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Biomonitoring of hair samples by laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS)

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Abstract

An analytical method for determining essential elements (Zn, Fe and Cu) and toxic elements (Cr, Pb and U) on single hair strands by laser ablation inductively coupled plasma mass spectrometry (LA-ICP-SFMS) using a double focusing sector field mass spectrometer was developed. Results obtained directly using LA-ICP-SFMS of hair were compared with those measured by inductively coupled plasma quadrupole mass spectrometry (ICP-QMS) of solutions of digested hair samples and the analytical methods were found to agree well.

Different quantification strategies for trace element determination in hair samples such as external calibration, standard addition and isotope dilution were compared and demonstrated for uranium. For uranium determination in powdered hair by LA-ICP-MS solution-based calibration was applied by coupling the laser ablation chamber to an ultrasonic nebulizer.

The significance of single hair analysis by LA-ICP-SFMS was demonstrated by a case study of a person who changed living environment. Differences in the uranium content observed along the single hair strand correlated with the changes in the level of uranium in drinking water. The uranium concentration in a single hair decreased from 212 to 18 ng g^{-1} with a change in the uranium concentration in drinking water from 2000 to 30 ng l^{-1}. In addition, measurements of uranium isotope ratios showed a natural isotopic composition throughout the whole period in the drinking water, as well as in the hair samples.

This paper demonstrates the potential use of laser ablation ICP-MS to provide measurements on a single hair strand and its potential to become a very powerful tool in hair analysis for biological monitoring.

Keywords: Biomonitoring of hair; Isotope ratio measurements; Laser ablation ICP-MS; Trace elements; Uranium

1. Introduction

Biomonitoring medical samples with respect to essential and toxic elements is gaining importance as a tool for studying the effect of deficiency or excess of elements on our health and their contribution to the development of different diseases [1–4]. In general, the majority of investigations of heavy metals in medical samples deal with blood and urine analysis but hair samples can also be used for the sensitive biomonitoring of environmental exposure or ingestion through food or drinking water. The analysis of hair samples has several advantages over the analysis of blood and urine. First, hair is stable and robust and its composition does not change over time. Second, it does not require special storage or handling. Third, unlike blood samples, sampling is painless, very easy and requires no specific professional skills. Finally, hair has the unique ability to reflect the total body intake over an extended period and it is possible to trace changes over time depending on the length of the hair. Therefore, hair is an excellent candidate for the biomonitoring of metals, particularly for the detection of trace impurities.

For the last two decades, hair analysis has been used for metal determination in medical research [1–5], where the concentration of elements in the hair of healthy subjects was compared to that of people with different diseases. Trace elements in hair were investigated in the criminology field [6,7] where it was analyzed for traces of cocaine. An interesting application is in archaeology [8], where the hair of an ancient iceman was analyzed and information on his diet, environmental [9–12] and occupational exposure [13,14] to different trace elements could be detected. Furthermore, hair analysis is also used in medical

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research to explore the connection between the concentration of metals in hair and different diseases such as autism [1] and epilepsy [3].

The determination of trace metals in hair has been performed using different analytical techniques, most of them involving bulk measurements after total digestion of the sample. Methods for the determination of metal concentrations in hair samples after digestion include atomic absorption spectroscopy (AAS) [15], inductively coupled plasma mass spectrometry (ICP-MS) [16,17] and inductively coupled plasma atomic emission spectrometry (ICP-AES) [18]. Another approach for the analysis of solid hair samples, or of samples after milling and homogenizing, is bulk analysis. One powerful technique for bulk analysis is laser ablation ICP-MS (LA-ICP-MS), as demonstrated by Rodushkin and Axelsson [16,17] for the determination of 71 elements in hair and nails samples. The multi-element analysis of trace elements in hair samples there was carried out after pretreatment of the samples. For quantification purposes, tablets from powdered certified reference material (from the Institute of Geophysical and Geochemical Exploration, Langfang, China) [19] were prepared. Other analytical techniques for the direct analysis of hair samples include X-ray fluorescence (XRF) [20], X-ray fluorescence excited by synchrotron radiation (SRXRF) [21], neutron activation analysis (NAA) [22] and instrumental neutron activation analysis (INAA) [23].

All of these analytical techniques can be used for hair analysis but they all have some disadvantages. The measurements reflect the average composition of trace elements in the hair sample and several strands of hair are required for each sample. As a result, spatially resolved information in one single hair is unavailable and therefore the data does reflect temporal changes in the intake of the analyte.

In the last 3 years, hair analysis has progressed and works investigating the transformation of hair content with time have been published. Highly sensitive methods have been utilized to achieve this goal. Induction heating electrothermal vaporizer ICP-MS (ETV-ICP-MS) [24] was applied for mercury analysis of single human hair strands. For quantification purposes, the analytical data were calculated relative to a standard of powdered hair used for external calibration. The reported limit of detection was in the range of 20–30 pg based on 0.6 mg sample. Time-of-flight secondary ion mass spectrometry (ToF-SIMS) was used to measure the elemental distribution in human hair [25]. The elements Na, K, Al, Si, Mg, Ca, Ti and Fe were analyzed and it was shown that the surface of hair samples can act as a sink for species to which it is exposed to in the environment. SIMS with multiple ion collectors (NanoSIMS 50, CAMECA) was used for imaging arsenic traces in hair [26]. A cross-section scanning of hair was performed. The analysis allowed distinguishing of intoxication from surface pollution of the sample. With synchrotron radiation, the cross-sectional and longitudinal distribution of some major and minor elements was determined in hair samples taken from haemodialysed patients [27] and laser ablation ICP-MS with dynamic reaction cell (DRC) was used to measure Hg and Pt in one individual hair after these elements were ingested [28]; the hair samples analyzed reflected the forensic evidence in both cases. It was also found that the concentration in the outer and inner part of hair varied similarly with time.

