—Technical Note—

Complementary analysis of trace elements in nail samples using instrumental neutron activation analysis and inductively coupled plasma mass spectrometry

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(Received 16 December 2013; accepted 7 May 2014)

Instrumental neutron activation analysis (INAA) and inductively coupled plasma-mass spectrometry (ICP-MS) were applied to determine minor and trace elements in human nail samples. Validation of each method was performed by measuring NIES No. 5 Human Hair certified reference material. Good agreements were observed between certified and measured values for these methods. Nail samples collected from 18 healthy volunteers were washed, dried and cut into small pieces. The samples were divided into two portions, one of which was analyzed by INAA and the other by ICP-MS. In INAA, Na, Mg, Al, Cl, K, Ca, V, Mn and Cu were recognized and the concentrations of these elements were calculated. On the other hand, peaks of Cu, Zn, Sr, Ag, Sn, Sb, Ba, Ce Hg and Pb were observed in the spectrum by ICP-MS. Copper, Zn, Sr, Ag and Pb were determined by ICP-MS because these elements showed sufficiently high intensity without spectrum interference. Nine and five elements were determined by INAA and ICP-MS. Copper was determined by both methods, and good correlation was observed. Combination of INAA and ICP-MS has an advantage to provide more information of elemental contents for nail samples if more than only one of the two methods is employed.

Key words: Nail, ICP-MS, INAA

Introduction

Determination of inorganic elements in biological samples is important from the viewpoint of nutritional status or criminal investigation, because elemental concentrations in these samples reflect the intake of biologically essential or toxic elements. Several studies have

been performed for this purpose using atomic absorption spectrometry^{1–3)}, instrumental neutron activation analysis (INAA)^{4,5)} and inductively coupled plasma-mass spectrometry (ICP-MS)^{6–10)}. Among these methods, INAA has advantages of no complicated pretreatment and nondestructive during analysis. The application of this method has been spread for

various samples such as foodstuff¹¹⁾, blood sera¹²⁾ and concrete^{13,14)}. Inductively coupled plasma-mass spectrometry has advantages that it doesn't require special facility such as nuclear reactor and has a potential for high sensitivity, multi-elemental analysis. This method has been also widely used for determination of trace elements in glass^{15,16)}, steel wires¹⁷⁾ or lead shotgun pellets¹⁸⁾.

One of the problems with inorganic element analysis in human nail is that there is no certified reference material (CRM). The accuracy and precision of analytical results for human nail have to be estimated by the comparison of results obtained by at least two analytical methods based on completely different principles. It is preferable to combine more than two methods for the assessment of analytical results or more number of elements to be analyzed.

In this work, combination of INAA and ICP-MS was investigated after each method was validated by measuring NIES No. 5 Human Hair¹⁹⁾ CRM. These methods were applied to the analysis of minor and trace elements in a series of human nails.

Materials and Methods

Sample preparation

A total of 18 fingernail samples were collected from healthy individuals (male 11, female 7) using procedures approved by the Institutional Review Board of the Tokyo Metropolitan University, Japan. There was no sample which wore nail polish. The samples were then transferred to clean beakers and washed with acetone for 20 minutes in ultrasonic bath, with purified water for 20 minutes twice, and finally with acetone for 20 minutes. After the nail samples were dried on a clean bench overnight, they were cut into small pieces and kept in acid-cleaned glass bottles. The samples were divided into two portions, one of which was analyzed by INAA and the other by ICP-MS.

Instrumental neutron activation analysis

Approximately 10–40 mg of each sample was weighed in acid-cleaned polyethylene bags

Table 1 Elements and short-lived radionuclides used for their determinations.

Elements	Radionuclides	Half-life	γ -ray energy (keV) ^{a)}
Na	²⁴ Na	15.02 h	1368.5, 2754.1
Mg	²⁷ Mg	9.46 min	1014.4
Al	²⁸ Al	2.24 min	1778.7
Cl	³⁸ Cl	37.24 min	1642.2, 2167.6
K	⁴² K	12.36 h	1524.6
Ca	⁴⁹ Ca	8.72 min	3084.4
V	⁵² V	3.76 min	1434.1
Mn	⁵⁶ Mn	2.58 h	1810.7
Cu	⁶⁶ Cu	5.10 min	1039.2

^{a)} γ -ray energy used for the determination of corresponding elements.

