International Journal of Mass Spectrometry 307 (2011) 142–148

Contents lists available at ScienceDirect

International Journal of Mass Spectrometry

journal homepage: www.elsevier.com/locate/ijms

Preparation of stable standards of biological tissues for laser ablation analysis

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ARTICLE INFO

Article history:
Received 28 November 2010
Received in revised form 18 January 2011
Accepted 18 January 2011
Available online 26 January 2011

Keywords:
Biological samples
Sol–gel matrix
Laser ablation ICP-MS
Trace elements
Standards

ABSTRACT

A novel method for preparation of durable standards of biological samples for quantitative analysis by laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS) has been developed. Rat brain tissue samples were ground and then spiked by aqueous solutions containing controlled amounts of trace metals of interest. The spiked rat brain samples were then encapsulated in a sol–gel matrix produced by tetraethyl orthosilicate (TEOS). The spiked standards were prepared by addition of 0–50 $\mu g\,g^{-1}$ of eight elements (Li, Co, Cr, Cu, Fe, Mn, Pb and Zn) to the biological samples. The correlation coefficients of the calibration curves for all elements tested were better than 0.97. Carbon-13, found in all biological samples, was used as an internal standard to correct for variations in the amount of material ablated and carried to the ICP-MS plasma torch, a common problem in LA-ICP-MS analysis. The results of analysis of the standards showed very good repeatability and homogeneity as well as long room temperature shelf life.

1. Introduction

Laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) is a powerful tool for *in situ* analysis of trace elements in a wide variety of solid samples such as ceramic materials, metals, glass and solid biological media like teeth enamel, hair and bones. This method allows qualitative, semi-quantitative and quantitative analyses as well as isotopic ratio measurements and requires very little sample preparation. The LA-ICP-MS technique can be applied to analyze the elemental and isotopic composition of a small spot on a solid sample or to image a bulk sample with a resolution better than 100 μm. The apparent advantages of this technique are the variability of sample materials that can be examined, the fact that sample preparation is minimal and the reduction in the level of interferences from polyatomic ions. In addition, separate optimization of the sampling and ionization processes [1] allows for more degrees of freedom in the calibration and enables fine-tuning of the laser ablation and ICPMS system However, carrying out quantitative analysis by LA-ICP-MS requires a rather complex calibration procedure. The main reasons are the inconsistent relationship between the signal response in the ICP-MS and the concentration of the analytes in the sample due to non-stoichiometric sampling during the ablation process [2,3] and the incomplete vaporization and ionization of large particles (>125 nm) in the plasma [4–6].

There are two approaches commonly used to deal with these problems: use of an internal standard (IS) and calibration with matrix matched standards. The internal standard compensates for different ablation yields from both the sample and the standard and enables the preparation of a calibration curve. IS also compensates for the shot-to-shot variation and therefore improves the precision of the measurement [7]. Carbon-13 has been used as an internal standard of laser ablation analysis in different applications [8–12]. For example, variations were observed in the intensity of ^{13}C signals in sections of the sample that had different thickness, but when the signal was normalized to the thickness a good linear correlation was found [9–11]. Due to the sensitivity and response, carbon-13 has been used to image the carbon distribution in biological samples with good resolution, and as an internal standard for the distribution of other elements [9–11]. Thus, carbon-13 is suitable and commonly used as an internal standard in laser ablation analysis of biological samples. Matrix matched standards for calibration techniques, if available, can provide a correction for the signal response disparity between different elements. These standards are usually a mixture of an appropriate matrix or binder material and the analytes, but their preparation is time-consuming and in some cases such a sample matrix may not be available.

There are several commercial certified reference materials (CRMs) for different types of solid matrices (glass, ceramic, cement, metals, etc.) but the range of matrices available does not cover every

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doi:10.1016/j.ijms.2011.01.022

type of sample (e.g., biological tissue and polymers) [13]. The most common CRM for use in laser ablation quantitative analysis are the silicate CRMs made by NIST. These standards are used in LA-ICP-MS studies for calibration of the measurement system in dry plasma condition [14], but, as mentioned above, the composition of the matrix may affect the signal intensity differently for each given element. Therefore those standards are not suitable for other types of samples when quantitative information is required. For this reason, several LA-ICP-MS laboratories now synthesize their own standards. One of the simplest approaches that has been proposed to resolve the calibration problems was on-line addition of aqueous standards for calibration LA-ICP-MS [15–17]. The application of this technique opened a path to practicable studies that use this method of calibration and offer their own variations to it [7,18–21]. A new approach was adopted during the last few years with the aim of developing solid matrix matched standards. Ideally, such an approach will provide an internal standard to correct for the shot-to-shot variability as well as a matrix matched standard to correct for the signal response discrimination.