This field of single hair analysis is continuing to expand, but until now the literature has not dealt with the quantification of elements in a single hair strand collected from a "normal" population; it has focused instead on special cases, where the amount of the tested element was high.

In the present work, the suitability of LA-ICP-MS for hair analysis will be considered by measuring essential elements and toxic elements at trace concentration levels in hair samples taken from a "normal" population. In addition, as a continuation of previous works on the biological monitoring of uranium [9–11,29], special attention will be focused on uranium determination in hair samples by LA-ICP-MS. Different analytical quantification procedures, such as standard addition and isotope dilution for external calibration and standard addition to the sample as previously reported by our laboratory [30–34] were used and evaluated in order to find the most suitable procedure for the quantification of uranium in hair. Due to the radioactive property of uranium and the potential use of the method in exposure monitoring, the ability to track differences in the $^{235}U/^{238}U$ isotope ratio in uranium will be discussed.

2. Experimental

2.1. ICP-MS instrumentation

A double focusing sector field ICP-MS (ICP-SFMS, ELEMENT, Thermo Electron GmbH, Bremen, Germany) coupled with commercial laser ablation system LSX-200 (CETAC LSX-200, CETAC Technologies, Inc., Omaha, NE, USA) was used for the analysis of human hair as powder or as single hairs. Mass spectrometric measurements were carried out at low mass resolutions ($m/\Delta m \approx 300$) for the isotope ratio study of uranium and medium mass resolutions ($m/\Delta m \approx 4400$) for quantification studies. In order to apply solution-based calibration as the quantification technique, an ultrasonic nebulizer (U-6000AT, CETAC Technologies, Inc.) was coupled to the LA-ICP-SFMS instrument. The experimental arrangement of solution-based calibration using a USN in LA-ICP-SFMS is shown in Fig. 1.

Multi-element analyses on digested human hair samples were performed by a quadrupole-based ICP-MS (ICP-QMS, ELAN 6000, Perkin-Elmer SCIEX, Concord, Ontario, Canada). Sulfur, which is a matrix element in hair, was selected as an internal standard element. Hair contains a high amount of sulfur because the amino acid cysteine is a key component of the keratin proteins in hair fiber (approximately 14%). Therefore, the experimental parameters were optimized to maximum $^{32}S^+$ ion intensity for quantitative measurements and with respect to $^{238}U^+$ for isotopic ratio studies of uranium. The optimized experimental parameters are summarized in Table 1.

2.2. Sample preparation and standard solutions

Hair samples were taken from three individuals without any history of environmental exposure. The protocol for the removal of external contamination from the hair samples involved rinsing

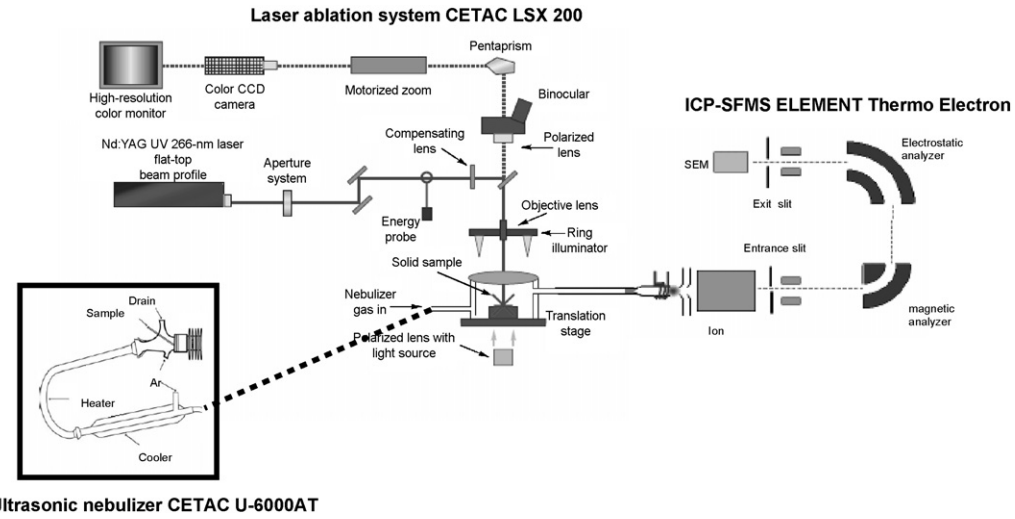

Fig. 1. Schematic of LA-ICP-SFMS by coupling a CETAC LSX-200 [32] with a double focusing sector field ICP-MS (Thermo Electron, Bremen [43]).

with 5 ml of acetone (HPLC grade, Merck, Darmstadt, Germany) for 10 min. The liquid was then decanted and the procedure was repeated twice with 5 ml of water, before the hair samples were washed with acetone and finally rinsed once again with water. After that the samples were left to dry.

