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ESTABLISHING A PROTOCOL FOR ELEMENT DETERMINATIONS IN HUMAN NAIL CLIPPINGS BY NEUTRON ACTIVATION ANALYSIS

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ABSTRACT

Human nail samples have been analyzed to evaluate occupational exposure, nutritional status and to diagnose certain diseases. However, sampling and washing protocols for nail analyses vary from study to study not allowing comparisons between studies. One of the difficulties in analyzing nail samples is to eliminate only surface contamination without removing elements of interest in this tissue. In the present study, a protocol was defined in order to obtain reliable results of element concentrations in human nail clippings. Nail clippings collected from all 10 fingers or toes were previously pre cleaned using an ethyl alcohol solution to eliminate microbes. Then, the clippings were cut in small pieces and submitted to different reagents for washing by shaking. Neutron activation analysis (NAA) was applied for nail samples analysis which consisted of irradiating aliquots of samples together with synthetic elemental standards in the IEA-R1 nuclear research reactor followed by gamma ray spectrometry. Comparisons made between the results obtained for nails submitted to different reagents for cleaning indicated that the procedure using acetone and Triton X100 solution is more effective than that of nitric acid solution. Analyses in triplicates of a nail sample indicated results with relative standard deviations lower than 15% for most of elements, showing the homogeneity of the prepared sample. Qualitative analyses of different nail polishes showed that the presence of elements determinated in the present study is negligible in these products. Quality control of the analytical results indicated that the applied NAA procedure is adequate for human nail analysis.

1. INTRODUCTION

The determination of elemental composition in human tissues such as fingernails has aroused great interest to assess the nutritional status, in the diagnosis of diseases, especially those systemic and to evaluate occupational exposure to toxic elements. There are 25 elements recognized as essentials for human and animal life, being those 11 of them are present at the trace levels [1]. The human nail tissue is composed of keratin and it acts as a final protection of the fingers and the nail helps to do delicate movements, to hold small objects [2]. Due to the slow growth of nails, they are not affected by transient factors that alter the levels of minerals in blood serum. However, the nails are exposed to soaps, nail polishes and other substances that may remain attached to its outer surface [3]. For nails to be useful as a trace element monitor factors, such as contamination by soil, nails polish, and the influence of age, sex, and longitudinal variation must be understood [4]. Therefore establishing a protocol for cleaning nails before the analyses is required to remove only the exogenous contaminant and for comparison the results between the studies. The purpose of this study was to establish adequate conditions for treatment and preparing of nail samples as well as the analytical procedure to obtain reliable results.

2. EXPERIMENTAL

2.1. Procedure for Neutron Activation Analysis

2.1.1. Preparation of synthetic standards of elements

Certified standard solutions of elements acquired from Spex CertiPrep USA were used to prepare synthetic element standards. From the stock standard solutions, diluted solutions containing one or more elements were prepared. Aliquots of 50 µL of these solutions were pippetted on small sheets of n°. 40 Whatman filter paper with the sizes 1.5 cm x 3.5 cm. These sheets were placed in a desiccator for drying the aliquots at room temperature. The micropipette used was previously checked in relation its calibration. The sheets were folded and placed in plastic bags. The plastic (polyethylene) foils used to prepare these bags were previously cleaned using dilute nitric acid solution and purified water.

2.1.2. Irradiation, measurements and calculations

The cleaned nails were dried at room temperature before the weighing. Each nail sample or certified reference material was weighed (140 to 200 mg) in polyethylene bags. Each bag containing the sample or standard was wrapped with aluminum foil. This set of samples and standards were placed in an aluminum irradiation device called "rabbit." Irradiation was performed in the IEA-R1 nuclear research reactor for 16 hours under a thermal neutron flux from 4.0×10^{12} n cm^2 s^{-1} to 5.0×10^{12} n cm^2 s^{-1}.