and sealed prior to irradiation. NIST 1566a Oyster Tissue was also prepared and used as comparison standard. NIES No. 5 Human Hair was analyzed as quality control material. The irradiation of the samples and standard was performed by using the pneumatic transfer system at JRR3 in Japan Atomic Energy Agency with a thermal neutron flux of about $10^{13} \text{ cm}^{-2} \text{ s}^{-1}$. The irradiation time of 2 minutes was employed. After appropriate decay times, gamma-ray measurements were performed using a high purity Ge detector. All samples were counted twice: first for 300 s and second 1000 s. Comparative method was used for the quantification of elemental concentration in the nail samples. Radionuclides used for the determination of elemental concentrations are listed in Table 1.

Inductively coupled plasma-mass spectrometry

Approximately 10 mg of each nail sample was accurately weighed on a microbalance with an accuracy limit of 100 μg , and put in a teflon vessel, to which 1 mL of nitric acid was then added. The teflon vessels were placed in a pressure tight case and heated by microwave oven with the power of 630 W for 10 minutes. After cooling to room temperature, the vessels were released and the sample solutions were diluted with purified water to 10 mL. All nail samples and NIES No. 5 Human Hair CRM were found to be completely digested by this

procedure. Standard solutions for calibration were prepared by mixing and diluting standard solutions (for atomic absorption spectrometry) of individual elements.

Results and discussion

Validation of analytical methods

The analysis of NIES No. 5 Human Hair CRM was performed by INAA in order to ascertain the accuracy of the method. Results are presented in Table 2 with literature values. The values for Mg and Cu are more accurate to the certified values than other work reported by Saiki et al.²⁰⁾ even though involving uncertainties of 25% and 24%, respectively. The difference

between certified and measured values for Na, Mg, Ca and Cu might be due to low counting statistics. Each element such as Al, Cl and V, whose certified value was not available, showed good agreement with those by Saiki et al.²⁰⁾.

NIES No. 5 Human Hair was also analyzed by ICP-MS. The results are summarized in Table 3. Values are expressed as mean $\pm$ standard deviation (SD; 1σ) of three independent measurements. Good agreements were observed between certified and measured values, indicating that the pretreatment and the determination by ICP-MS were appropriately performed. Detection limits listed in Table 3 were expressed as elemental concentrations in

Table 2 Analytical results of NIES No. 5 Human Hair CRM by INAA (in $\mu\text{g g}^{-1}$).

Element	Present work		Saiki et al. ²⁰⁾		Certified value ¹⁹⁾
	Measured (Conc. $\pm 1\sigma$) ^{a)}	Deviation (%) ^{b)}	Measured (Conc. $\pm 1\sigma$) ^{a)}	Deviation (%) ^{b)}	
Na	35.8 $\pm$ 3.6	+ 37.6	24 $\pm$ 4	− 7.7	26 $\pm$ 1
Mg	235 $\pm$ 59	+ 13.1	308 $\pm$ 10	+ 48.1	208 $\pm$ 10
Al	259 $\pm$ 21	+ 8.0	295 $\pm$ 45	+ 22.9	[240] ^{c)}
Cl	250 $\pm$ 3	− 0.2	251.6 $\pm$ 9.5	+ 0.6	[250] ^{c)}
K	35.1 $\pm$ 2.4	+ 3.3	30 $\pm$ 3	− 11.8	34 $\pm$ 3
Ca	620 $\pm$ 34	− 14.8	820 $\pm$ 44	+ 12.6	728 $\pm$ 30
V	0.549 $\pm$ 0.078	—	0.55 $\pm$ 0.07	—	—
Mn	5.12 $\pm$ 0.30	− 1.5	5.00 $\pm$ 0.02	− 3.8	5.2 $\pm$ 0.3
Cu	15.5 $\pm$ 3.7	− 4.9	13.4 $\pm$ 1.9	− 17.8	16.3 $\pm$ 1.2

a) Uncertainty calculated using statistical counting errors.

b) Deviation from a corresponding certified value.

c) Reference value.

Table 3 Analytical results of NIES No. 5 by ICP-MS (in $\mu\text{g g}^{-1}$).