In brief, 40–70 mg of the hair were weighed in a 15-ml screw-capped, polypropylene test tube (Blue Max, Becton Dickinson, USA) and the samples were digested by adding 2 ml of concentrated nitric acid (supragrade and purified by sub-boiling distillation, Merck) and 1 ml of 30% hydrogen peroxide (Suprapur, Merck). Placing the vials in a hot (80–90 °C) water bath expedited the digestion, which then took 10 min (caution: the screw cap must be placed loosely on the tube to allow the overpressure from digestion products to escape). The digestion procedure of hair samples was described in detail by Gonnen et al. [29]. Finally, the samples were diluted with water to yield a final volume of 15 ml. A blank vial that went through the whole process of rinsing and digestion was prepared for each batch of samples.

Table 1
Optimized operating parameters of the experimental systems

ICP-MS	ELEMENT ICP-SFMS	ELAN 6000 ICP-QMS
Nebulizer type	PFA-100	Micromist
RF power (W)	1250	1300
Cooling gas flow rate (l min^{-1})	18	14
Auxiliary gas flow rate (l min^{-1})	0.6	0.8
Nebulizer gas flow rate (l min^{-1})	1.2	0.88
Detection mode		Dual
Auto lens		On
Scanning mode	Peak hopping	Peak hopping
Extraction lens potential (V)	2000	
Mass window (%)	10	10
Mass resolution, $m/\Delta m$	300/4400	300
Ultrasonic nebulizer		**CETAC U-6000AT**
Sample uptake rate (ml min^{-1})		2
Heater temperature (°C)		140
Cooler temperature (°C)		3
Laser ablation		**CETAC LSX-200**
Wavelength (nm)		266 (of Nd-YAG laser)
Method		Single line scan
Pulse duration (ns)		20
Repetition frequency (Hz)		20
Spot size (μm)		300
Laser energy per pulse (mJ)		2.2
Scan speed (μm s^{-1})		30

Samples for single hair analysis were made by fixing rinsed hairs (1 cm in length) one by one on a carbon patch (description of the patch is given in the standard preparation section). Corrections for variations in ablation efficiency and plasma variation were made by internal standardization using the matrix element sulfur. A schematic of sample preparation procedures is summarized in Fig. 2.

For the quantification of Zn, Fe, Cu, Cr, Pb and U, multi-element standard solutions (ICP Multi Element Standard IV and VI from Merck) were used.

Samples for the standard addition mode were prepared by diluting the Merck uranium standard to the following concentrations: 100, 200 and 500 μg g^{-1} in 50 mg samples of rinsed and powdered hair.

All dilutions were made with high-purity deionized water (18 MΩ cm^{-1}), obtained from Milli-Q-Plus water purifier (Millipore Billerica, MA, USA). All chemicals used were of supragrade (Merck). Nitric acid was further purified by sub-boiling distillation.

2.3. Quantification strategies

Several quantification strategies for LA-ICP-MS, such as external calibration (with homemade standard), standard addition and solution-based calibration were developed. In addition, ICP-MS was used for trace analysis of essential and toxic elements in hair samples after digestion by ICP-MS.

A schematic summary of the quantification strategies is given in Fig. 3.

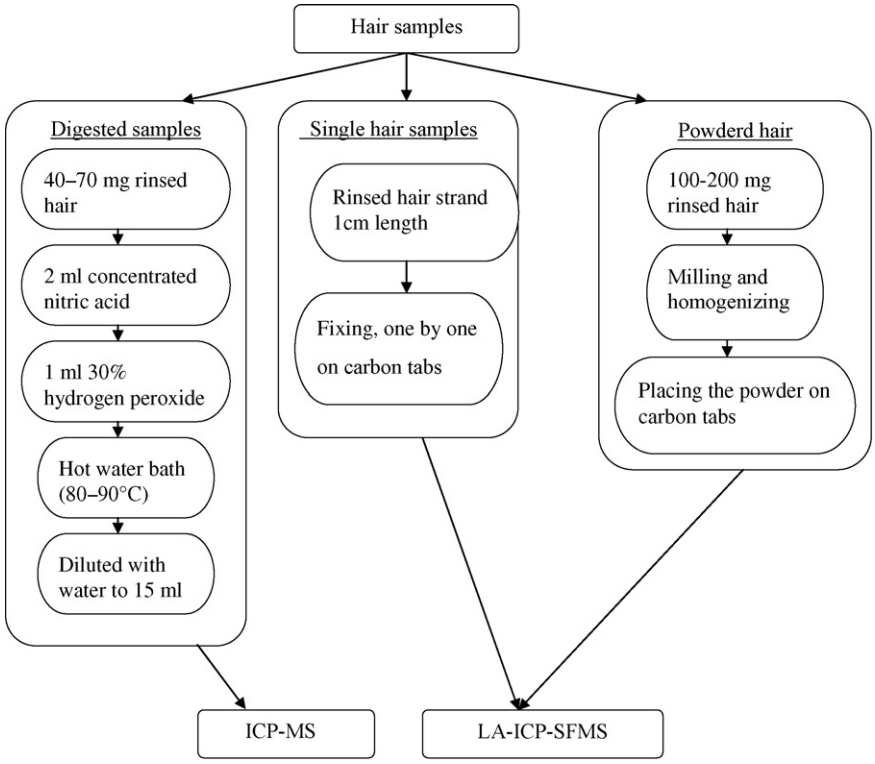

Fig. 2. Schematic of sample preparation of hair samples by digestion, of single hair fiber and of powered hair samples.