There are a few problems with these homemade standards. First, homogeneity, that is very important, is hard to achieve, especially when working with solid or semi-solid matrices as is the case for biological samples. Second, it is hard to find bulk material, in a pure state that does not contain unknown amounts of the analytes. Third, frequently the amount of bulk material is limited, like in the case of biological tissues [1].

Several techniques are reported for homemade standards for laser ablation analysis. In the field of geochemistry, for example, measurement of the abundances and distribution of trace metals in natural sulfide minerals has been investigated due to the economic and environmental importance of these elements [22]. Preparation of reliable standards for this kind of analysis is preformed using different methods. Among the techniques used one can find preparation of powdered standards [22], purification of sulfide salts [23] and melting followed by spiking of the matrix [13]. LA-ICP-MS analysis has also been applied for age determination of rocks, soil samples, fossils and trees and the variation of the homemade standards for these applications is wide. One study reported the use of different tree rings as matrix matched standards where width of the ring stands for the climate conditions and the chemical composition refers to the soil nutrients conditions [24]. In other strategies a spike solution is added to a powdered sample, then the solution is allowed to evaporate, and is blended and homogenized and then the powder is pressed to pellets [25]. Yet another method is to spray picoliter-droplets generated by inkjet printers and use the dry residual spots as a reference [26].

In recent years, the interest in LA-ICP-MS analysis of biological specimens, particularly for the measurement of trace metal concentration and distribution, has increased significantly. Some trace metals are considered as essential elements and are vital for many organisms. However, deficiency or excess may be indicative of pathological conditions, while high concentrations of toxic elements may cause disease.

Laser ablation combined with ICP-MS can provide information with high resolution on the concentration of metal elements in single cells. In addition, it can yield spatially resolved concentration maps of trace elements in cells or tissue samples, which can be very useful [27,9,28,29]. The use of LA-ICP-MS for quantitative evaluation of biological and medical samples requires suitable quantification procedures [27,30,31,1]. Strategies developed for quantification include; preparation of matrix matched laboratory standards, solution-based calibration [32–34], calibration using certified reference materials (CRMs) [35–37] and film coating of samples. However, each of these methods has its advantages and drawbacks.

Solution-based LA-ICP-MS calibration for biological samples is easily carried out but does not account for variations that may occur during the laser sampling process [34]. Additionally, in studies of silicate samples it has been found that combined sample introduction procedures have some restrictions. First, it requires careful gas flow optimization; second, applying this analysis strategy requires that all major element components are contained in the sample. Third, unknown amounts of water, small amounts of chlorine or CO_2, and any major element not measurable by LA-ICP-MS, can contribute to the uncertainty in the measurement. Despite these drawbacks, it still can be used for the quantification of geological materials where all matrix elements are measurable and opens ways of quantifying samples of completely unknown composition [38].

Preparation of matrix-matched standards, or film coated standards, is time consuming and sometimes difficult to carry out because, as mentioned above, it is almost impossible to find certified, or very high purity, matrix materials. Moreover, the biggest problems of homemade matrix matched biological standards are to ensure sample homogeneity and the fact that biological material tend to spoil so the standards may have a short shelf life.

A sol–gel process is defined as "the preparation of ceramic materials by preparation of a sol, gelation of the sol, and the removal of the solvent" [39]. The technique makes use of the fact that certain SiO_2-liquids may be made to set very rapidly producing a stiff 'gel'. The structure of a pure SiO_2-gel consists of a 3D-network of Si–O linkage with a high porosity; the pores will be filled with the liquid used in the gel preparation, normally water and/or alcohol [28]. The use of sol–gel as matrix to produce sturdy stable standards is a developing area of research [40–43].

The aim of this work is to present a procedure for preparation of rat brain standards, based on the sol–gel procedure, to be used in LA-ICP-MS analysis. These standards serve as a model for homogenized, stable biological standards with a long shelf life. The preparation procedure of these biological standards and their characterization will be described in this article. Due to the lack of certified biological standards the validation of these standards is based on their measured homogeneity and the response of the signal intensity to the known amount of added spike materials.