After adequate decay time of 4 days, samples and standards were placed individually in stainless steel planchets for gamma ray measurements. The induced gamma ray activities measurements were carried out using a hyperpure germanium detector from EG & ORTEC connected to a gamma ray spectrometer and associated electronic system. The system used had a resolution (FWHM) of 0.81 keV for 122.06 keV peak of ^{57}Co and of 1.97 keV for 1332.50 keV peak of ^{60}Co. The analysis of gamma spectra was performed using a computer program VISPENB2 [5] in TURBO BASIC language. The samples and standards were measured in three different decay times of about 5, 12 and 19 days in order to eliminate the problem of interferences and to increase the number of elements to be determined. In the first measurement, the As, Br, Ca, K, La, and Na were determined, in the second the elements Cr, Fe, Rb, Sb and Sc, and in third Co, Cs, Se and Zn. The radioisotopes measured were identified according to their half-lives and gamma-ray energies. The calculations for obtaining the element concentrations were performed by the comparative method.

2.2. Analysis of Certified Reference Materials

For quality control in relation to the precision and accuracy of the results reference materials NIST SRM 1577b *Bovine Liver* and NIST SRM 1566b *Oyster Tissue* provided by the National Institute of Standards & Technology, USA were analyzed.

Percentages of humidity of these certified reference materials were determined in order to obtain the results on a dry basis. For this determination approximately 250 mg of each reference material were basis weighed drying in an Universal oven at a temperature of 85 °C, for 24 hours. The percentages of humidity obtained in this drying process were 4.71 % for the certified reference material NIST 1577b *Bovine Liver*, 3.2 % for NIST 1566b

Oyster Tissue. These percentage values were used to calculate the element concentrations on a dry basis.

2.3. Analysis of the Polyethylene Material used for Sample Irradiation

This test was performed since the samples are irradiated in the plastic bags and they are not removed for counting. Elemental analysis of plastic samples pre-cleaned with dilute nitric acid solution and purified MILLIQ water was performed by neutron activation analysis. For this assay a polyethylene foil of 10 cm x 10 cm and weighing 0.33305 g was irradiated for 16 hours.

2.4. Qualitative Analysis of Nail Polish Samples

As some clipping nails presented with nail polishes before their cleaning, nail polishes were analyzed to verify the possible presence of their chemical elements absorbed into the nail samples. Nail polishes were analyzed qualitatively by NAA.

Three samples of nail polishes with different colors and following characteristics were analyzed: 1) with anti-allergy component, sparkling pearl nail polish, 2) red nail polish of known brand in the market and a relatively high commercial value and 3) the orange nail polish with a little commercial value brand. For these tests, samples of nail polish were placed in plastic bags, where they kept for 24 hours at room temperature for drying. After drying the samples these plastic bags were heat sealed and irradiated at the nuclear research reactor.

2.5. Collection of Clipping Nail Samples for Cleaning Assays

Clipping nail samples were obtained from adult individuals of both genders of a population considered "healthy" residing in the metropolitan region of São Paulo, Brazil. The nail clippings of the hands and feet were collected using a clipper or a pair of scissors and they were placed in plastic bags. The present study was previously approved by the internal ethical committee of Faculty of Public Health, University of São Paulo.

2.5.1. Treatment of nail samples for the analyses

Initially the clipping nail samples were kept immersed in a solution of 70 % ethanol without stirring for a period of 10 minutes to reduce risks to the microbiological contaminants such as fungi and bacteria during the handling of samples by the analyst. Then the nails were cut into small pieces (fragments) using a pair of scissors with titanium coating to prevent sample contamination.

Regarding to the washing procedure for removing impurities from exogenous origin, several studies are published and using different reagents are used such as hydrogen peroxide + methanol + acetone [6], 1 % Triton X100 + deionized water [7], 2 % Triton X100 + distilled water + acetone [8], and 10 % (v/v) HNO_3 + distilled water [9].

The nail treatment procedure proposed and in this study consists of the following steps: three consecutive washings with acetone p.a, with mechanical shaking for 1 minute, 3 consecutive treatments with 2 % Triton X100, with mechanical shaking for 1 minute, followed by two

washings with MILLIQ purified water, with mechanical shaking for 20 seconds. A shaker with agitation frequency of 3 rpm was used for shaking. The separation of the nail fragments from the washing solution was performing by the process of filtration using n°. 41 Whatman filter paper. The filtrate was also washed twice using MILLIQ water, and finally two washes with acetone p.a. The filter paper was placed with the nails in Petri dishes. The cleaned samples were kept at room temperature for a period from 24 to 48 hours for drying.