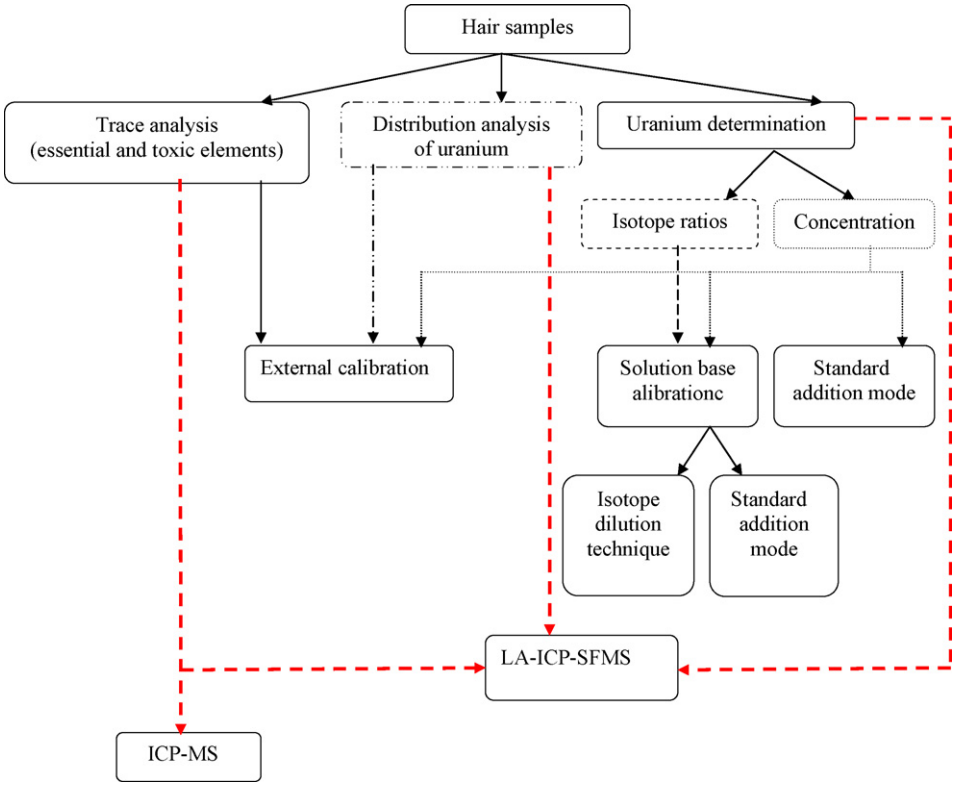

Fig. 3. Schematic of the analytical procedures used in this work including trace analysis of essential and toxic elements, distribution analysis, determination of the concentration and isotope ratios of uranium.

2.3.1. Standard addition calibration in LA-ICP-MS

The standard addition mode was applied for the trace analysis of uranium in hair samples whereby the laboratory standards (with different uranium concentrations from 100 to 500 $\mu g\,g^{-1}$ to cover the calibration range) were prepared by adding solution to powdered hair samples, homogenizing and drying. Then, these samples for calibration were analyzed directly by LA-ICP-MS. The calibration curve for uranium was obtained from these measurements.

2.3.2. Solution-based calibration in LA-ICP-MS

2.3.2.1. Standard addition technique for uranium determination. The standard addition technique was developed in our laboratory for the multi-element trace analysis of high purity metals, semiconductors and insulators, where no matrix-matched standard reference materials were available [35]. Thereby, the uranium standard solutions (with different concentrations of analytes) were nebulized successively using an ultrasonic nebulizer (USN) with desolvator during the laser ablation of the powdered hair samples. The concentration of trace elements was determined by means of the calibration curves, in our case for uranium.

The standard solutions for calibration were prepared by diluting the uranium calibration standard from Merck to the following concentrations: 0.003, 0.006 and 0.012 ng ml^{-1}.

2.3.2.2. Isotope dilution technique. In addition, uranium was determined in the powdered hair sample by isotope dilution technique using an isotope-enriched tracer solution ($^{235}U/^{238}U$—0.9996980). As an isotopic-enriched reference material, uranium CCLU-500 solution was applied (this laboratory standard was established by calibration against the NIST-500 SRM by TIMS [36]).

The concentration of uranium in the solution was chosen so as to obtain an isotope ratio as near as possible to one in the mixture of sample and tracer solution.