2. Material and methods

2.1. Instrumentation

A quadrupole-based ICP-MS (ICP-QMS, ELAN DRC-e, Perkin-Elmer Sciex) coupled with the LSX-213 commercial laser ablation system (CETAC LSX-213, CETAC Technologies, Inc., Omaha, NE, USA) was used for the analysis of sol–gel rat brain standards. Mass spectrometric measurements were carried out for the following essential and toxic elements: Li, Cu, Fe, Mn, Zn, Co, Pb, and Cr. Some of these elements are present at trace levels in rat brain. The analyses were carried out at the Geological Survey of Israel (GSI), Jerusalem.

In order to apply calibration curves as a quantification technique, sol–gel capsules consisting of ground rat brains were prepared. Carbon (the isotope ^{13}C was used), which is a matrix element in brain tissues, was selected as the internal standard element. Brain cells contain a high amount of carbon, the basic building material of biological matter. Therefore, it was quite easy to optimize the experimental parameters to obtain maximum $^{13}C^+$ ion intensity for quantitative measurements and the parameters were tuned to obtain optimal signal intensities of the trace metals we were interested in. The optimized experimental parameters are summarized in Table 1.

Table 1
Optimized operating parameters of the experimental systems.

ICP-MS	
Perkin-Elmer ELAN DRC-e	
Plasma conditions	
Incident RF power	1150 W
Cooling gas flow rate	Ar 10.0 L min^{-1}
Auxiliary gas flow rate	Ar 0.95 L min^{-1}
Nebulizer gas flow rate	Ar 0.70 L min^{-1}
Detection mode	Dual
Auto lens	On
Scanning mode	Peak hopping
Laser ablation	
Laser ablation parameters	
Laser	Nd:YAG
Wavelength	213 nm
Repetition rate	20 Hz
Energy	750 V
Spot size	25 μm
Scan rate	40 μm s^{-1}
Carrier gas flow rate L min^{-1}	0.4 L min^{-1}

2.2. Test animals

Male Sprague-Dawley rats weighing 200–250 g were habituated to housing conditions for at least seven days, housed four/cage in a vivarium with stable temperature and a reversed 12-h light/dark cycle, with unlimited access to food and water. Animals were handled once daily.

2.3. Preparation of brain samples

Whole Brain tissue was first chopped by a Teflon knife and then ground in an agate shatter box to obtain a homogenized sample.

Li, Cu, Fe, Mn, Zn, Co, Pb, and Cr aqueous solutions were added from 1000 mg mL^{-1} standard solution (ICP IV Multi-element, Merck). Multi-element solutions were prepared and mixed with 0.1 g processed brain tissue to get 0, 10, 20, 40, 50 μg g^{-1} nominal addition of each of the elements listed above. After the spiking, the homogenization process was repeated to get homogeneously spiked tissue samples.

2.4. Preparation of xerogels

The sol–gel process for the production of a silica glass begins with hydrolysis of silica alkyloxide and subsequent polymerization to particles (sols). The sols grow and aggregate to chains and 3D network structures that encase the fluids (gel). Addition of an acid or a base catalyzes the process and silica linkage is increased with the addition of a simple alcohol. The net reaction using tetraethyl orthosilicate (TEOS) [42] is: $C_8H_{20}SiO_4 + 2H_2O \leftrightarrow 4C_2H_5OH + SiO_2$.

The mixture of reagents used in the present work was nominally: TEOS:water:ethanol:HNO_3:HF molar ratio of 1:4:4:0.8:0.06.

Tetraethyl orthosilicate (TEOS, 99.999%, Aldrich) and ethanol (Analytical grade, Merck) were stirred together. After one hour, an aliquot of water and one drop of concentrated HNO_3 (69–70% super pure, J.T. Baker) were added and stirred for 15 min. Four drops of HF (40% super pure, Merck) concentrated were added and 10 min stirring was carried out. Deionized (>18.2 MΩ) water is used for all reagent preparations.

After preparation of the sol–gel solution and the spiked tissues samples, 0.1 g aliquot of the ground brain tissue was added to 1 mL of the TEOS–xerogel solution. The resulting solution is shaken gently by hand for 30 s and poured into molds. These molds are then covered with perforated parafilm and placed in a fume hood at room temperature. The extent of perforation of the parafilm affects the evaporation rate. The filled molds are considered ready when the resulting xerogels achieve a constant mass (<0.5% daily change in weight), typically after about one week.

