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STUDY ON TRACE ELEMENT DETERMINATION IN HUMAN HEAD HAIR USING NEUTRON ACTIVATION ANALYSIS

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ABSTRACT

Trace element determination in human hair has become increasingly popular for monitoring environmental exposure, assessing nutritional status, evaluating intoxication and diagnosing diseases. However, there are controversies of this use due to the difficulty in the removal of only exogenous origin elements from the hair, the small correlation data between elements contents in the hair and other tissues and the poor quality of analytical results for certain elements. In this study, adequate experimental conditions have been established for human scalp hair analysis in order to obtain further reliable reference value ranges. Neutron activation analysis (NAA) was used for the determination of fourteen trace elements. Irradiations were performed at the IEA-R1 nuclear research reactor. Aliquots of samples from three individuals were analyzed and the results presented good reproducibility, indicating the sample homogeneity. The quality control of the results was assessed analyzing certified materials. The relative errors lower than 8% and relative standard deviations varying from 1.2 to 15% were obtained for most of elements in the reference materials analysis. Hair samples from voluntary donors from São Paulo State, aged from 15 to 60 years were studied and the results obtained indicate that As, Co, Cr, Cs, La, Sb, Sc and Se are present in the hair at a low level of μg kg^{-1} and the elements Br, Ca, Fe, K, Na and Zn, at mg kg^{-1} level. There is a necessity of obtaining reliable reference values or intervals for hair trace elements for a defined healthy population.

1. INTRODUCTION

Analyses of human hair samples have received much attention lately for studying trace elements in vivo. This biological material has been analyzed to be used for monitoring environmental exposure to pollutants as well as for evaluating poisoning by metals. Besides, the trace element composition of hair has been investigated for assessing nutritional status and human health or disease [1]. It is considered that hair of normal and healthy individuals generally contains each trace element within a well-defined concentration range. It is interesting thus to establish a trace elemental concentration range for a given population. Marked deviations from these values indicate physiological or environmental disorder [2]

Hair analysis is a non-invasive diagnosis method, with samples easily collected, transported and stored. Moreover, it provides a long-term information. The elements concentrations in hair are higher when compared with those obtained in body tissues or fluids [3]

Difficulties with the proper interpretation of hair analysis results are owing to the absence of well defined reference concentration ranges, problems associated with differentiating between endogenous and exogenous deposition and inconsistency of hair concentration anomalies with nutritional status and clinical symptoms [4]. The difficulties in establishing normal or reference ranges are also due to the natural variance or hair composition as a

possible consequence of age, sex, hair color, ethnical and geographic origin, dietary factors, and so on [5].

In the present study, adequate experimental conditions have been established for human hair analysis in order to obtain further reliable reference value ranges.

Instrumental neutron activation analysis (INAA) was used to determinate elements As, Br, Ca, Cd, Co, Cr, Cu, Fe, K, Na, Sb, Sc, Se and Zn in hair samples from donors living in São Paulo State. The quality assurance of the analytical results was evaluated by analyzing the certified Standard Reference Materials INCT-TL-1 Tea Leaves acquired from the Institute of Nuclear Chemistry and Technology (INCT) [6] and IAEA-85 Human Hair, prepared by the International Atomic Energy Agency (IAEA) [7].

2. EXPERIMENTAL

2.1. Hair Sample Collection and Preparation

Samples of head hair (approx 500 mg) were cut from the scalp of the occipital region using a pair of stainless steel scissors. The age range of donors was 15 to 65 years old. During the collection of the hair samples, each individual was asked to complete a questionnaire detailing name, sex, age, occupation, dietary habits, and so on. In laboratory, the samples were cut in lengths smaller than 2 mm using a par of stainless steel scissors and then washed to remove exogenous contaminants from the surface. The hair was washed four times in 2% Triton X100 solution, once in acetone (p.a.- Merck), three times in Milli Q water and, in the end, washed twice in acetone [8]. The washed samples were placed on a CAAL n^{o} 1541 filter paper and dried at room temperature.

Sample homogeneity tests for chemical elements were done, using a mixture of hair samples from three donors. Seven aliquots from this sample were analyzed to evaluate reproducibility of the results.

2.2. Reference Materials and Standards Preparation

The accuracy and precision of hair analysis results was verified by analyzing certified reference materials INCT-TL-1 Tea Leaves and IAEA-85 Human Hair. In the certificate, the informative and recommended values are expressed on dry material basis. Therefore, for the results comparison, it was necessary to determine the humidity of these reference materials Percentages of weight loss were determined as recommended in their respective certificates, drying the materials in an oven at 85 ^{0}C for INCT-TL-1 Tea Leaves and 80 ^{0}C for IAEA-85 Human Hair until a constant weight is obtained. The following values of the weight losses were found, in percentages, 4.2 for INCT-TL-1 Tea Leaves and 7.3 for IAEA-85 Human Hair. These reference materials were analyzed in the same experimental conditions of the hair sample analyses.