Element	Present work			Certified value ¹⁹⁾
	Measured (Conc. $\pm 1\sigma$) ^{a)}	Deviation (%) ^{b)}	Detection Limit	
Cu	16.5 $\pm$ 0.3	+ 1.2	0.0084	16.3 $\pm$ 1.2
Zn	168 $\pm$ 2	− 0.3	0.1365	169 $\pm$ 10
Sr	2.35 $\pm$ 0.17	+ 2.1	0.0057	2.3 $\pm$ 0.2
Ag	0.121 $\pm$ 0.016	—	0.0017	—
Pb	5.98 $\pm$ 0.14	− 0.3	0.0037	[6.0] ^{c)}

a) Uncertainty calculated using standard deviation. (n = 5)

b) Deviation from a corresponding certified value.

c) Reference value.

hypothetical hair samples equivalent to three times of the statistical uncertainty (1σ) of counting obtained by five measurements of blank solution.

Analysis of the nail samples using INAA and ICP-MS

The results obtained by INAA are presented in Table 4. Magnesium, Cl and Ca were found as minor elements ($100\text{--}10000\ \mu\text{g g}^{-1}$), while Na, Al, K, V, Mn, Cu as trace elements (less than $100\ \mu\text{g g}^{-1}$). Instrumental neutron activation analysis has the advantage over ICP-MS in the determination of light elements and halogen such as Na, Mg, Al and Cl.

In the determination using ICP-MS, the qualitative analysis was performed by measuring mass spectrum prior to the quantitative analysis. A typical spectrum is shown in Fig. 1. Measuring point of mass-to-charge ratio 80 was excluded for detector protection. Some background peaks derived from Argon dimer, Xe and I were observed in blank solution, and these corresponding peaks were shown with parenthesis in Fig. 1. Peaks derived from Cu, Zn, Sr, Ag, Sn, Ba, Hg and Pb could be observed in all samples. Antimony was found in N6, N7, N9, N10 and N18. Cerium was found in

all samples except N14 and N17. Among these elements found in qualitative analysis, five trace elements such as Sn, Sb, Ba, Ce and Hg didn't show enough intensity to perform determination. No useful information was obtained from these five trace elements. In this work, Cu, Zn, Sr, Ag and Pb were quantitatively determined in further experiments because these elements had enough intensity for quantification and spectrum interference was negligible. The results are listed in Table 5. Values are expressed as mean $\pm$ SD of five measurements using one sample solution. In the calculation of Sr in sample N2 and N11, one data of the five measurements showed an extremely high value, therefore it was excluded as an outlier.

Zinc is an essential element for humans and it was found at higher concentrations in nails when compared with other four elements, as shown in Table 5. Zinc concentrations for these samples ranged from 83 to $158\ \mu\text{g g}^{-1}$. Strontium and Ag were determined as trace elements. High Ag concentration was observed in sample N2 that derived from the person whose hobby was photograph. It was thought that Ag included in the developing solution adhered to the fingernail and which was not removed by

Fig. 1 Mass spectrum of sample N2 by ICP-MS.

Table 4 Analytical results of nail samples by INAA.

Sample	Concentration ($\mu\text{g g}^{-1}$)								
	Na	Mg	Al	Cl	K	Ca	V	Mn	Cu
N1	15.8	71	21	54	13.3	781	0.024	1.62	4.0
N2	8.1	92	14	33	2.5	495	0.019	1.05	7.3
N3	21.8	80	2	100	11.0	871	0.005	0.46	4.4
N4	8.2	65	12	30	5.8	971	0.011	0.89	5.2
N5	25.3	112	1	57	22.7	116	0.009	0.50	3.8
N6	12.1	46	20	60	17.3	413	0.082	0.84	6.9
N7	26.6	117	50	180	2.6	638	0.049	1.76	6.4
N8	58.1	65	13	409	54.4	188	0.026	0.62	6.0
N9	26.2	114	4	228	16.8	1037	0.029	0.38	10.4
N10	84.6	64	12	138	82.6	639	0.022	0.79	5.3
N11	12.8	69	9	118	13.5	496	0.018	0.12	4.3
N12	23.7	124	4	98	25.5	864	0.011	0.22	4.8
N13	9.9	106	19	78	13.1	675	0.043	0.72	6.8
N14	11.7	28	9	127	9.9	447	0.003	0.18	4.9
N15	110.0	174	9	37	66.5	134	0.028	0.63	4.6
N16	30.9	64	4	502	29.4	315	0.005	0.23	4.0
N17	2.0	45	5	75	11.2	428	0.010	0.23	5.3
N18	20.5	85	20	125	28.5	336	0.040	0.18	3.0

Table 5 Analytical results of nail samples by ICP-MS.

