ARTICLE



Qualitative Imaging of Elemental Spatial Distribution in Stalagmites through Laser Ablation Inductively Coupled Plasma Mass Spectrometry Analysis

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The laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) technique is considered versatile for multielemental analysis and imaging because it is easy to handle, compatible with different types of solid samples, requires minimal sample preparation, and provides high spatial resolution and sensitivity. One of the challenges of imaging analysis is to obtain accurate and precise spatial

information of elements distribution in the sample, so the optimization of the laser ablation (LA) parameters is essential. In this context, this study aimed to optimize the LA parameters for direct analysis of speleothem samples. Laser intensity, frequency, and spot diameter were evaluated through multivariate experimental design and multi-response data, the influence of ablation scan speed and the use of ⁴⁴Ca as an internal standard (IS) for the qualitative image of ⁶⁶Zn, ¹³⁷Ba, ⁵⁵Mn, ⁵⁷Fe, ⁸⁸Sr, ⁶⁰Ni, and ²⁶Mg distribution in the sample were also evaluated. The multivariate optimization revealed positive interactions between the parameters evaluated, i.e., the greater the laser intensity, LA frequency, and spot diameter, the greater the analyte signal and, thus the sensitivity. Therefore, 90% laser ablation intensity, 20 Hz repetition rate, and 100 µm spot diameter were selected. In the scan speed evaluation, the images obtained with 40 and 20 µm s⁻¹ were very similar for all isotopes. The use of ⁴⁴Ca as IS did not impact the resolution of the images. The use of ⁴⁴Ca can provide important information about the speleothem formation and paleoclimate changes.

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INTRODUCTION

Different techniques for elemental distribution imaging are available, such as laser-induced breakdown spectroscopy (LIBS),¹ X-ray fluorescence (XRF),² secondary ion mass spectrometry (SIMS),³ and laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS)⁴. These techniques possess advantages and limitations, regarding the mechanism of action and interaction with the sample matrix, sensitivity, and spatial resolution.^{5,6}

In this context, LA-ICP-MS is considered versatile, allowing to high spatial resolution and sensitivity, thus enabling access to spatial distribution of chemical elements in solid samples qualitatively and quantitatively.⁷ The technique consists of a laser source, an ablation cell, a transport path, and a detector. The interaction between the laser source and the sample surface promotes the sample melting and evaporation, which creates a dry aerosol that will be transported to ICP-MS by an inert gas flow, where the elements of interest are detected. More details about the LA-ICP-MS principle can be found in the review reported by Francischini and Arruda (2021).⁷

Image acquisition through LA-ICP-MS analysis is another potentiality of this technique. The image acquired by the correlation between x,y laser coordinates and the signal intensity of each analyte (z coordinate), using an appropriate software,^{7,8} such as LA-iMageS⁹ and Matlab¹⁰. The elemental image is formed by innumerous pixels that are directed related to the image resolution, and spatial information from the ablated sample surface.⁸ The image must provide real and/or similar information of the sample, so the highest spatial resolution is desired, which is directly correlated to the pixels size; they depend on the laser ablation parameters, such as scan speed, spot diameter, distance between ablation lines or spots, and integration time.^{7,8,11} Therefore, the optimization of laser parameters is mandatory to obtain adequate sensitivity and high spatial resolution images.

Chemometrics, through mathematical and statistical tools, allows to identify relevant experimental information and the optimal conditions of analysis. Thus, through multivariate optimization and factorial planning, the number of tests can be reduced, saving time and costs.¹² In a multivariate optimization approach, the factors are varied simultaneously, through experimental planning, allowing the analyst to observe the interaction between them and find the best analysis condition. This approach can be advantageous, but the data treatment is more laborious and the use of specific software is needed.^{12–14} In multivariate analyses, the multi-response (MR) function has shown great applicability: it proposes the optimization of parameters in a multi-elemental determination, through a common response, at a compromised condition.¹³ Kötschau et al. (2013) used a 2⁴⁻¹ fractional factorial design to optimize LA-ICP-MS experimental conditions for the imaging of P, S, K, Ca, Cr, Mn, Fe, Ni, Cu, Zn, Cd, La, and Ce in sunflowers leaves, enabling high resolution images.¹⁵ However, the use of MR function in LA-ICP-MS analysis is still scarce in the literature.

