Available online at www.sciencedirect.com
ScienceDirect
International Journal of Mass Spectrometry 264 (2007) 175–180
International Journal of Mass Spectrometry
www.elsevier.com/locate/ijms

Imaging of elements in thin cross sections of human brain samples by LA-ICP-MS: A study on reproducibility

Myroslav V. Zoriy, J. Sabine Becker *

Central Division of Analytical Chemistry, Research Centre Jülich, D-52425 Jülich, Germany

Received 22 March 2007; accepted 13 April 2007
Available online 20 April 2007

Abstract

Laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) developed for the imaging of elements in thin cross sections of biological tissues was investigated with respect to its reproducibility. A commercial laser ablation system was coupled to a double-focusing sector field ICP-SFMS. Five neighboring sections from the same human brain tissue were cut to a thickness of 20 μm and scanned (raster area ∼1 cm^2) with a focused laser beam (wavelength 266 nm, diameter of laser crater 50 μm, and laser power density 1×10^9 W cm^{-2}). The obtained 2D images of adjacent sections were compared with each other. For all adjacent slices of human brain tissue, similar spatial distributions of the elements of interest (C, Cu and Zn) were found. The calibration of analytical data (e.g., Cu and Zn) was performed using a matrix-matched standard prepared synthetically. The reproducibility of the developed method was quantified as the relative standard deviation of the concentration of elements in the same regions in tissue analyzed for five adjacent sections. Using this approach, three different zones (A, B and C) were selected in the scanned sample. The following values were obtained for reproducibility in these zones: 5.4–6.5% for the $^{13}C^+$ measurements, 5.8–8.2% for Cu concentrations and 5.1–6.7% for Zn concentrations.

Keywords: Human brain; Imaging; Laser ablation inductively coupled plasma mass spectrometry; Reproducibility

1. Introduction

In recent few years, the quantitative imaging (mapping) of elements in different types of biological samples has grown to be a very challenging task in analytical chemistry [1–6]. The distribution profiles of essential elements such as Fe, Cu, Se, Zn, Mn, Mo, Co, Ni through the cross section of analyzed tissue (e.g., brain, liver, plant section, etc.) can reveal an unique information (e.g., unknown regions in the analyzed tissue with the enrichment/depletion of element of interest [2,7–9]) about many open questions in different areas of biological and medical research. Moreover, the determination of other toxic elements, such as Pb and U, could be of additional interest, because Pb and other divalent cations of heavy metals have been shown to impair calcium-channel proteins and affect neuronal axons and neurotransmitter release. All of these elements are involved in various biological processes, and their distribution and quantity has been analyzed using different kinds of tracer [4,5,10]. Thus, it is not only the concentration of elements in analyzed tissue, but also their precise spatial distribution (mapping or imaging) in organs, tissues and even single cells is of great importance [2,5].

At present, a number of analytical techniques are available for the imaging of elements in biological tissues [11–14]. Some of these techniques offer excellent spatial resolution but poor detection limits. However, for imaging studies of biological tissue, a powerful analytical technique with both good spatial resolution and high sensitivity is necessary. Commonly used methods in biological and medical research for the visualization of element distribution in tissues are histochemical staining techniques [15], which however do not provide multi-element capability. Other surface analytical techniques, such as scanning electron microscopy with energy dispersive X-ray analysis (SEM-EDX) [11], proton-induced X-ray emission (PIXE) [12] or autoradiography [16] are generally not sensitive enough for trace analysis. Using secondary ion mass spectrometry (SIMS), it is possible to produce ion images of the distribution of chemical elements in tissue with a spatial resolution in the low μm and sub-μm range, but quantification is difficult due to inherent matrix effects of up to six orders of magnitude [17–19].

* Corresponding author. Tel.: +49 2461 612698; fax: +49 2461 612560.
E-mail address: s.becker@fz-juelich.de (J.S. Becker).