3. Results and discussion

The brain tissue standards were prepared by the sol–gel technique described above with five different concentrations of the analytes: a blank (no addition of analytes) and spiking with 10, 20, 40 and 50 μg of analyte per gram of brain tissue. The signal intensity of eight analytes (^{7}Li, ^{63}Cu, ^{66}Zn, ^{208}Pb, ^{55}Mn, ^{59}Co, ^{57}Fe and ^{53}Cr) as well as that of ^{13}C were measured by LA-ICPMS with the operational parameters presented in Table 1.

Fig. 1 is a photograph of the pellets that were produced in the sol–gel process. The objective of the production process is to manufacture homogeneous and stable standards with a long shelf life. The homogeneity and the shape of each pellet were tested visually using a microscope, followed by LA-ICPMS investigation. A typical light microscope image of standard of sol–gel matrix matched standards is shown in Fig. 1(d–f). For comparison, images of bulk sol–gel pellets are shown in Fig. 1(a–c). The pellets were round and their texture was uniform. Comparison of Fig. 1(a–c) to Fig. 1(d–f) shows that the biological matter is concentrated at the top of the pellet. This is due to the fact that it is not fully liquefied in the sol–gel pro-

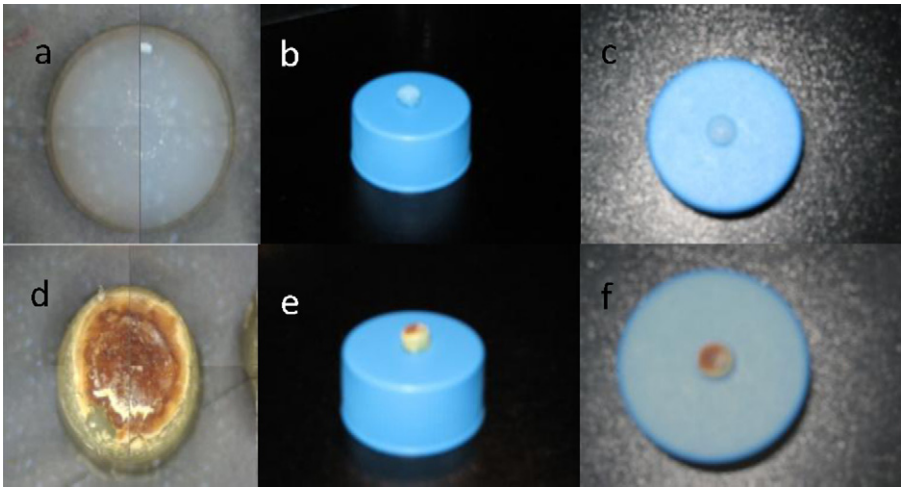

Fig. 1. Sol–gel brain standard and blank pellets. (a)–(c) are sol–gel blank pellets and (d)–(f) are pellets of brain tissue standards. (a) and (d) are close-up photos of the blank and the sample. The diameter of the sample is ~5 mm.

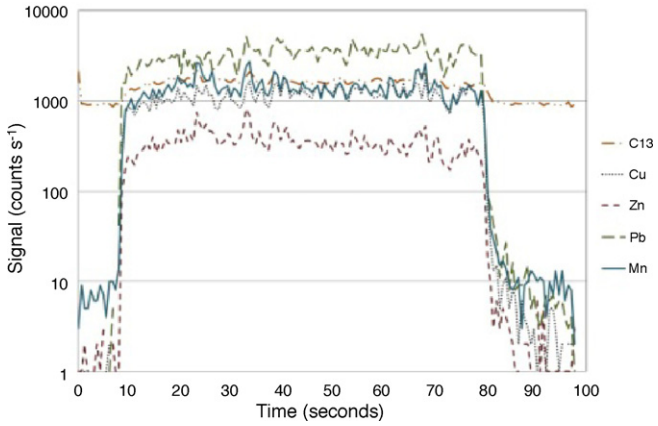

Fig. 2. The stability and homogeneity of the Carbon-13, copper, zinc, lead and manganese signals during a scan of ~2.5 mm at a rate of 40 μm/s of a sol–gel brain tissue pellet standard.

cess and its density is higher than that of the sol–gel bulk, hence, it sinks to the bottom of the production mold. Consequently, when it is removed from the mold the biological matter is centered at the top of the pellet.