Sample	Concentration ^{a)} ($\mu\text{g g}^{-1}$)				
	Cu	Zn	Sr	Ag	Pb
N1	4.1 ± 0.0	102 ± 1	0.37 ± 0.01	0.020 ± 0.003	0.50 ± 0.01
N2	5.3 ± 0.0	83 ± 3	0.12 ± 0.00 ^{b)}	2.913 ± 0.071	4.89 ± 0.07
N3	4.3 ± 0.0	99 ± 1	0.08 ± 0.01	0.030 ± 0.004	2.01 ± 0.01
N4	4.7 ± 0.0	158 ± 1	0.60 ± 0.01	0.161 ± 0.002	0.31 ± 0.01
N5	4.7 ± 0.0	122 ± 2	0.53 ± 0.01	0.076 ± 0.004	1.18 ± 0.02
N6	7.2 ± 0.0	116 ± 1	0.09 ± 0.05	0.111 ± 0.009	0.59 ± 0.03
N7	6.8 ± 0.1	95 ± 1	0.34 ± 0.01	0.187 ± 0.005	3.33 ± 0.05
N8	4.5 ± 0.1	97 ± 1	— ^{c)}	0.026 ± 0.001	0.74 ± 0.01
N9	6.8 ± 0.1	143 ± 1	0.24 ± 0.01	0.159 ± 0.003	4.03 ± 0.04
N10	8.0 ± 0.1	106 ± 1	0.24 ± 0.01	0.471 ± 0.010	2.63 ± 0.03
N11	4.6 ± 0.0	105 ± 3	0.09 ± 0.01 ^{b)}	0.210 ± 0.054	0.54 ± 0.16
N12	3.9 ± 0.0	97 ± 1	0.60 ± 0.01	0.057 ± 0.004	0.14 ± 0.00
N13	4.9 ± 0.0	115 ± 1	0.68 ± 0.01	0.016 ± 0.001	0.28 ± 0.01
N14	4.9 ± 0.0	92 ± 1	0.15 ± 0.01	0.849 ± 0.008	0.36 ± 0.01
N15	4.6 ± 0.0	94 ± 1	0.37 ± 0.01	0.057 ± 0.003	0.64 ± 0.01
N16	4.7 ± 0.0	99 ± 1	— ^{c)}	0.011 ± 0.002	0.16 ± 0.01
N17	3.8 ± 0.0	145 ± 1	0.14 ± 0.01	0.106 ± 0.004	0.41 ± 0.01
N18	3.3 ± 0.0	87 ± 1	0.27 ± 0.01	0.081 ± 0.003	1.37 ± 0.02

^{a)} Mean ± SD (n=5). ^{b)} Mean ± SD (n=4). ^{c)} Under detection limit.

washing procedure. Lead contents differed widely among the samples. As the differences were observed between the samples, its elemental concentrations in nails can be used as an indicator of contaminations in individuals.

Copper was determined by both ICP-MS and INAA. The concentration of Cu determined by ICP-MS ranged from 3.3 to 8.0 $\mu\text{g g}^{-1}$, and it was close to the results obtained by INAA. A reasonably good correlation was observed despite the narrow range of concentration values.

In this study, nine and five elements were determined by INAA and ICP-MS, respectively. They are not overlapped except Cu, so INAA and ICP-MS can be effectively combined to obtain much information from nail samples.

Conclusion

Trace and minor elements in NIES No. 5 Human Hair and fingernails were determined by INAA and ICP-MS. Verification of the analytical accuracy by these methods was performed using this CRM. Most of the results obtained by these two methods showed good agreement with certified values. Despite INAA require special facility such as nuclear reactor, it has advantages for light elements and halogen such as Na, Mg, Al and Cl, those determinations are difficult for ICP-MS. Combination of INAA and ICP-MS has a merit to provide sufficient information of elemental contents for nail samples, showing that they can work complementarily.

As the variation of the elements determined in this study reflects environmental factor or daily intake of an individual, nail analysis can be an important indicator for nutritional status and forensic investigation. One's profession or hobby might be presumed by the characteristic element contained in nail, or forensic discrimination could be performed by the comparison of elements with control samples. Our work clearly verified that well-calibrated measurements by a combination of INAA and ICP-MS can be suitable to provide such data.

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