1387-3806/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijms.2007.04.009

Application of laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) for the imaging of elements possesses several advantages compared to the other surface analytical techniques, due to its high sensitivity, minor matrix effects (compared to SIMS), absence of or only simple sample preparation, as well as easy quantification procedures [20–23]. When no suitable standard reference material is available, several strategies can be applied for quantification purposes in LA-ICP-MS, including a preparation of matrix-matched laboratory standards [8,21] or the use of solution-based calibration [24,25]. Recently, we used LA-ICP-MS for the imaging of elements (such as P, S, Cu, Zn, Th and U) in microtome sections of rat [9] and human brains [7,8]. The mass spectrometric analysis yielded an inhomogeneous, site-specific distribution for P, S, Cu, and Zn in 20 μm thin brain sections of the human brain's hippocampus or in tumor infected regions, suggesting that these elements have a physiological role. In contrast, Th and U were more homogeneously distributed at a low-ng g^{-1} concentration level. Furthermore, LA-ICP-MS was extensively used to determine the concentration of elements in selected protein spots derived from 2D gel electrophoresis [4,26,27]. Hutchinson et al. [28] used LA-ICP-MS for the qualitative imaging of β-amyloid protein in the brain of a transgenic mouse model of Alzheimer's disease. A correlation between the Aβ deposits and the concentration of trace elements was found.

However, at present, in the literature exist only a few numbers of works, devoted to the rigorous study of the figures of merits of the imaging LA-ICP-MS procedure. For example, Feldmann et al. [6], investigated the different laser ablation cell geometries to find a cell design with a constantly high signal intensity across the cell together with short wash-out times. A cell design with a volume of about 11.3 cm^3 was found to be optimum and its geometry was adapted for the analysis of membranes with the separated by SDS-PAGE proteins.

The aim of the present work is to study the reproducibility of the recently developed [7–9] imaging LA-ICP-MS method for the analysis of thin cross sections of biological tissues. For this purpose, several adjacent sections of a thickness of 20 μm were cut from the same tissue and screened to compare the distribution of the elements of interest (e.g., C, Cu and Zn) and to find out how reproducible these profiles are within the neighboring sections. This study on reproducibility of imaging mass spectrometry of elements in thin cross sections of human brain was performed as further investigation of LA-ICP-MS technique established recently in our lab [7].

2. Experimental

2.1. Instrumentation

A double focusing sector field ICP-MS (ICP-SFMS, ELEMENT, Thermo Electron Corporation, Bremen, Germany) coupled to a laser ablation system CETAC LSX 200 (Cetac Technologies, Inc., Omaha, NE, USA) was used to produce images of C, Cu and Zn distributions in thin adjacent cross sections of brain samples (thickness of slices −20 μm). The ablation of biological tissue was performed using an Nd:YAG laser (wavelength: 266 nm, repetition frequency: 20 Hz, spot diameter: 50 μm; laser power density: 1×10^9 W cm^{-2}). Schematic arrangement of the instrumentation used is presented in Fig. 1. The ablated material was transported by argon as a carrier gas into the inductively coupled plasma (ICP). The ions formed in the ICP were extracted in the sector field mass spectrometer and separated according to their mass-to-charge ratios. The ICP torch was shielded with a grounded platinum electrode (GuardElectrode, Thermo Electron Corporation). All measurements were performed in the low mass resolution mode of ICP-SFMS. All polyatomic ions interferences (containing, e.g., Na, Ca and Mg) were carefully checked in pre-investigation study of the samples and ensured that their influence can be negated due to the relatively high signals intensities of the analytes. Further details about the instrumentation used can be found elsewhere [7,24,29,30].

2.2. Samples and sample preparation

All of the samples analyzed were 20 μm thick and all were neighboring sections. These serial sections were prepared from