The homogeneity of the analyte distribution in the brain tissue standards prepared using the sol–gel technique is presented in Fig. 2. Only a depth of a few microns were ablated from the standard pellet. Scans were performed for about 1 min at a scan rate of 40 μm/s so that a length of 2.4 mm was scanned across the standard pellet. The pellet diameter is about 5–6 mm so this scan is a good representation for the whole standard pellet. The stability of the signals of copper, zinc, lead and manganese, as well as that of ^{13}C that serves as an internal standard for normalization and correction of variations in the laser pulse, is evident. Note that the background signal level of the analytes is below 10 cps, while the carbon-13 background level is close to 1000 cps. Nevertheless, the signal intensity of the analytes during ablation of the sample is sufficiently above the background, and this has been used to create a full image of the sample [11].

The stability of the ^{13}C signal shows that the biological matter is uniformly distributed across the standard pellet since carbon is the main matrix component of the sample. All the other elements have been originally added to the brain tissue sample. The fact that the signal level of all the elements is stable across the sample indicates that all steps of standard production: the addition of elements solution, the homogenization of the tissues samples and the pellet preparation were successful.

There was a concern that during the preparation of the standards some of the spiking solution could migrate from the ground brain tissue and reach the sol–gel matrix. This would affect the concentration true value of the spike and distort the calculations.

Table 2
Results for the concentration (μg/g) of Cr, Co, Fe, Cu, Pb, Mn, Zn and Li in brain tissues: the digestion procedure and laser ablation calibration with sol–gel standards.

Element	LA-ICPMS concentration in the sol–gel std (μg/g)	ICPMS concentration in solution (μg/g)
Cr	1.8 ± 0.5	2.2 ± 0.5
Co	2.9 ± 2.8	0.08 + 0.01
Fe	12.7 ± 3.7	13.3 + 2.4
Cu	5.5 ± 1.5	14.2 + 5.4
Pb	<loq	0.59 + 0.55
Mn	1.2 ± 1.5	3.7 + 1.7
Zn	36.8 ± 4.1	12.1 + 4.6
Li	6.3 ± 3.5	3.8 + 0.5

We have several reasons to believe that this is not the case. First, the spiking solution is added to the brain tissue, homogenized and after an adsorption period the tissue is added to the sol–gel solution. Second, the observed linear response (see below) indicates that the spike solution was not transport in to the sol–gel matrix. Third, as part of the process of challenging the method we analyzed the sol–gel side of the standard pallet. Fig. 3 shows the intensity versus time response, of analysis of two standards, a blank brain and 50 ppm spiked standard that represent the concentration extremes of the method. The elements measured represent one element that exists in the brain at low levels, Mn, and an element that is present in the brain at a high level, Zn. In both cases, as seen in Fig. 3, there is no difference between the responses of the two standards. The three arguments described above, together with the measured data, clearly suggest that the spike solution is not transported to the sol–gel matrix.

Fig. 4 depicts the calibration curves for the eight analytes: ^{7}Li, ^{63}Cu, ^{66}Zn, ^{208}Pb, ^{55}Mn, ^{59}Co, ^{57}Fe and ^{53}Cr and Table 3 summarizes the limits of detection (LOD) and quantitation (LOQ) as well as the R^2 of the calibration curves for these elements. The data was derived from measurement of matrix matched biological standards prepared in this work. In general, for solid standards in LA-ICPMS studies, the sample must be ablated at several sites in order to give a representative response. In the present work each standard was ablated five times along a path of 2.4 mm. The intensity of the analytes ablation signal was divided by the intensity of the ^{13}C signal that served as an internal standard. The blank standard, sol–gel pellet containing rat brain tissue with no additional spike, was considered as the zero concentration point in the calibration curves. A weighted regression line was calculated using the calibration data for each analyte. Regression lines are presented for each element in Fig. 4. The intercepts of the calibration curves with the vertical axis in Fig. 4 represent the actual (natural) concentration of the analytes in the brain tissue. Thus, lead and manganese have an intercept close to zero, indicating that their concentration in the brain tissue is negligible, while the other elements are present in very small quantities in the brain tissue. Table 2 sum-