2.4. Isotope ratio measurements of uranium in hair samples by LA-ICP-MS

Uranium isotope ratio measurements were carried out for some of the hair samples by LA-ICP-MS in a line scan. A laboratory standard solution of uranium NBS-3164 (0.5 ng ml^{-1}) with natural isotopic composition was used to optimize the experimental parameters for isotope ratio measurements.

2.5. Preparation of synthetic laboratory standards

Since no commercial, certified, matrix-matched reference material with all of the elements measured was available and since it was decided to use a single hair as a reference standard in this work, a synthetic standard was prepared. A 2 cm long hair sample from the scalp of a healthy male adult was treated according to Section 2.2. Part of the sample was dissolved, analyzed by ICP-MS and all elements were quantified and compared to the certified solution standard as mentioned below. A powder standard was prepared from another part of the hair sample after rinsing by milling 100–200 mg of the hair with SPEX 6700 FREEZER/MILL (SPEX Industries, Inc., Metuchen, NJ, USA). The homogeneous powder was placed on carbon tabs (PLANO GmbH, Wetzlar, Germany). In addition, the single hair sample was fixed on carbon tabs for LA-ICP-SFMS studies. Both solid samples and the laboratory standards were analyzed by LA-ICP-SFMS.

3. Results and discussion

3.1. Trace analysis in a single hair strand by LA-ICP-SFMS

For the quantification of analytical data, solid standard reference materials were applied, using a homemade laboratory standard and sulfur as an internal standard matrix element.

The essential and toxic elements in single hair samples taken from three volunteers were analyzed and the results obtained are summarized in Table 2. The measured concentrations of Zn, Fe, Cu, Cr, Pb and U were compared with data from the literature. In the hair samples investigated, the concentration of essential elements in hair was in 10–50 $\mu g\,g^{-1}$ range, while for toxic elements it varied from 0.08 to 5 $\mu g\,g^{-1}$. The results were found to be within the range of concentrations published in the literature. However, for five of the elements, the measured concentrations had a lower range, except for zinc, which was found to be even lower in two samples than in the literature. It should be noted that the published range includes different populations, while the present study focuses on three random, "normal", unexposed people and these differences are therefore reasonable. The mean relative standard deviation of the results from the five replicate analyses of single hairs lies in the range of 10–25%, which is similar to the RSD reported by Rodushkin and Axelsson [19] for wisps of hair analysis by LA-ICP-MS. In the present work, the diameter of the laser spot used was 300 μm. The diameter of the hair samples varied between 50 and 70 μm. Therefore, all material was ablated. The position of the ablated point along the hair strand was not cross-section dependent.

Another aspect that should be borne in mind is that only a small section from the same part of the scalp and length of the hair is analyzed in single-hair measurements, and this part may not be representative of the entire strand. Nevertheless, good agreement was found between laser ablation of single hairs and solution analysis of digested hair for the same elements, as shown in Figs. 4 and 5. Thus, it may be expected that the level of trace elements in hair samples collected from people who do not change their dietary habits or living environment will have quite

Table 2
Concentration of Zn, Fe, Cu, Cr, Pb and U in single hair strands from three volunteers ($\mu g\,g^{-1}$)

	Person A	Person B	Person C	Published range [17]
Zn	32.7 ± 4.0	26.4 ± 2.1	44.9 ± 5.3	40–327
Fe	52.8 ± 3.1	28.3 ± 3.1	26.1 ± 1.0	3–900
Cu	13.5 ± 4.0	10.1 ± 1.0	25.6 ± 0.3	0.3–293
Cr	1.0 ± 0.1	0.5 ± 0.04	0.7 ± 0.05	0.03–33
Pb	4.7 ± 0.6	1.6 ± 0.07	2.0 ± 0.2	0.22–7.26
U	0.1 ± 0.04	0.06 ± 0.03	0.25 ± 0.01	0.005–1.28

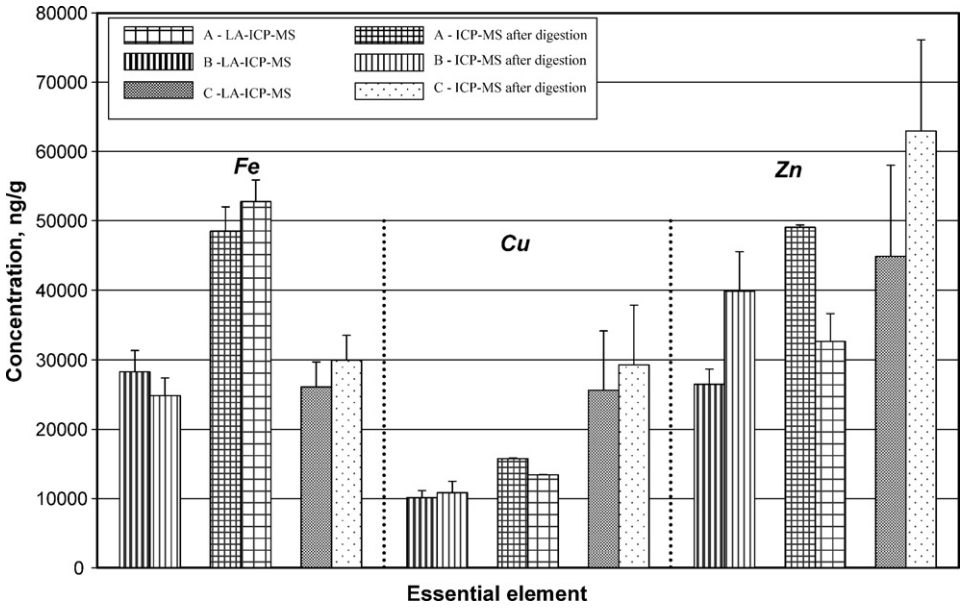

Fig. 4. The average concentration of essential elements in single strands of human hair from three subjects.

a uniform distribution even if they were collected from different parts of the scalp. Once again it should be emphasized that the results presented above were for people who are not occupationally or environmentally exposed, i.e., "normal" levels of the trace elements.