The efficiency of washing nails procedure with acetone p.a + 2% Triton X100 + water proposed for this work was compared with that with acetone p.a + 2 % Triton X100 + 10 % nitric acid + water and also with the procedure using acetone p.a + 10 % nitric acid + water.

2.5.2. Analysis of clipping nails in triplicate

The homogeneity of nail samples prepared was verified by the analysis of a sample in triplicate by neutron activation analysis.

3. RESULTS AND DISCUSSION

3.1. Results of Certified Reference Materials

Tables 1 and 2 show the results obtained in the analysis of certified reference materials NIST 1577b *Bovine Liver* and NIST 1566b *Oyster Tissue* respectively.

Table 1. Concentrations of elements in certified reference material NIST 1577b *Bovine Liver*.

Elements	M±SD	RSD (%)	RE (%)	Certificate values [10]
Br	10.1±0.3[a]	2.6	-	(9.7)[b]
Co	0.24±0.010	5.8	-	(0.25)[b]
Fe	186±7	3.6	1.2	184±15
K	10306±644	6.3	3.7	9940±20
Na	2504±42	1.6	3.4	2420±60
Rb	13.1±0.5	4.0	4.5	13.7±1.1
Se	0.75±0.03	4.3	4.4	0.73±0.06
Zn	124±4	2.8	2.7	127±16

[a] Arithmetic mean and standard deviation obtained from 2 to 4 determinations.
[b] Number in parentheses indicates informative value
Results in mg kg^{-1} .

Table 2. Concentrations of elements in certified reference material NIST 1566b *Oyster Tissue*.

Elements	M±SD	RSD (%)	RE (%)	Certificate value [11]
As	7.19±0.29[a]	4.0	6.0	7.65±0.65
Br	51.85±0.98	1.9	-	-
Ca	909±79	8.6	8.4	838±20
Co	0.36±0.01	3.4	2.4	0.371±0.009
Fe	195.8±8.6	4.4	4.8	205.8±6.8
K	6287±555	8.8	3.5	6520±90
Na	3329±72	2.1	1.0	3297±53
Rb	3.137±0.090	1.6	2.3	3.262±0.145
Se	2.10±0.10	5.0	2.0	2.06±0.15
Zn	1352±19	1.4	5.0	1424±46

[a] Arithmetic mean and standard deviation obtained from 3 to 4 determinations. Results in mg kg^{-1}.

Results obtained in these reference materials are in good agreement with their respective certified values, showing that the activation analysis procedure used was adequate for the determination of several elements. The percentages of relative errors (RE) of the results obtained for the material NIST 1577b *Bovine Liver* were lower than 4.5 % and the relative standard deviations (RSD) were lower than 6.3 %. The percentages obtained for the RE for NIST 1566b *Oyster Tissue* were lower than 8.4 %, and RSD were lower than 8.8 %.

3.2. Results Obtained in the Preliminary Assays

3.2.1. Analysis of plastic material and nail polishes

Table 3 shows the concentrations of elements found in the plastic material used for sample irradiation as well as those obtained in nail samples. From these data the amounts of elements present in plastic bags and in nail samples were calculated. The comparison between the masses of the elements present in the nail and those in a plastic bag indicated that the quantities of elements of a plastic bag are very low and can be considered negligible. In the third column of Table 3 the masses of the elements in a plastic bag of 22.24 mg used for irradiation are shown. In the fourth column are the masses of the elements present in about 200 mg of nail.

The nail polish samples were irradiated for 16 hours and the qualitative analysis indicated the presence of radioisotopes of various elements, but at very low counting rates, similar to the counts obtained in plastic bags. The sample which presented peaks more intense in the gamma spectrum was the orange nail polish. The radioisotopes ^{131}Ba, ^{47}Ca and ^{46}Sc due to these low counting rates obtained in the analysis of nail polish samples were detected the

contribution of absorption of the element of nail polishes after washing was considered negligible.

Table 3. Concentrations of elements in a sample of treated plastic.