Element standards were prepared using certified stock solutions (SpexCertip). Aliquots of diluted solutions containing one or more elements with known concentrations were prepared and pipetted onto small sheets of Whatman nº 40 filter paper using an Eppendorf pipette. These sheets were dried in a desiccator at room temperature. Then they were finally placed in polyethylene bags that had been cleaned using diluted HNO_3 p.a and Milli Q water.

2.3. Irradiation and Measurement

About 180 mg of each hair sample and reference material, weighed in clean polyethylene bags, were irradiated together with elemental standards in the IEA-R1 swimming pool-type reactor of IPEN-CNEN/SP for 16 hours, with a thermal neutron flux of approximately $5x10^{12}$n $cm^{-2}s^{-1}$. After adequate decay times, the gamma ray activities were measured using an EG&G ORTEC HPGe detector, model GEM 60210. The resolution (FWHM) of the system used was of 1.2 keV for 121.9 keV gamma-ray of ^{57}Co and 2.1 keV for 1332.5 keV gamma-ray of ^{60}Co. Samples and standards were measured three times according to the conditions presented in Table 1. The element concentrations were calculated by the comparative method [9]. The gamma spectrum obtained was processed using the VISPECT2 software [10].

Table 1. Counting conditions according and elements determined

Counting	Decay times	Determined element
First	2 Days	As, Br, Cd, Cu, K, La, Na
Second	14 Days	Ca, Cr, Fe, Rb, Sb
Third	21 Days	Co, Cs, Sc, Se, Zn

3. RESULTS AND DISCUSSION

Table 2 shows the average concentrations of elements obtained in the analyses of INCT-TL-1 Tea Leaves reference material. The relative standard deviations of the results obtained varied from 5 to 13% and the relative errors were lower than 15%. The standard difference or Z-score value [11] of the results was also calculated. The |Z-score| values obtained were lower or equal to 1, indicating that the results obtained are within the range of certified value at the 99% of confidence level.

Results obtained in the analysis of IAEA-85 Human Hair reference material are presented in Table 3. A good precision expressed as relative standard deviations varying from 0.1% to 15% was obtained for most elements. The less precise results were obtained for the elements Cs, Cu and K, due to low statistical counting in the measurements. The relative errors obtained were lower than 8%, indicating that the results obtained are in good agreement with

their respective certificate values. The results of the |Z-score| (Table 3) present values lower than 2, thus the results obtained can be also considered satisfactory for the certified elements.

Table 2. Elemental concentrations obtained in INCT-TL-1-Tea Leaves certified reference material

Element mg kg^{-1}	Average ± SD (n)[a]	RSD (%)	RE (%)	Z-score	Values of Certificate [6]
Br	12.41 ± 0.01(n=5)	5.5	1.2	0.12	12.3 ± 1.0
Ca[c]	0.616 ± 0.004 (n=4)	8.4	5.8	0.46	0.582 ± 0.052
Co[b]	373 ± 2 (n=4)	7.7	3.4	-0.26	387 ± 42
Cr	1.79 ± 0.01 (n=4)	9.1	6.1	-0.43	1.91 ± 0.22
Cs	3.67 ± 0.03 (n=4	10.6	1.5	0.10	3.61 ± 0.37
Fe	495 ± 1 (n=5)	11.6	14.8	-	432[d]
K[c]	1.52 ± 0.01 (n=4)	11.8	10.5	-0.2	1.70 ± 0.12
La	0.929 ± 0.003 (n=5)	5.0	7.1	-0.85	1.00 ± 0.07
Na	21.9 ± 0.2 (n=4)	10.1	12.0	-0.76	24.7 ± 3.2
Rb	85.4 ± 0.3 (n=4)	8.7	4.7	0.39	81.5 ± 6.5
Sc[b]	241 ± 1 (n=5)	13.1	0.1	-0.61	266 ± 24
Zn	35.5 ± 0.2 (n=5)	9.7	6.2	-0.5	34.7 ± 2.7

[a] Arithmetic mean and Standard Deviation, n indicates number of determination.
[b] Results given in μg kg^{-1} .
[c] Values in percentages of mass.
[d] Informative values

Table 3. Elemental concentrations obtained in IAEA-85 Human Hair certified reference material

Element mg kg^{-1}	Mean ± SD (n)[a]	RSD (%)	RE (%)	Z-score	Values of Certificate [7]
As[b]	95.4 ±3.0 (n=6)	7.3			
Br	1.78 ± 0.03 (n=7)	15.2			
Ca	922 ± 13 (n=7)	7.1	3.0	-0.3	847 – 1010
Co[b]	103.1 ± 1.5 (n=7)	5.6			
Cr	1.49 ± 0.01 (n=7)	3.7			
Cs[b]	16.2 ± 0.9 (n=7)	16.5			
Cu	16.1 ± 0.2 (n=5)	16.6	4.1	-0.2	15.7 – 17.8
Fe	73.3 ± 1.2 (n=7)	5.3	7.5	-1.4	71.0 – 87.8
K	6.2 ± 0.1 (n=4)	18.7			
La[b]	48.8 ± 0.2 (n=7)	9.4			
Na	19.36 ± 0.01 (n=7)	7.5			
Sb[b]	35.8 ± 0.6 (n=7)	8.1			
Se	1.02 ± 0.02 (n=7)	2.9	4.4	-0.4	0.96 – 1.17
Sc[b]	8.8 ± 0.1 (n=7)	8.6	4.2	-0.3	0.0084 – 0.0100
Zn	168.0 ± 0.3 (n=7)	3.0	0.42	-0.1	156 – 170