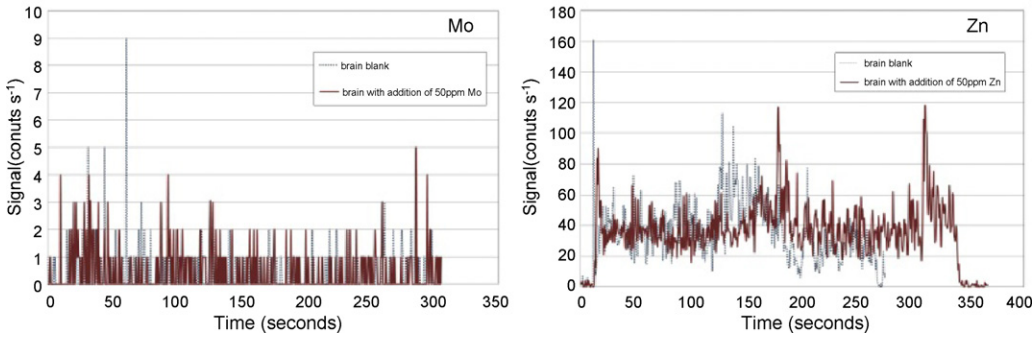

Fig. 3. Distribution of Zn and Mo signal intensities in the sol–gel area of the blank and the standard with highest addition (50 μg/g).

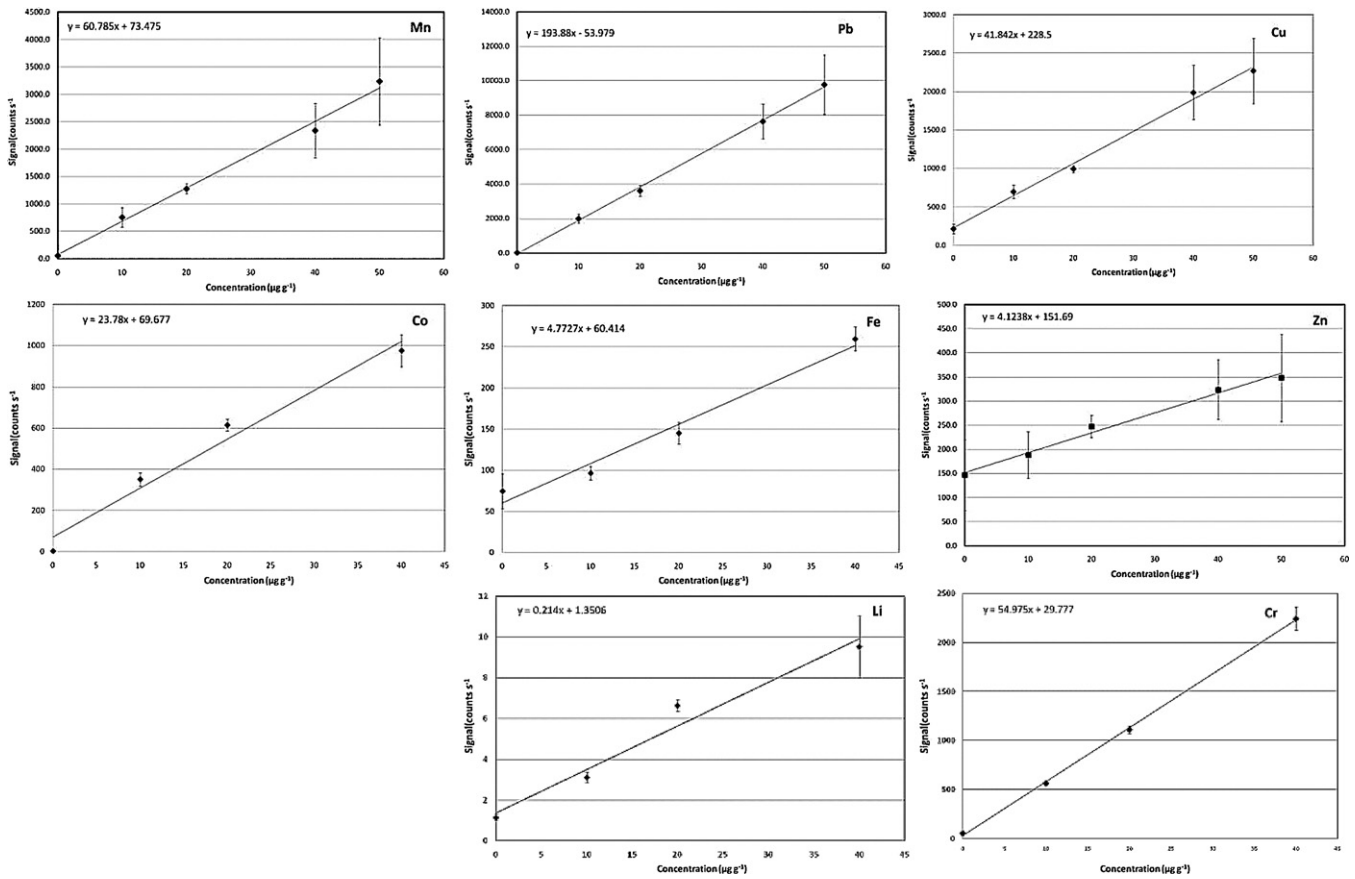

Fig. 4. Calibration curves, normalize to Carbon-13, obtained for the different elements from ablation of brain tissue sol–gel standards containing the original ground brain sample and the same with addition of 10, 20, 40 and 50 μg/g of manganese, lead, copper, cobalt, iron, zinc, lithium and chromium.