Elements	Concentration in the plastic (mg kg^{-1})	Mass (in mg) of element in 22.24 mg of plastic bags	Concentration in nail (mg kg^{-1})	Mass (in mg) of element in 200 mg of nail
Br	0.1420±0.0011[a]	$3.1x10^{-6}$	1.7480±0.0078[a]	$3.5x10^{-4}$
Ca	26.0±2.9	$5.8x10^{-4}$	958±37	$1.9x10^{-1}$
Co	(21.09±0.63) 10^{-3}	$4.7x10^{-7}$	(31.6±1.6) 10^{-3}	$6.3x10^{-6}$
Cr	0.0516±0.0043	$1.1x10^{-6}$	0.251±0.015	$5.0x10^{-5}$
Cs	10.22±0.63	$2.3x10^{-4}$	7.7±2.1	$1.5x10^{-3}$
Fe	2.77±0.29	$6.2x10^{-5}$	34.6±1.0	$7.0x10^{-3}$
K	21.21±0.84	$4.7x10^{-4}$	114.9±4.3	$2.3x10^{-2}$
La	(3.35±0.13) 10^{-3}	$7.4x10^{-8}$	(134.7±1.3) 10^{-3}	$2.7x10^{-5}$
Na	12.960±0.040	$2.9x10^{-4}$	309.89±0.44	$6.2x10^{-2}$
Sb	(8.10±0.26) 10^{-3}	$1.8x10^{-7}$	(24.1±3.7) 10^{-3}	$4.8x10^{-6}$
Sc	(0.340±0.050) 10^{-3}	$7.5x10^{-9}$	(2.64±0.15) 10^{-3}	$5.2x10^{-7}$
Zn	11.000±0.060	$2.4x10^{-4}$	117.09±0.63	$2.3x10^{-2}$

[a] Results of one determination. The uncertainties of the results were calculated using statistical counting errors of the sample and standart.

3.2.2. Results of a nail analysis in triplicate

Table 4 shows the results obtained in the analysis of a nail sample in triplicate. For most of elements, the results presented relative standard deviations lower than 15 % indicating the homogeneity of the prepared sample. For the elements As, Cs, La an Rb results showed relative standard deviations higher than 17.7 % due to low concentrations of these elements in the sample and to poor statistical counting obtained in the gamma ray measurements.

Brockman *et al* [9] obtained large variation in duplicate analysis of the nails. The average of relative standard deviations was 17 % for Se and 4 % for Zn. This variation was attributed to the heterogeneous distribution of the elements in nails before or after cleaning procedure.

Table 4. Results (in mg kg^{-1}) of a nail analysis in triplicate.

Elements	M±SD	RSD (%)
As	$(28.3 \pm 6.2^{a}) \times 10^{-3}$	22.0
Br	1.848±0.025	1.6
Ca	862±48	5.6
Co	$(14.8 \pm 1.1) \times 10^{-3}$	7.6
Cr	0.214±0.014	6.7
Cs	11.4±4.0	35.0
Fe	18.4±2.5	13.8
K	402±56	14.0
La	$(12.7 \pm 2.6) \times 10^{-3}$	20.2
Na	324±12	3.8
Rb	$(1.17 \pm 0.21) \times 10^{-3}$	17.7
Sb	$(12.5 \pm 1.9) \times 10^{-3}$	14.8
Sc	$(2.52 \pm 0.32) \times 10^{-3}$	12.7
Se	0.3728±0.0059	1.6
Zn	79.6±1.5	1.8

[a] Arithmetic mean and standard deviation

3.3. Results Obtained for Nails Cleaned Using Different Procedures

The results obtained in the analysis of washed and unwashed fingernail samples presented in Table 5 indicate that the concentrations of most elements in washed sample are lower or at the same order of magnitude of those unwashed sample. Arsenium was not detected in unwashed nails due to the high activities of others radioisotopes that mask the peak of ^{75}As. The elements Co, K and Na presented in bold in Table 5 indicate that they were removed during the washing procedure.