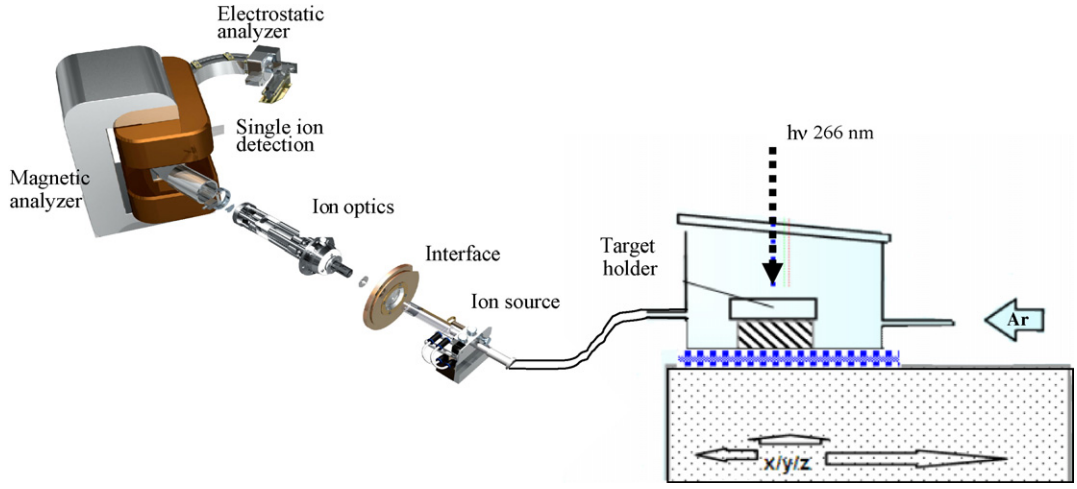

Fig. 1. Schematic arrangement of LA-ICP-MS for the imaging of element distribution in thin neighboring sections of human brain samples.

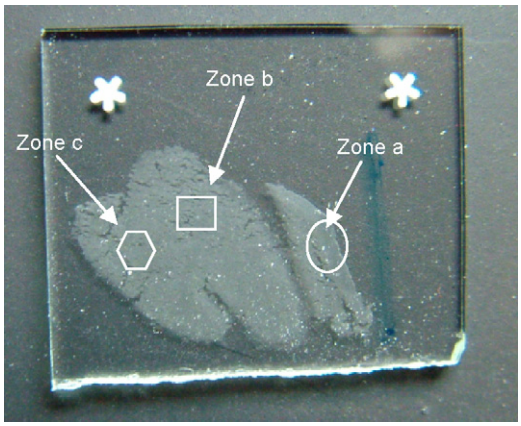

Fig. 2. Photograph of one of the analyzed sections with the zones of interest marked.

the same human brain tissue using a Leica 3010 cryostat microtome at −20 °C. The size of the samples was approximately 3.6 cm^2. Following sectioning, the tissue was mounted to sodium-free glass slides, air-dried and stored. As an example, in Fig. 2, the photo image of one of the analyzed sections is shown. Prepared in this manner adjacent sections were further considered to have the "quasi-same" lateral distribution of elements of interest and were used to determine the reproducibility of the developed imaging LA-ICP-MS method.

2.3. Preparation of laboratory standards for LA-ICP-MS measurements of brain samples

Matrix-matched laboratory standards with well-defined concentrations of the elements of interest were prepared for the calibration of analytical data. For this purpose, three slices of the same brain tissue (each ∼0.65 g) were spiked with 100 μL of standard solutions containing known concentrations of selected elements (Cu, Zn). The final concentrations in the brain tissue after spiking were 10, 5, and 1 μg g^{-1} for Cu and Zn. The fourth brain slice was spiked with 100 μL of 2% HNO3 and was used for blank correction. All brain samples were carefully homogenized and centrifuged for 5 min. Samples were then frozen at a temperature of −50 °C. Frozen matrix-matched synthetic laboratory standards of human brain tissues from the hippocampus were cut into sections 20 μm in thickness and placed onto the glass substrate.

2.4. Measurement procedure

The experimental parameters of LA-ICP-MS were optimized with respect to the maximum ion intensity of $^{63}Cu^+$ using the synthetically prepared laboratory standard with a concentration of copper of 5 μg g^{-1}. The maximum ion intensity was observed at a carrier gas flow rate of 1.2 L min^{-1} that transports the ablated material to the ICP-MS. Further optimized experimental parameters for the analytical method are summarized in Table 1. In order to study the reproducibility of the method, under these conditions thin brain tissue sections were investigated by LA-ICP-MS with respect to the spatial distribution of C, Cu and Zn.

Table 1
Optimized operating conditions for LA-ICP-MS procedure developed for the determination of the lateral distribution of C, Cu and Zn in thin adjacent sections of human brain tissue

	LA-ICP-SFMS
Laser ablation system	CETAC, LSX-200
Wavelength of Nd-YAG laser (nm)	266
Laser power density (W cm^{-2})	1×10^9
Laser scan speed (μm s^{-1})	40
Number of lines per analyzed sample	150
Repetition frequency (Hz)	20
Laser beam diameter (μm)	50
Inductively coupled plasma mass spectrometer	Element (Finnigan)
RF power (W)	1200
Cooling gas flow rate (L min^{-1})	18
Auxiliary gas flow rate (L min^{-1})	0.65
Nebulizer gas flow rate (L min^{-1})	1.2
Sampler cone	Nickel, 1.1 mm orifice diameter
Skimmer cone	Nickel, 0.9 mm orifice diameter
Mass resolution, m/Δm	300
Mass window (%)	10
Runs	12,000–14,000
Passes	1
Scanning mode	Peak hopping
Analysis time (h)	5–6

To obtain two-dimensional images of element distribution, the region of interest was systematically screened (line by line). The spot size of laser craters was 50 μm. In total, about 50 lines were scanned through the each of analyzed cross sections. ICP-MS data files were converted into *txt* file and were used to produce 2D images of element distribution. The images were plotted using a programming script in MATLAB® 6.5 computing software. Further details about the measurements procedure used can be found elsewhere [8,30,31].