The above facts and conclusions are true for iron, copper, chromium, lead and uranium. Zinc was exceptional due to the fact that the measured concentration was not only lower than the range quoted in the literature, but the standard deviation of the results was higher than for the other five elements. This could be due to the unique behavior and role of zinc in hair [37,38] or possibly to cross contamination in the experimental system. Its metabolism and function in the human body and has been widely studied. As reported, zinc is an essential element in the human body and plays an important role in both activating enzyme systems and as a cofactor of several metallo-enzymes [37,38]. It has been previously noted that the concentration of zinc in hair depends on sex, age and seasonal variations, and therefore results are sensitive to such variations [39,40]. The data shown in Figs. 4 and 5 and the results given in Table 2 demonstrate the potential of using laser ablation ICP-MS technique for single hair analysis not just in special cases where the concentration is high but also for unexposed "normal" persons.

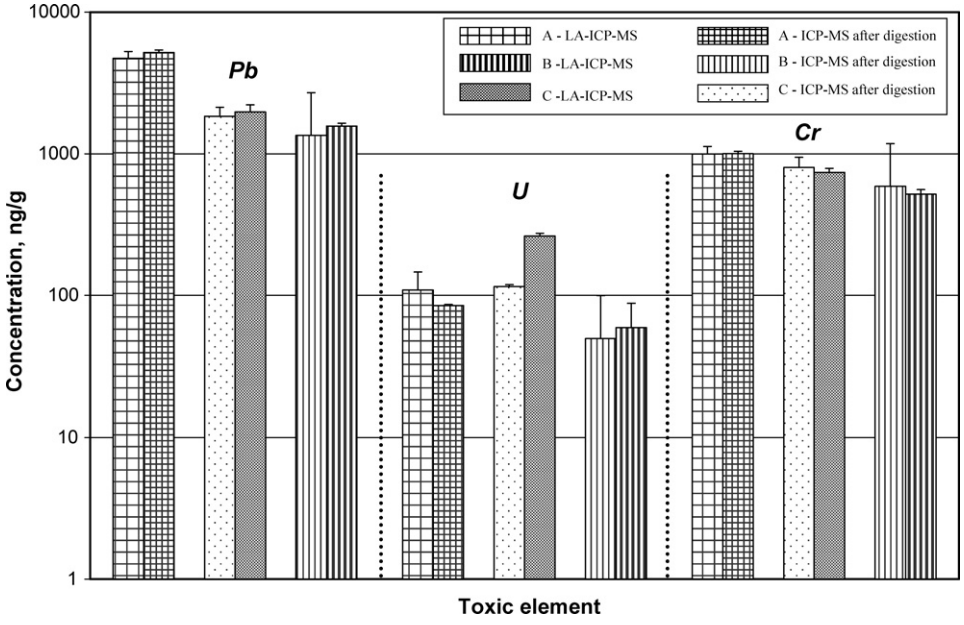

Fig. 5. The average concentration of toxic elements in single strands of human hair of three subjects.

3.2. Uranium analysis

This part focuses on the ability to measure uranium concentrations in hair using laser ablation ICP-MS. The question whether a change in dietary habits or living environment, that involves a change in the intake of trace elements, would be reflected in hair analysis is especially intriguing. This assessment made it possible to detect changes in the concentration of uranium in hair in correlation with changes in the uranium level in the environment, as discussed below.

3.2.1. Uranium concentration determination

Different quantification strategies in LA-ICP-MS, which were developed for geological samples [41] were applied to powdered hair standards. In addition, quantification methods using solid homemade standard reference materials, as previously described, combined with an internal matrix standard, were also tested.

3.2.2. The standard addition technique

In this quantification method, standard solutions (with different concentrations of the analyte) were added to the sample (powdered hair) in the sample preparation stage and were homogenized well in the sample. The concentration of the trace element (uranium in this case) was determined from the calibration curve, which was based on the standard addition samples that were ablated according to the same procedure. The normalized ion intensity of uranium agreed well with its concentration and the correlation coefficient (R^2) was found to be 0.9975.

3.2.3. Solution-based calibration in LA-ICP-MS by single gas flow injection

The main advantage of this arrangement, as shown previously [41], is the possibility of optimizing the gas flow rate to obtain maximum signal intensity. This was observed at a carrier-gas flow rate of 1 l min^{-1} for the transport of ablated material to the ICP-MS, and an optimal mixing of nebulized standard solution and laser-ablated solid sample directly in the ablation chamber. Two strategies for solution-based calibration in LA-ICP-MS were used in this work: standard addition (since no blank sample exists) that is useful when the amount of sample is large, and isotope dilution, which is commonly used for the analysis of small sample amounts. Both techniques are similar to the calibration methods used in solution analysis by ICP-MS.