Table 5. Concentrations (in mg kg^{-1}) of the elements in a fingernail sample with and without cleaning before the analysis

Elements	Cleaning with Triton X100+acetone	Without cleaning
As	(32.5±2.8[a]) 10^{-3}	ND[b]
Br	2.3340±0.0080	3.131±0.011[a]
Ca	805±18	930±20
Co	**(27.71±0.87) 10^{-3}**	**(84.9±1.7) 10^{-3}**
Cr	0.219±0.018	0.274±0.019
Cs	7.8±1.6	8.8±1.7
Fe	33.02±0.66	33.29±0.70
K	**582.8±1.9**	**1209±47**
La	(18.06±0.90) 10^{-3}	(16.89±0.98) 10^{-3}
Na	**671.30±0.95**	**1171.2±1.7**
Rb	(1.200±0.030) 10^{-3}	(2.090±0.040) 10^{-3}
Sb	(65.6±4.3) 10^{-3}	(85.6±7.1) 10^{-3}
Sc	(2.69±0.12) 10^{-3}	(3.46±0.13) 10^{-3}
Se	0.469±0.013	0.419±0.012
Zn	123.47±0.66	124.03±0.67

[a]Results of a single determination**.** The uncertainties of the results were calculated using statistical counting errors of the sample and standard.
[b]ND: Not detected

The results of element concentrations obtained in a toenail sample with and without cleaning with Triton X100 + acetone, presented in Table 6, also indicated that there was the removal of various elements when the sample was cleaned, excepting in the case of Se.

Table 6. Concentrations (in mg kg^{-1}) of elements in a toenail sample with and without cleaning before the analysis

Elements	Cleaning with Triton X100+acetone	Without cleaning treatment
As	$(41.6 \pm 2.4^{a}) \times 10^{-3}$	$(53.5 \pm 3.9^{a}) \times 10^{-3}$
Br	1.2830 ± 0.0057	2.1460 ± 0.0077
Ca	1109 ± 19	1301 ± 19
Co	$(19.45 \pm 0.79) \times 10^{-3}$	$(49.9 \pm 1.4) \times 10^{-3}$
Cr	0.166 ± 0.015	0.224 ± 0.015
Cs	4.6 ± 1.1	13.5 ± 1.8
Fe	14.77 ± 0.73	25.74 ± 0.97
K	752.1 ± 9.1	1617 ± 22
La	$(10.41 \pm 0.76) \times 10^{-3}$	$(58.5 \pm 1.1) \times 10^{-3}$
Na	376.73 ± 0.53	734.5 ± 1.0
Rb	$(1.280 \pm 0.050) \times 10^{-3}$	$(2.760 \pm 0.070) \times 10^{-3}$
Sb	$(28.4 \pm 2.5) \times 10^{-3}$	$(51.8 \pm 4.5) \times 10^{-3}$
Sc	$(1.95 \pm 0.14) \times 10^{-3}$	$(3.67 \pm 0.17) \times 10^{-3}$
Se	**$(472 \pm 14) \times 10^{-3}$**	**$(418 \pm 14) \times 10^{-3}$**
Z	96.46 ± 0.43	89.41 ± 0.45

[a] Results of a single determination. The uncertainties of the results were calculated using statistical counting errors of the sample and standard.

The results of Table 7 indicate that the nails submitted to two washing procedures A and B presented lower element concentrations or in the same order of magnitude of those of the unwashed one. On the other hand the results of nails submitted to the washing with Triton X100+acetone and that with Triton X100+HNO_3+acetone indicated that the HNO_3 solutions causes removal of some elements.

Table 8 shows the results obtained in nails (of an individual) washed using Triton X100 + acetone and those with 10 % HNO_3 solution + acetone. The elements Cr, K, La, Na, Rb, Sb and Zn were more removed when HNO_3 solution was used instead of Triton X100. For the elements Br, Sc and Se both procedures presented concentrations at the same order of magnitude. It was also verified that the washing with HNO_3 solution was quite drastic, resulting in slight dissolution of the sample. Also reported about the nail amount loss when diluted HNO_3 solution was used for cleaning [9]. Consequently the use of HNO_3 solution for cleaning nails was discarded in our study.