[a] Arithmetic mean and Standard Deviation, n indicates number of determination.
[b] Results given in μg kg^{-1}

In the homogeneity study, seven aliquots of a sample were analyzed and these results are presented in Table 4. The relative standard deviations of these results are lower than 7%, indicating that the results present a good reproducibility and the sample can be considered homogeneous for the elements analyzed.

In Table 5 are presented preliminary results obtained in the analysis of hair samples collected from a group of voluntary people, and the element concentrations obtained revealed great variations. For the element Na, for example, the values found varied from 3.8 mg g^{-1} to 2.179 mg g^{-1}.

Table 4. Concentrations of trace elements in hair obtained in replicate analysis

Element mg kg^{-1}	Mean ± SD	RSD (%)	Element mg kg^{-1}	Mean ± SD	RSD (%)
As[a]	3.8 ± 0.5	12.4	K	12.1 ± 0.8	6.9
Br[a]	609 ± 4	0.7	La[a]	14.68 ± 0.4	2.8
Ca	3.653 ± 35	0.9	Na	17.30 ± 0.2	1.2
Co	409 ± 2	0.5	Sb[a]	10.8 ± 0.7	6.9
Cr[a]	63.06 ± 4	6.0	Sc[a]	1.20 ± 0.05	3.8
Cs	7.81 ± 1.1	13.0	Se[a]	225 ± 5	2.4
Cu	18 ± 1	8.0	Zn	132.02 ± 0.3	0.2
Fe	11.7 ± 0.2	1.4			

a. Results given in μg kg^{-1}

Table 5. Elemental concentrations in hair and literature data

Element mg kg^{-1}	This study		Values of literature	
	Geometric Mean (n=20)[a]	Range	Saiki et al[b] [12] (n=30)[a]	Chatt & Katz[b] [13] (n= 50)[a]
As[c]	13.3 x÷ 1.2	4.7 – 76.7	6.7 – 126	
Br	0.86 x÷ 0.09	0.289 – 1.75	0.42 – 85.4	5.7 x÷ 2.0
Ca	1101 x÷ 77	373 – 5.452	118 – 1788	410 x÷ 2.0
Cd	0.31 x÷ 0.02	0.09 – 0.98	0.044 – 1.22	
Co	0.85 x÷ 0.05	22.5 – 173.1	0.008 – 0.325	
Cr	0.080 x÷ 0.006	0.042 – 0.460	0.068 – 0.753	
Cu	24.7 x÷ 2.2	6.7 – 169	4.0 – 5.6	870 x÷ 2.2
Fe	10.1 x÷ 0.5	7.1 – 20.3	7.2 – 36.8	10 x÷ 1.8
K	5.9 x÷ 1.4	0.32 – 274	0.53 – 25.7	17 x÷ 2.2
Na	13.3 x÷ 5.8	2.89 – 2.180	1.50 – 29.7	
Sb[c]	13.5 x÷ 1.0	7.6 – 165.2	3.1 - 848	
Sc[c]	1.3 x÷ 0.12	0.35 – 11.1	1.18 – 5.70	
Se	0.28 x÷ 0.01	0.115 – 0.562	0.009 – 0.869	0.69 x÷ 1.6
Zn	123.5 x÷ 6.4	69.0 – 244.6	106 – 264	159 x÷ 1.2

a. n Indicates number of determination
b. Instrumental neutron activation analysis (INAA)
c. Results given in μg kg^{-1}

As can be seen, the data of some elements showed considerable intersubject variability. These results indicate that biological factors such as gender, age as well as health status and nutritional habits might be responsible for the variations in elemental compositions of hair.

The elemental concentrations found in hair were also compared with those presented by Saiki et al. [12] and Chatt and Katz [13]. Our results are of the same order of magnitude as the literature values. Data of hair analysis fit to a log normal distribution therefore geometric mean was calculated instead of arithmetic mean. Furthermore, the study presented by Carneiro et al. [14] indicates that the age factor influences in the concentration range for some elements in the hair.

4. CONCLUSIONS

From the preliminary results obtained in this study it can be concluded that the procedure used in the sample treatment allows to obtain homogenous samples in relation to elements determined in this work. Besides, the data obtained in the analysis of certified reference materials indicates that NAA provides good precision and accuracy of the results.

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