marizes the concentration found for the eight elements by the laser ablation technique, using the set of standards, after extrapolating each calibration curve to zero added analyte concentration. These results were compared with those obtained from complete digestion of brain tissue and using the ICPMS to measure the concentration of each element in the resultant liquid sample. In most cases the results are similar, within the statistical error, except for lead that was below the quantitative limits of detection, and for Cu and Zn for which there was a difference between the procedures. Both Cu and Zn are essential elements in the body and have relatively high concentrations in the brain. However, the distribution of these elements in the brain is not uniform. These two metals are concentrated in some parts of the brain in vesicles or organs. It is very difficult to separate these brain organs and since their dimensions are small it is hard to homogenize them in a mix of the whole brain. We believe that this is the reason to the results with different concentrations of Cu and Zn analysis. In support of this hypothesis note the similarity in the results for iron (Table 2). Iron is essential to the function of the brain and has a high concentration in the brain, but unlike zinc and copper it is uniformly distributed in the brain. As a result, for iron, both analytical techniques yield similar results while for Cu and Zn there are noticeable differences.

The validity of the sol–gel standards was examined in two levels. First, the limit of detection (LOD) of several trace elements was established (Table 3). The reported values, with exception of cobalt, are lower than those reported for the human body [44]. Since cobalt has very low levels in the body, 1.1 mg for all body, the LOD values obtained in this work are very reasonable, clearly indicating that the standards are suitable for analytical work with biological samples. The second parameter for testing the validity of the standards is the linearity of response. As shown in Fig. 4 and by the R^2 values in Table 3, the calibration curves for the eight elements in the sol–gel standards of rat brain tissues were very good.

Sample homogeneity can be expressed as the deviations between measurements in different scans of the same standard pellet expressed as the relative standard deviation (RSD) in percent. Fig. 5 depicts the RSD values for all measured trace elements added to the sol–gel standards. The calculated RSD values constitute a good indication of the homogeneity degree since it is assumed, based on the laser ablation spectrum of ^{13}C shown in Fig. 2, that the brain tissue is homogeneously distributed in the pellet. Typical variations of normalized raw intensity across line scans of the standard pellets were found to correspond to RSD of less than 10%. In this range, the values of the RSD on the high standards, 40 and 50 ppm spiked brain tissue, are higher than the lower ones, 10 and 20 ppm spiked brain tissue, possibly due to the difficulty in the homogenization of the biological samples. The highest variation across the tissue line scans was in the blank samples (without any addition of trace element) possibly due to low counting statistics in the analysis. It is therefore assumed that strict control over the preparation procedure of the standard pellets will improve the RSD and resolve this problem.

The method validation parameters are compatible with the analysis conditions, the tissue properties and shape. Therefore, when looking at published results of laser ablation-ICPMS imaging fields we have to compare with studies that were carried out on similar biological samples. In the literature there are many studies carried out using laser ablation, and laser ablation calibration techniques, but only few of them related to soft tissues where the parameters of the analytical method are given. In the work

Table 3
The limit of detection (LOD) and limit of quantification (LOQ) for different elements in ablation of brain tissue sol–gel standards (μg/g) and the correlation coefficient obtained for the calibration curves.

	^{7}LI	^{63}Cu	^{66}Zn	^{208}Pb	^{55}Mn	^{59}Co	^{57}Fe	^{53}Cr
LOD	1.6	2.7	11.7	0.05	0.8	0.1	8.9	2.8
LOQ	5.2	9.0	39.1	0.2	2.7	0.3	29.7	8.4
R^2	0.968	0.994	0.998	0.987	0.992	0.974	0.974	0.999

