol\,g^{-1}$)	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

[10]Vasconcellos, M.B.A., et al. (2000).

Table 6 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.

In Table 7 are presented the results obtained for selenium in nail samples of Brazilian subjects. 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.

Table 5. Determination 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

Table 6. Determination 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/Pills in μg (^{77m}Se)	Relative Standard Deviation (%)	Mass of Se/Pills in μg (^{75}Se)	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
	$n = 10$		$n = 10$	
	$\bar{x} = 190.4$ (μg)		$\bar{x} = 178.8$ (μg)	
	$s = 9.5$		$s = 8.7$	
	$s_{rel} = 5.0\%$		$s_{rel} = 4.9\%$	
	μg Se/pills = 200 μg nominal			

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. Statistical tests applied have shown that the results obtained using both radioisotopes do not differ significantly.

Table 7. Determination of Selenium in Nail Samples by Neutron Activation Analysis, Using the Radioisotopes ^{77m}Se and ^{75}Se

Sample Code Number	Se ($\mu g\,g^{-1}$) Using ^{77m}Se	Relative Standard Deviation (%)	Se ($\mu g\,g^{-1}$) 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

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, since fish are known to concentrate mercury from water, specially in the form of methylmercury.

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 important 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.

The analysis of selenium in vitamin supplement using the short-lived radioisotope can provide a quick and reliable method of assessing the quality of this kind of supplement.

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