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Table 7. Concentrations (in mg kg^{-1}) of elements in a fingernail sample cleaned using different procedures

Elements	Cleaning Procedure		
	A[a]	B[b]	C[c]
As	(29.1±2.4) 10^{-3}	(25.3±2.2) 10^{-3}	(40.0±2.6) 10^{-3}
Br	1.7480±0.0070	1.6190±0.0080	2.2210±0.0070
Ca	957±36	623±42	916±27
Co	(31.6±1.6) 10^{-3}	(25.5±1.4) 10^{-3}	(30.0±0.50) 10^{-3}
Cr	(251±14) 10^{-3}	(235±15) 10^{-3}	(499±17) 10^{-3}
Cs	7.8±1.6	ND[d]	8.8±1.7
Fe	34.6±1.0	27.70±0.90	36.6±1.0
K	114.9±4.3	144.4±2.5	229.1±6.2
La	(134.7±1.3) 10^{-3}	(52.2±1.2) 10^{-3}	(169.0±1.3) 10^{-3}
Na	309.89±0.44	297.23±0.42	491.53±0.70
Rb	(0.290±0.040) 10^{-3}	(0.250±0.030) 10^{-3}	(0.570±0.040) 10^{-3}
Sb	(24.0±3.7) 10^{-3}	(26.8±2.7) 10^{-3}	(28.7±3.8) 10^{-3}
Sc	(2.64±0.15) 10^{-3}	(1.84±0.13) 10^{-3}	(2.52±0.13) 10^{-3}
Se	(587±19) 10^{-3}	(632±19) 10^{-3}	(656.5±2.7) 10^{-3}
Zn	117.09±0.63	88.93±0.52	109.50±0.56

[a] Procedure A: Triton-X100 + acetone + water; [b] Procedure B: Triton-X100 + HNO_3 + acetone + water; [c] Procedure C: Not washed, submitted only for microbiological decontamination treatment; [d] Not detected

Table 8. Concentrations (in mg kg^{-1}) of elements in a fingernail sample cleaned using different procedures

Elements	Cleaning Procedure	
	A[a]	D[b]
As	ND[d]	(23.9±3.9) 10^{-3}
Br	1.990±0.015	2.090±0.012
Cr	(294±17) 10^{-3}	(217±16) 10^{-3}
Cs	10.0±2.1	15.2±2.1
Fe	32.11±0.99	26.26±0.94
K	231.8±3.0	137.9±3.8
La	(14.3±2.0) 10^{-3}	(9.0±1.3) 10^{-3}
Na	242.4±1.2	118.23±0.60
Rb	(0.350±0.040) 10^{-3}	(0.190±0.030) 10^{-3}
Sb	(72.4±1.4) 10^{-3}	(52.7±1.1) 10^{-3}
Sc	(2.40±0.50) 10^{-3}	(2.68±0.14) 10^{-3}
Se	(416±18) 10^{-3}	(397±17) 10^{-3}
Zn	99.06±0.66	63.80±0.43

[a] Procedure A: Triton-X100 + acetone + water;
[b] Procedure D: HNO_3 + acetone + water.

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4. CONCLUSIONS

The results obtained for certified reference materials indicated good agreement with the certificate values, demonstrating the accuracy of the data. Results obtained also presented good precision, evaluated by the relative standard deviation values obtained.

With regard to the plastic used for sample irradiation it was possible to verify that it is resistant to activation conditions, not suffering any changes that may cause leakage of the sample. The results of this plastic bag analysis showed that the elements Br, Ca, Co, Cr, Cs, Fe, K, La, Na, Sb, Sc and Zn are present at low levels and could be considered negligible in the nail analysis.

The results of washing tests showed that the procedure using the solutions Triton X100 + acetone+ water allows the removal of various contaminants and nail polish too.

The results obtained in triplicate analysis of a nail sample showed homogeneity of the prepared sample indicating that sample preparation adopted was adequate.

On the basis of the data obtained it can be concluded that the experimental procedure could be applied in nail element determinations for the elements As, Br, Ca, Co, Cr, Cs, Fe, K, La, Na, Rb, Sb, Sc, Se and Zn. The preliminary data encourage further study for a large population to obtain reliable data.

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