Different types of samples can be analyzed by LA-ICP-MS concerning environmental samples,⁷ such as tree rings,^{16,17} leaves and grain to observe metal accumulation and translocation of elements,¹⁸ or to evaluate the impact of nanoparticles in homeostasis changes¹⁹. The reconstruction of the climate for a given period is an important demand of the Climate and Earth Science community.²⁰ In this regard, speleothems feature as of the main natural archives used by geoscientists to reconstruct past variations of local climate.^{21,22} Essentially, speleothems are cave mineral deposits, largely formed by the carbonate precipitation, usually calcite and aragonite, from saturated seepage water. In general, variation in the concentration of some elements in the speleothem mineral matrix, such as Mg, Sr, and Ba, are largely dependent on processes related to the water-rock interaction, which ultimately results from the variation of local rainfall.^{23–25}

Since speleothems are stalagmites characterized by regions distinguished by microlayers of carbonate deposition, analysis techniques that enable high sensitivity and spatial resolution are appropriate. Furthermore, the improvement of high-resolution sampling analytical methods allows us to perform

annual-resolution records. Therefore, this study aimed optimize the laser ablation parameters through a multivariate approach, as well as evaluating the impact of ablation speeds and the use of an internal standard on the spatial resolution in the analysis of stalagmites by LA-ICP-MS. The obtained images can contribute for the understanding of environmental issues.

MATERIALS AND METHODS

Instrumentations and measurements

The spatial distribution of ⁴⁴Ca, ²⁶Mg, ⁶⁶Zn, ¹³⁷Ba, ⁵⁵Mn, ⁵⁷Fe, ⁸⁸Sr, and ⁶⁰Ni was evaluated using a New Wave UP-213 LA system (Nd:YAG laser at 213 nm) and a quadrupole-based inductively coupled plasma mass spectrometer (PerkinElmer ELAN DRC-e). All LA-ICP-MS measurements were performed in a class 10,000 cleanroom and the ICP-MS operations conditions were verified daily using an Mg, In, Be, Ce, and U solution to check the production of oxide, double and mono-charged ions, to keep them as recommended by the instrumental manufacturer.

Sample preparation

The LA parameters optimization was performed using the National Institute of Standards and Technology (NIST) 612 (trace element in glass) certified reference material (CRM). A real stalagmite sample with a thickness or 3 mm was obtained from the Geosciences Institute of University of São Paulo (São Paulo, SP, Brazil) and used in the resolution optimization.

Optimization of laser ablation instrumental parameters

The evaluation of the laser parameters was performed through a multivariate approach, in which the instrumental parameters were varied according to experimental planning. Parameters, such as laser spot diameter (µm), frequency (Hz), and laser intensity (%), were studied according to the levels shown in Table I using the NIST 612 CRM, and monitoring the isotopes ⁶⁶Zn, ¹³⁷Ba, ⁵⁵Mn, ⁵⁷Fe, ⁸⁸Sr, ⁶⁰Ni, ⁴⁴Ca, and ²⁶Mg. The LA-ICP-MS parameters are summarized in Table II.¹⁷

	Levels			
Parameters	-1	0	1	
Spot (µm)	20	60	100	
Frequency (Hz)	10	15	20	
Intensity (%)	30	60	90	

Table I. Experimental levels of the analytical parameters evaluated for laser ablation

Table II. Operational conditions of by LA-ICP-MS for speleothem analysis

Instrumental settings				
Radiofrequency power (W)	1300			
Nebulizer gas flow (L min ⁻¹)	1.2			
Auxiliary gas flow (L min ⁻¹)	1.6			
Data acquisition parameters				
Reading mode	Peak hopping			
Detector mode	Dual			
Sweeps	3			
Dwell time (ms)	20			