3. Results and discussion

3.1. Two-dimensional mapping of adjacent brain tissue sections

Five adjacent sections of the same human brain tissue were scanned using developed mapping LA-ICP-MS procedure in order to determine the reproducibility of the method. In total, 50 lines through the each of the analyzed sections were performed, so the time needed for one successful measurement was about 6 h. The measured ion intensity of selected elements *versus* time was further used to produce their two dimensional distributions. The set of figures obtained for C, Cu and Zn distributions through the analyzed sections is shown in Fig. 3a–c, respectively. Generally, in all of these sections, similar layered structures were found for each of the analyzed elements, along with the same localization of defined regions enriched or depleted with the element of interest. In contrast to this, the spatial distributions for different elements within one section present different patterns. For example, in zone A, a relatively low concentration of Cu was observed, while the

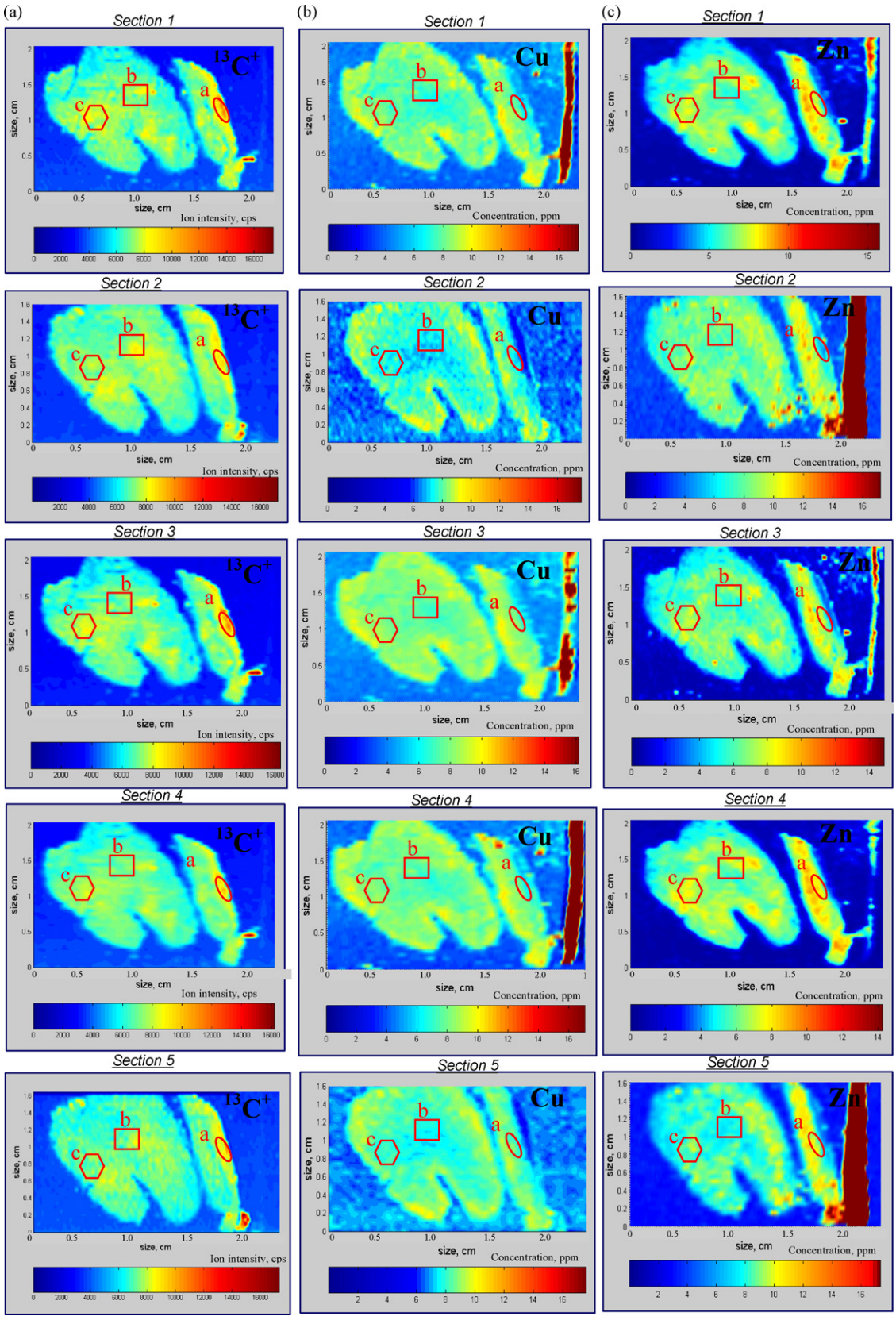

Fig. 3. Spatial distribution of elements of interest ($^{13}C^+$, Cu and Zn) in five adjacent sections from the same human brain tissue with the zones of interest marked.

carbon ion intensity in this region was found to be in some extent.

3.2. Quantification of analytical data measured by LA-ICP-MS

Using the synthetically prepared matrix-matched laboratory standards with concentrations of the element of interest of 1, 5 and 10 μg g^{-1}, the distribution profiles of Cu and Zn in the adjacent sections of human brain tissue measured by LA-ICP-MS were quantified (see Fig. 3b and c, respectively). The observed