3.2.3.1. The analyte standard addition mode. This quantification method was developed for multi-element trace analysis of high-purity metals, where neither matrix-matched standard reference materials nor high purity blank samples were available [41]. In the present study, the analyte addition technique was used and the standard solutions (with different concentrations of analyte) were nebulized successively using an USN and the sample (powdered hair) was ablated with a focused laser beam during solution calibration. The concentration of the trace element (uranium in this case) can be determined from the intercept of the calibration curve. For this experiment, the correlation between the measured uranium ion intensity and its concentration was found to have a coefficient (R^2) of 0.9915.

Table 3
Comparison of different methods for measurement of uranium in powdered hair standard (ng g^{-1})

Analysis method	Calibration strategy	U concentration (ng g^{-1})	LOD (ng g^{-1})
ICP-MS	External calibration of digested samples	22.6 ± 2.5	0.2
LA-ICP-MS	Standard addition	19.4 ± 4.1	1.2
LA-ICP-MS	Isotope dilution	20.2 ± 0.3	2.0
LA-ICP-MS	Solution-based calibration	22.1 ± 1.9	2.0

3.2.3.2. The isotope dilution technique. The calibration technique chosen for the determination of trace impurities in small sample sizes using LA-ICP-MS was the isotope dilution technique. It was applied in the present work to determine the concentration of uranium in small amounts of powdered hair samples. In on-line isotope dilution with LA-ICP-MS, an isotope-enriched spike ^{235}U solution (CCLU-500, ^{235}U spike sample) was nebulized using the ultrasonic nebulizer (USN) for 1 min, then the hair sample was ablated (to evaluate the results) and an additional ablation of the hair sample together with nebulization of the spike solution was carried out. The change in isotope ratios was measured using mass spectrometry.

Table 3 compares the results for uranium content in the powdered hair standard obtained for LA-ICP-MS using different calibration modes and measured by ICP-QMS after digestion. Each result represents the average and standard deviation of measurements of three samples from the same batch. The detection limits for different analytical procedures were compared. The lowest detection limit for uranium determination (0.2 ng g^{-1}) was observed for ICP-MS analysis of digested hair samples. The detection limits for LA-ICP-MS were one order of magnitude higher than for ICP-MS of the digested samples. However, the analysis time and sample preparation of the laser ablation techniques were shorter then the solution-based analysis. In addition, the digestion procedure requires larger sample quantities. If the results from the digested samples are taken as the target value, then the solution-based calibration gives the closest value, although the results from all three laser ablation methods are within the standard deviation of the measurements.

3.2.4. Uranium isotope ratio measurements

Isotope ratio measurements of uranium were performed for the homemade standard. The standard was analyzed by ICP-MS, as a solution after digestion. Mass discrimination correction was made and the mass bias factor (assuming an exponential correction [41]) was determined using a laboratory-standard solution of uranium NBS-3164 (0.5 ng ml^{-1}) with a natural isotopic composition. The homemade powdered standard was used in later experiments as a natural isotopic composition standard for the isotopic determination along a single hair by LA-ICP-SFMS.