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Data acquisition parameters					
Integration time (ms)	60				
Isotopes	⁶⁶ Zn, ¹³⁷ Ba, ⁵⁵ Mn, ⁵⁷ Fe, ⁸⁸ Sr, ⁶⁰ Ni, ⁴⁴ Ca, ²⁶ Mg				
Laser conditions					
Wavelength of Nd:YAG laser (nm)	213				
Laser ablation intensity (%)	30, 60, 90				
Frequency (Hz)	10, 15, 20				
Spot size (µm)	20, 60, 100				
Scan speed (µm s⁻¹)	60				
Average energy output (mJ)	1.80				
Average fluence (J cm ⁻²)	25.6				
Warm-up time (s)	7				
Wash-out time (s)	10				

Table II. Operational conditions of by LA-ICP-MS for speleothem analysis (continuation)

A complete factorial design 2³ was applied, in which three variables were studied at 3 levels (-1, 0, and +1), as shown in Table III. The central point was performed in triplicate, totaling 11 experiments performed in random order.

Table III. Experimental Design 2° for laser ablation optimization					
Experiment	Spot (µm)	Frequency (Hz)	Intensity (%)		
1	-1	-1	-1		
2	-1	-1	1		
3	-1	1	-1		
4	-1	1	1		
5	1	-1	-1		
6	1	-1	1		
7	1	1	-1		
8	1	1	1		
9	0	0	0		
10	0	0	0		
11	0	0	0		

 Table III. Experimental Design 2³ for laser ablation optimization

Laser ablation acquisition was conducted in line scan mode after laser warm-up (7 s), to obtain the background signal in the initial and the final ablation process, with parallel lines with 0.2 mm separated (distance between each center line) and 60 μ m s⁻¹ scan speed. The ablated material was transported to the ICP using argon as the carrier gas. The chamber was purged for 10 s with argon for clean-up after each ablation step.

All ICP-MS data were exported and processed using the Microsoft Office Excel 2010 (Microsoft Corporation) software. The multi-response function (MR) was obtained according to Equation 1.¹³ The response surface was obtained by the free available Chemoface software.¹⁴

$$MR = \Sigma\left(\frac{I_i}{I_{max.i}}\right)$$

(Equation 1)

l_i = signal intensity of individual element I_{maxi} = maximum signal intensity of each element in the experimental design

Imaging resolution

After optimization of the parameters of laser ablation using the CRM NIST 612, the scan speed was evaluated for the speleothem sample (Figure 1). Thus, ten ablation lines with 3 mm length, 137.5 µm distance between each line were carried out by means of 100 µm spot diameter, 20 Hz of frequency, and 90% laser intensity. The ⁴⁴Ca isotope was evaluated as an internal standard to observe for correction of signal drift during analysis.¹⁹ All data were processed using the freely available LA-iMageS software to obtain the elements distribution image.⁹



Figure 1. Speleothem (stalagmite) surface with the ablation region highlighted.

Three scan speeds were evaluated (20, 40, and 60 μ m s⁻¹), providing the images obtained by the scan speed and total integration time correlation according to Equation 2.¹¹

 $Pixel\ size = v_a t_{in}$

 v_a = scan speed (µm s⁻¹) t_{in} = total integration time (s) (Equation 2)

RESULTS AND DISCUSSION

Optimization of laser ablation instrumental parameters

The laser ablation process involves heating, melting and evaporation at the laser incidence site on the sample due to the high temperatures and pressures.²⁶ As such, the ablation parameters will influence the amount of sample that will be ablated, sensitivity, and accuracy. The LA parameters vary according to the type of sample due to the different interactions laser-sample surface.^{7,27}

To choose the optimum conditions of the laser system and determine different elements in each experiment, a single response (global response) obtained through the individual MR was used to define a compromised condition among the variables;¹³ the best condition of ablation was the one that provide the highest analyte signal intensity. The signal intensity and MR for each element can be seen in Table IV, revealing that experiment 8 (highlighted) has led to the highest signal intensity for all analytes.