concentrations of Cu in the analyzed sample were in the range of 8–12 ppm, while the Zn concentrations ranged from 6 to 10 ppm. The concentration of carbon was not evaluated due to the difficulty of the preparing a suitable standard. Therefore, only its ion distribution profiles are presented in the current study.

3.3. Determination of reproducibility of the method

In order to qualitatively evaluate the reproducibility of the developed LA-ICP-MS method, three different zones (A, B and C) were selected in the analyzed sample (see Fig. 2). In all of these zones, an average was determined for the elements measured and their relative standard deviations for the five adjacent sections were further defined as reproducibility *R* of the method. In Fig. 4a–c, the calculated reproducibility for $^{13}C^+$, Cu and Zn, respectively, are presented. Due to the lack of a suitable carbon standard, its reproducibility was evaluated using the measured ion intensity. For the Cu and Zn calculations, the determined concentration was applied. The values obtained for the reproducibility in the three selected zones were in the range of 5.4–6.5% for the $^{13}C^+$ measurements, while for the Cu and Zn concentrations, the reproducibility's in the range of 5.8–8.2% and 5.1–6.7%, respectively, were found.

It should also be mention here that the determined reproducibility values may not correspond to the true reproducibility of the method due to the "quasi-similarity" of the measured samples. The thickness of the analyzed sections was 20 μm, which means that there could be small differences in the concentrations of defined elements even in between such neighboring sections, and would, therefore, affect the reproducibility of the method.