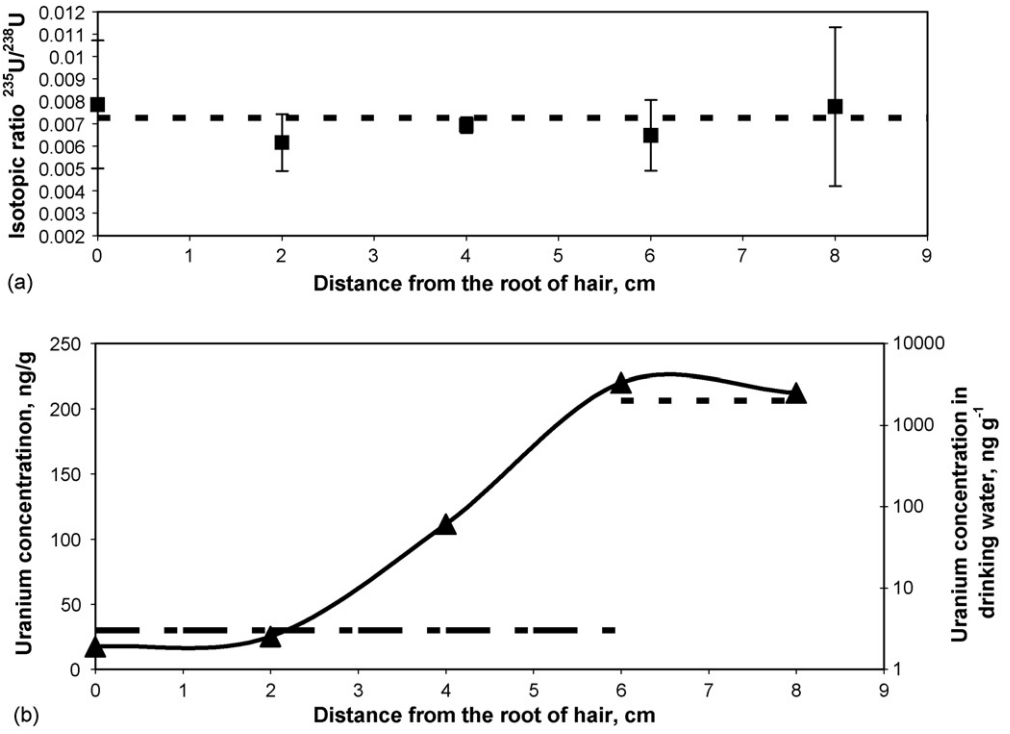

Fig. 6. (a) The $^{235}U/^{238}U$ isotope ratio measured along a single hair strand collected from an individual who changed residence area. (b) Changes in uranium concentrations along a single hair strand collected from an individual who changed residence area in relation to uranium content in drinking water.

3.3. Distribution measurements of uranium along a hair strand by LA-ICP-MS

The suitability of LA-ICPMS for the investigation of variations in concentration of uranium throughout the length of a hair strand was tested. For this purpose, the test subject was a person who changed living environments and who experienced a sharp decrease in the uranium content of drinking water from 2 to 0.035 $\mu g\,l^{-1}$. A single hair, including its root, was washed according to the procedure described above, and cut to 1 cm long sections. The pieces were placed on a carbon pad and each one was analyzed by LA-ICP-SFMS. The experiment was repeated for a few hair strands. Fig. 6b presents the amount of uranium in the hair (triangles and solid line) and its concentration in water (dotted lines) as a function of the distance from the scalp or the root of the hair. It was assumed that the hair growth rate is 1 cm per month, as reported by Lafleur [42]. By converting the distance from the scalp into time (hair closer to the scalp is newer), the transition from a high uranium concentration in drinking water to a low concentration can be clearly seen. After 2 months (6 cm from the scalp), the drinking water containing 2 $\mu g\,l^{-1}$ uranium was replaced by water with a low uranium content (0.035 $\mu g\,l^{-1}$) and the effect on the uranium hair content started almost immediately, reaching a stable low level after 4 months. The $^{235}U/^{238}U$ isotopic ratio in the hair and in drinking water was determined for the period of the experiment. The mass bias correction (assuming an exponential correction [41]) was determined using a natural isotopic composition homemade standard for single hair analysis by LA-ICP-SFMS and using a laboratory standard solution of uranium NBS-3164 (0.5 ng ml^{-1}) with a natural isotopic composition for the water investigated by ICP-MS [43]. The ratio along the hair was analyzed, and as shown in Fig. 6a, the isotopic composition did not change during this period and was natural. This indicates that the source of uranium in the hair was mainly in the drinking water.

4. Conclusions

Laser ablation ICPMS has been proven to be a very useful tool for direct measurements of trace elements in a single hair strand. The limit of detection for digested hair samples, as shown in Table 3, was better than the LOD obtained by the other laser ablation techniques. However, laser ablation has several advantages that make it a very unique and useful technique. First, the laser ablation technique does not require sample preparation (except for the removal of surface contamination). Second, the measurements are simple to perform and less time consuming than digestion and the analysis of liquid samples. Third, there is need for only one hair fiber for the analysis, and finally and most importantly, this approach makes it possible to conveniently track changes in concentration that occur over time. Thus, intake effects of toxic elements on the human body can be monitored using hair samples as a bioassay medium, even several weeks or months after the exposure. In addition, chronic exposure due to changes in dietary habits or environmental factors can be distinguished from acute, or accidental, exposure incidents.

The results show good agreement between ablated and digested hair samples for essential and toxic elements. This provides a new approach to biological monitoring by hair analysis,

since it makes the comparison of different components possible using the same technique for hair from the "normal" population, as well as special cases and exposed individuals. Thus, elements that differ by several orders of magnitude may be measured simultaneously.

A change in uranium along hair was also observed by LA-ICP-MS measurement. The change in uranium contents with the distance from the scalp correlates well with the intake of uranium in drinking water. It is important to note that the isotope ratio ($^{235}U/^{238}U$) was constant and natural [44], since the source was urban tap water. The results show natural isotopic ratio which is not surprising, as mentioned above, but the ability to measured isotopic ratio was demonstrated here. This ability opens a new way to isotopic forensics investigation and monitoring. Another important application of this powerful tool is supervision over time after exposure accidents.

Comparing different quantification strategies of laser ablation ICP-MS analysis, e.g., solution-based calibration, external standardization and standard addition, led to the conclusion that standard addition is the preferred calibration method for uranium determination in hair by laser ablation ICP-MS. This was proven for a "normal" homemade laboratory standard, where the uranium content was low. Therefore, we can assume that it is the best choice if a sensitive, easy and quick method is required.

The power to measure elements of different magnitudes in the same sample at the same time in a single hair fiber and the ability to derive time-resolved information lends this method great potential, and makes it a very strong tool in hair analysis, for biological monitoring in a variety of research fields. In view of these advantages, laser ablation ICP-MS has the potential to become the method of choice for hair analysis, as well as a routine research tool for the assessment of a "normal" and exposed population.

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