Signal intensity (cps)									
Exp.	⁶⁶ Zn	¹³⁷ Ba	⁵⁵Mn	⁵⁷ Fe	⁸⁸ Sr	⁶⁰ Ni	⁴⁴Ca	²⁶ Mg	MR
1	450	2	360	46	4	13	17075	1566	1.43
2	506	34	496	70	356	49	23906	2073	1.97
3	351	3	314	54	5	17	13791	1126	1.17
4	418	43	439	68	430	41	19719	1243	1.49
5	445	6	353	55	4	24	16086	1301	1.36
6	871	459	2195	133	4869	359	89517	1547	4.17
7	425	5	390	60	6	20	17623	1715	1.55
8	1224	1056	4924	231	11450	827	190947	1900	7.82
9	533	90	651	84	824	74	26215	1453	1.94
10	525	64	549	78	549	63	22011	1271	1.73
11	670	76	703	91	617	73	27504	2307	2.44

Table IV. Signal intensity and multi-response (MR) results for NIST 612, following a 2³ full factorial design LA-ICP-MS analysis

The MR data were treated using the software Chemoface whereas the effect of variables and the interactions between them was calculated. Figure 2 shows the result for the significance of variables through the Pareto's chart, considering 95% confidence level. In Figure 2 it is possible to verify that the effects of laser intensity (3) and spot size (1), as well as the interactions between them, and between spot size and frequency, are positive. Therefore, the higher the laser intensity, frequency and spot size, the higher the analyte signal, as indicated by the response surfaces in Figure 2.



Figure 2. Pareto's chart of the effects and the response surface for the full factorial design 2³ and multiple responses in LA-ICP-MS analysis.

This is explained by considering the laser intensity and fluence (energy per area) on the sample surface: higher laser intensity and fluence results in larger amounts of sample ablated, which will impact proportionally on the increase of the analytical signal observed. Regarding the ablation frequency, the number of pulses per unit of time, its increase (from 10 to 20 Hz) promoted an increase of the analyte signal intensity. A higher frequency made it more feasible for the laser beam to ablate the material from the sample impacting the sensitivity.¹⁰ It is worth highlighting that the changes in the laser ablation analytical parameters do not influence the background signal, which is obtained by the argon gas analytical signal.

There is a relationship between the spot size diameter, scan speed, and laser frequency that must be considered in LA-ICP-MS analysis to obtain sensitivity, according to the expected concentration of the analyte;¹⁰ some elements at low concentrations require the highest sensitivity while the opposite is desired for elements in high concentration. As expected, the increase in the spot diameter improved the sensitivity because a larger sample area (and a larger amount of analyte) was ablated. Therefore, the conditions selected for LA of the speleothems sample were: 90%, 20 Hz and 100 µm as the laser ablation intensity, ablation frequency and spot diameter, respectively.

Imaging resolution

In LA-ICP-MS imaging, the space between the center of each ablation line (y-resolution) and the correlation between scan speed and integration time (x-resolution)⁷ are important for the image resolution. As highlighted by Francischini and Arruda (2021), the ablation speed is even more important in the analysis of samples with high heterogeneity in the ablated area speleothems, for example, because it impacts on the amount of sample that is ablated and the pixel size.⁷ The smaller the pixel, the more pixels will be formed in the same sized image, increasing information about the ablated sample area and, consequently, the image resolution.⁸ Three scan speeds (20, 40, and 60 μ m s⁻¹) were evaluated in the analysis of the speleothem (stalagmite) sample surface. The obtained images for each isotope, using LA-iMageS software, can be seen in Figure 3.

As observed in Figure 3, by increasing the scan speed, the pixel size increase, worsening the image resolution in general. This can be clearly observed for ¹³⁷Ba, for example and, when looking at the other isotopes such as Ni, Mn, Zn and Fe, there seems to be an overlap of the data in the spatial distribution when there are higher scan speed. So, at 20 μ m s⁻¹ scan speed the pixels size is lower, increasing the image resolution as a consequence.