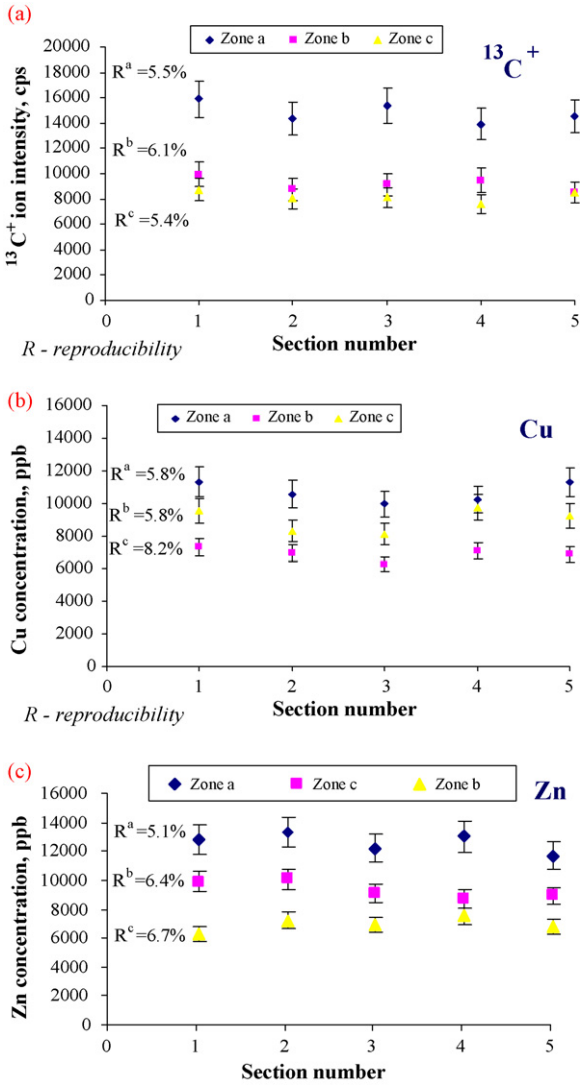

Fig. 4. Determined reproducibility for the developed method, calculated as the relative standard deviation of the average element concentration in three selected (R^a, R^b and R^c) zones for the five adjacent sections analyzed.

In contrast to this, the reproducibility of the imaging LA-ICP-MS method was studied on the synthetically prepared matrix-matched brain standards, where the distributions of the elements of interest (e.g. Cu and Zn) were considered to be

Table 2
LA-ICP-MS ion intensities of $^{63}Cu^+$ and $^{64}Zn^+$ measured in 6 different regions (average from three neighbouring lines, length about 400 μm) and their dependencies on the laser beam diameter measured on homogeneous prepared matrix-matched brain standard

	$^{63}Cu^+$ ion intensity [a] (10^4 cps)					$^{64}Zn^+$ ion intensity [a] (10^4 cps)				
	35 μm[a]	50 μm[a]	70 μm[a]	90 μm[a]	110 μm[a]	35 μm[a]	50 μm[a]	70 μm[a]	90 μm[a]	110 μm[a]
1	6.3	11.4	17.9	34.5	47.1	5.2	8.2	14.1	25.7	36.2
2	6.1	11.9	18.8	35.1	51.2	5.2	7.9	14.3	26.5	35.9
3	5.7	12.0	18.5	34.9	48.7	4.9	8.4	14.8	25.5	34.2
4	6.3	12.5	18.0	33.8	49.9	4.9	8.2	14.3	25.4	35.3
5	6.3	11.9	17.8	35.4	48.9	5.3	8.2	14.9	26.5	36.9
6	6.3	12.0	18.7	32.3	48.8	5.3	8.2	13.9	26.8	35.6
R.S.D. (%)	3.5	2.9	2.2	3.3	2.7	3.1	2.2	2.6	2.4	2.6

[a] Laser beam diameter.

homogeneous. The results for the $^{63}Cu^{+}$ and $^{64}Zn^{+}$ ion intensities measured in 6 different places within such homogeneous laboratory brain standard (concentrations of Cu and Zn were 10 mg g^{-1}) with the dependence on the laser beam diameter are summarized in Table 2. The intensities presented are the average of three neighboring lines, each with a length of 400 μm. Reproducibility's obtained in these measurements were found to be in the range of 2.2–3.5% and 2.5–3.1%, and were dependent on the laser beam diameter used.

4. Conclusions

LA-ICP-MS represents a powerful analytical tool permitting quantitative two-dimensional screening (mapping) of C, Cu and Zn in thin sections of biological tissues. Using the developed procedure, five adjacent thin sections of human brain tissue (thickness: 20 μm) were analyzed in order to determine the reproducibility of the method. In all of the measured sections, inhomogeneous distributions (layered structure) of the elements of interest (C, Cu and Zn) were found. The distributions obtained were similar for all neighboring sections. The reproducibility of the method was determined for three different zones in the analyzed tissue. The reproducibility values calculated were in the range of 5–8% and were dependent on the element analyzed proving that the developed LA-ICP-MS screening method is sufficient reliable. However, in this case a "quasi-similarity" between the samples measured should be taken into account. The reproducibility on homogeneous synthetic standard varied between 2.2 to 3.5%.

Acknowledgements

The authors are grateful to Prof. Dr. K. Zilles (Institute of Medicine, Research Centre Jülich) for support of this work and Dr. M. Dehnhardt for providing of brain samples.