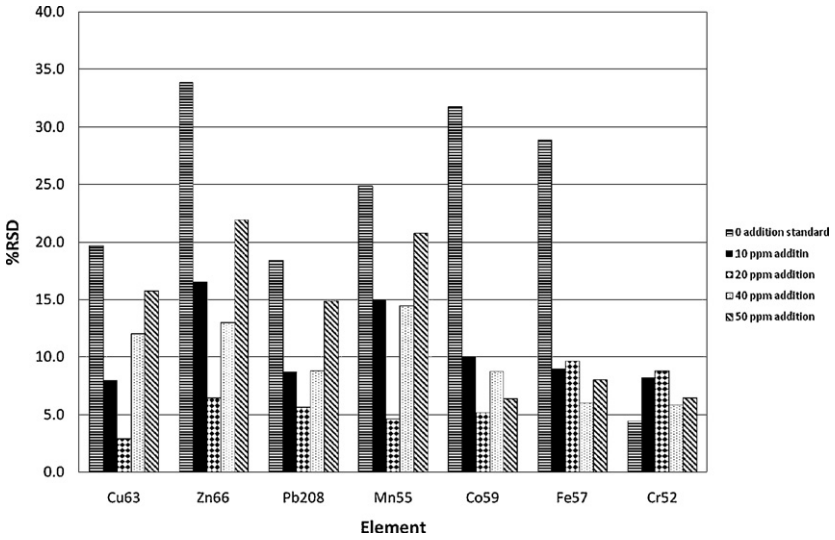

Fig. 5. Homogeneity of added elements in different preparations of the standards as relative standard deviation (%RSD).

reported in the literature we can find information on Cu, with LOD of 0.1–0.34 μg g^{-1}, Zn with LOD of 0.14–0.5 μg g^{-1} [28,45] and precision in the range of 20–30%. Toxic elements such as Pb, U and Th [28,46] were investigated and LOD values of 10 ng g^{-1} were given while for Pt the LOD was estimated to be 20 pg g^{-1} [47].

It is worth mentioning that as part of the method development we first tried to measure chromium at mass 52. The linearity of the standard calibration curve for this mass was good but the background level in the blank brain sample was very high (about 5000 cps). This high signal could probably be attributed to isobaric ion ^{40}Ar^{12}C, which is produced in the plasma when biological samples are analyzed. Due to this reason we chose to measure the element Cr at mass 53. In this mass there is also isobaric interference of ^{40}Ar^{13}C but since the abundance of ^{13}C is two orders of magnitude lower than that of ^{12}C, the background interference is much smaller.

4. Conclusions

A novel method for producing standards of biological materials for quantification by LA-ICPMS was developed and its validity was demonstrated. The calibration standards were prepared by a simple technique based on addition of a solution with a known and controlled amount of the analytes, mixed with homogenized rat brain tissue, into sol–gel matrix matched pellets. A variety of standards that contain known amounts of essential and toxic elements, that can be major, minor or trace elements, in standardized, homogenized, soft tissue samples were prepared. The chosen elements were among the most common ones found, or known, in trace amounts in biological soft tissues; however the method is applicable to any element available in an organic medium.

The requirements of standard materials for LA-ICP-MS analysis of biological samples have been defined before [1]. In the present work, five different standards with different additional concentration of eight trace elements were prepared. The use of ^{13}C as an internal standard, that is implicitly matrix matched, enables practical analysis to be performed and affords an easy way for making fine intensity corrections, in addition to correcting effects and variations in laser ablation efficiency.

Calibration curves that were obtained by the five standards show a good linear correlation. The precision of the results was good indicating a high level of homogeneity of the standards. Another advantage of these standards is their long shelf life that is due to the stabilization by the sol–gel matrix. We have observed at least three months stability (at room temperature) of the standards pellets between their preparation and until the present time when this manuscript is being written.

The data presented here demonstrates that sol–gel standards can be used in the analysis of biological matter by LA-ICPMS. Samples with different types of matrices, such as animal tissues or plants can be made practical utilizing this technique.

As discussed above, internal standard (IS) can be a powerful tool in the calibration of laser ablation data analysis. The ultimate internal standard in biological sample, based on tissue composition is ^{13}C, since carbon is the basic element in every living cell. We decided to combine matrix matched technique with internal standard procedure to get the best performance from the sol–gel standards. In the present case, one should bare in mind that brain tissue is inherently not a homogeneous organic material as the brain is divided into sections with different consistencies like porous parts and sections with white and gray matter, but the results shows that the homogenization procedure effectively overcomes this problem.

Acknowledgements

The authors would like to acknowledge the support from the Geological Survey of Israel (GSI), Jerusalem for letting us use their facilities. Special thank given to Dr. Ludwik Halicz for his help and support throughout the project.

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