However, the time required in the analysis must be considered when the scan speed is low. Considering a sequential ablation of 10 lines of 0.2 mm length and scan speed of 60 μ m s⁻¹, the time spent in the ablation is approximately 9 min. When the scan speed is 40 or 20 μ m s⁻¹, the time required increases to 13 and 26 min, respectively. Therefore, LA-ICP-MS imaging analysis involves more cost due argon consumption. In the present study, 40 and 20 μ m s⁻¹ as scan speeds provided similar resolution, especially for Sr, Mg, and Ba in the same sample region. These three elements are very important in environmental and climate change studies. Vansteenberge et al. (2020) also observed the same covariation of Sr, Ba, and Mg in a speleothem sample from the Han-sur-Lesse cave located in Belgium and concluded that the covariation reflects the prior calcite precipitation (PCP), correlated to the lower water availability in the summertime.²⁸ Vadillo et al. (1998) obtained Mg and Sr images by LIBS and highlighted their importance for speleothems since elemental concentration variation observed throught the images can be correlated to the deposition temperature and time.²⁹

In LA-ICP-MS analysis, internal standardization is usually necessary to correct signal drift. The internal standard (IS) must be homogeneously distributed in the sample matrix and should have characteristics similar to those of the analytes.^{27,30} Chacón-Madrid & Arruda (2018) evaluated the use of ¹²C, ¹³C, ²⁸Si, and ³¹P as IS in the acquisition of images of Ag, Mn and Cu distribution in soybean and concluded that ¹³C led to better linearity and precision when compared to the other isotopes used as IS.¹⁹ Amais et al. (2021) used ¹³C as IS in imaging of elements distribution in tree rings samples, which allowed to observe chemical differences among cells type.¹⁷ In the case of speleothems, that have a carbonate matrix, a Ca isotope could be considered for internal standardization. Calcium is a major element well distributed in the sample

(see Figure 3), which is a requirement of an IS in LA-ICP-MS. However, depending on the type of ICP-MS instrument employed and its operation mode, interference of ⁴⁰Ar and ⁴⁰Ca occurs. Therefore, internal standardization using the ⁴⁴Ca isotope was evaluated and the images generated can be seen in Figure 4.



Figure 3. Isotopes distribution on the speleothems (stalagmite) sample surface according to each scan speed evaluated.

As can be seen in Figure 4 the internal standardization seems to have not affected the images for all monitored isotopes. In this case, a qualitative analysis the use of IS can be considered optional. However, in environmental studies, the ratio element concentration/Ca concentration, can provide important information about the speleothem formation and paleoclimate changes without impairing the image resolution.^{24,31} In addition, internal standardization in LA-ICP-MS is quite necessary in obtaining images of elements in quantitative analysis of the sample.



Figure 4. Effect of internal standardization in LA-ICP-MS on the images of elements distribution in the surface of stalagmite.

CONCLUSIONS

The experimental design applied in this work assisted the optimization of the LA parameters for speleothem analysis by LA-ICP-MS. It was possible to confirm that the larger the spot diameter and the higher the laser intensity, the larger the analytes signals, thus resulting in improvements of sensitivity. The influence of the ablation scan speed on the imaging resolution was demonstrated, where there is an increase in resolution when the laser scan speed in lower, despite the longer analysis time required.

Regarding the use of IS, it was observed that internal standardization with ⁴⁴Ca did not affect the images of the elements distribution.

The results obtained in this work contribute to LA-ICP-MS advancements for imaging, showing the importance of the LA parameters optimization, especially in the analysis of samples containing microlayers, such as speleothems. In fact, this kind of samples requires high image resolution to be compared with the real sample under analysis, contributing to environmental researches.

Conflicts of interest

The authors declare that they have no competing interests. The funders had no role in the design of the study, the collection, analyses, or interpretation of data, in the writing of the manuscript, or in the decision to publish the results.

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