References

[1] S.F. Durrant, N.I. Ward, J. Anal. At. Spectrom. 20 (2005) 821.
[2] J. Feldmann, A. Kindness, P. Ek, J. Anal. At. Spectrom. 17 (2002) 813.
[3] T. Prohaska, C. Latkoczy, G. Schultheis, M. Teschler-Nicola, G. Stingeder, J. Anal. At. Spectrom. 17 (2002) 887.
[4] J.S. Becker, M.V. Zoriy, C. Pickhardt, M. Przybylski, J.S. Becker, Int. J. Mass. Spectrom. 242 (2005) 135.
[5] J.S. Becker, J. Anal. At. Spectrom. 20 (2005) 1173.
[6] I. Feldmann, K.U. Koehler, P.H. Roos, N. Jakubowski, J. Anal. At. Spectrom. 21 (2006) 1006.
[7] M.V. Zoriy, M. Dehnhardt, G. Reifenberger, K. Zilles, J.S. Becker, Int. J. Mass. Spectrom. 257 (2006) 27.
[8] J.S. Becker, M.V. Zoriy, C. Pickhardt, N. Palomero-Gallagher, K. Zilles, Anal. Chem. 77 (2005) 3208.
[9] J.S. Becker, M.V. Zoriy, C. Pickhardt, M. Dehnhardt, K. Zilles, J. Anal. At. Spectrom. 20 (2005) 912.
[10] J.T. Elliston, S.E. Glover, R.H. Filby, J. Radioanal. Nucl. Chem. 263 (2005) 301.
[11] V. Mizuriha, H. Hasegawa, M. Notoya, Acta. Histochem. 30 (1997) 3125.
[12] J. Mesjasz-Przybylowicz, W.J. Przybylowicz, Nucl. Instr. Method. Phys. Res. B. 189 (2002) 470.
[13] P.J. Todd, T.G. Schaaf, P. Chaurand, R.M. Caprioli, J. Mass. Spectrom. 36 (2001) 355.
[14] S.L. Luxembourg, T.H. Mize, L.A. McDonnell, R.M.A. Heeren, Anal. Chem. 76 (2004) 5339.
[15] A. Bauer, K.J. Langen, H.J. Bidmon, M.H. Holschbach, S. Weber, R.A. Olson, H.H. Coenen, K. Zilles, J. Nucl. Med 46 (2005) 450.
[16] A. Takeda, H. Tamano, S. Enomoto, N. Oku, Cancer Res. 61 (2001) 5065.
[17] S. Chandra, Appl Surf Sci 679 (2003) 203.
[18] G.H. Morrison, I. Gay, S. Chandra, Scanning Microsc. 8 (1994) 359.
[19] D. Touboul, F. Halgand, A. Brunelle, R. Kersting, E. Tallarek, B. Hagenhoff, O. Laprevote, Anal. Chem. 76 (2004) 1550.
[20] J.S. Becker, J. Anal. At. Spectrom. 17 (2002) 1172.
[21] J.S. Becker, Spectrochim. Acta B 57 (2002) 1805.
[22] D. Guenther, H. Cousin, B. Magyar, I. Leopold, Int. J. Mass. Spectrom. 12 (1997) 165.
[23] D. Guenther, B. Hattendorf, Tr. Anal. Chem. 34 (2005) 255.
[24] C. Pickhardt, J.S. Becker, H.-J. Dietze, Fresenius J. Anal. Chem. 286 (2000) 173.
[25] S.F. Boulyga, C. Pickhardt, J.S. Becker, At. Spectr. 25 (2004) 53.
[26] J.S. Becker, M.V. Zoriy, J.S. Becker, C. Pickhardt, M. Przybylski, J. Anal. At. Spectrom. 19 (2003) 149.
[27] N. Jakubowski, R. Lobinski, L. Moens, J. Anal. At. Spectrom. 19 (2004) 1.
[28] R. Hutchinson, A.G. Cox, C.W. McLeod, P. Marshall, A. Harper, E. Dawson, D. Howlett, Anal. Biochem. 346 (2005) 225.
[29] J.S. Becker, H.-J. Dietze, Spectrochim. Acta B 53 (1998) 14751506.
[30] M.V. Zoriy, M. Kayser, A.V. Izmer, C. Pickhardt, J.S. Becker, Int. J. Mass. Spectrom. 242 (2005) 297.
[31] J.S. Becker, M.V. Zoriy, M. Dehnhardt, C. Pickhardt, K. Zilles, J. Anal. At. Spectrom. 20 (2005) 912;
J.S. Becker, A. Gorbunoff, M.V. Zoriy, A.V. Izmer, M. Kayser, J. Anal. At. Spectrom. 21 (2006) 19.