Quantitative in situ microanalysis of minor and trace elements in biogenic calcite using infrared laser ablation – inductively coupled plasma mass spectrometry: a critical evaluation

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Abstract

This study evaluates the possibilities and limitations of infrared laser ablation – inductively coupled plasma mass spectrometry, using \(^{43}\text{Ca}\) as internal standard and the silicate glass reference materials NIST 610/612 as calibration standards, for quantifying the spatiotemporal variations of Mg, Mn, Sr, Ba and Pb in the calcite layer of *Mytilus edulis* shells. It demonstrates that the ablation behaviour of Ca relative to those of the analytes is dependent on the ablation time and substrate matrix (inorganic and biogenic calcite versus NIST 610/612); however, for ablation times \(\leq 80\) s, internal standardization with Ca improves precision to \(<10\%\) relative standard deviation (RSD) for all tested substrates. Variations in the Ca distribution in the studied shells are shown to be comparable to this precision, implying that we can assume a constant Ca concentration. When using NIST 610/612 as calibration standards for the analysis of inorganic calcite, an accuracy better than 10% and a precision \(<8\%\) RSD (not evaluated for Pb) are obtained for ablation times \(\leq 40\) s. For shell calcite a comparable reproducibility is obtained (\(<11\%\) average difference between overlapping analysis series) but since no homogeneous shell reference material is available the accuracy cannot be determined. Experimental results indicate that for certain instrument operating conditions accuracy could be deteriorated by differences in ablation characteristics between samples and standards, so that matrix matching is preferable. Owing to the lack of a matrix matched standard for the analysis of shell calcite, we decided to use NIST 610/612 as standards and to assure intercomparability of our results by normalizing all data with respect to one selected shell. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Infrared laser ablation–inductively coupled plasma mass spectrometry; In situ microanalysis; Biogenic calcite; Internal standard; Calibration standard

1. Introduction

In laser ablation–inductively coupled plasma mass spectrometry (LA–ICP-MS), in situ sampling of solids at high spatial resolution is combined with rapid and
sensitive multi-element analysis, allowing studies of the distribution of minor and trace elements in different sample types. A promising field of application is the elemental analysis of the successively deposited calcium carbonate layers of mollusc shells. For decades, researchers have been trying to correlate the minor and trace element contents of shells or shell fragments with environmental parameters such as temperature, salinity, productivity and pollution (e.g. critical review by Rosenberg [1]). However, up till now, few results valid for different species or even for different populations of the same species, have been obtained. According to Rosenberg [1], one of the explanations for the discrepancy in the results could be that by analysing whole shells or (apparently) randomly taken shell fragments, seasonal and ontogenetic trends in the element distribution have been masked, leading to misinterpretation of the data. Analysis of these trends is necessary to get a better insight into the different environmental and biological factors controlling shell chemistry. In recent studies addressing this subject it has already been shown that the variations of Sr, Mg and Ba concentrations in shells of certain bivalve species are linked to environmental parameters [2–4]. Sensitive microanalysis techniques such as LA–ICP-MS show great potential for this kind of research, as is demonstrated by e.g. Stecher et al. [2].

LA–ICP-MS requires the use of one or more internal standards to correct for instrument instability and drift. Furthermore, at least one reference material is necessary to correct for day-to-day changes in working conditions and to obtain (semi)quantitative results. In the case of in situ microanalysis, the selection of suitable internal and external standards is the main obstacle to be overcome. When selecting an internal standard, the major criterion is that its signal behaviour is similar to that of the analytes. Unlike for bulk analysis, where the homogenized samples can be spiked with the element(s) of choice, an element occurring naturally in the sample has to be used as internal standard in the case of in situ analysis. This greatly limits possibilities. When analysing calcium carbonates, an isotope of Ca is generally used as internal standard [5]. In an ultraviolet (UV) LA–ICP-MS study of the silicate glass standard NIST 612, it has been demonstrated that Ca shows a signal behaviour that is similar to that of a wide range of elements such as Sr, Ba, Mn and Mg, but is different from that of some other elements, e.g. Pb [6]. However, these results are not necessarily applicable for the analysis of calcite substrates or when infrared (IR) instead of UV laser ablation is being used. In order to obtain absolute concentrations, the concentration of the internal standard has to be known at each analysis site. In LA–ICP-MS analysis of shells, a constant Ca concentration is often assumed [7,8]. Since Ca is not necessarily uniformly distributed in shells [9], the former assumption could have an effect on the accuracy of the results. When selecting an external standard for in situ microanalysis, matrix matched reference materials that are sufficiently homogeneous are often very hard to find. Pearce et al. [10] prepared matrix matched standards by pressing CaCO3 powder, mixed with standard analyte solutions, into pellets. These standards were used in a number of IR–LA–ICP-MS studies of shells [7,8]. However, such reference materials are not commercially available. Non-matrix matched reference materials, such as NIST 612, have also been used for the IR–LA–ICP-MS analysis of biogenic carbonates [2]. Feng [11] has shown that calibration with NIST 612 guarantees accurate and precise results for the IR–LA–ICP-MS analysis for Sr, Y, Ba and rare earth elements (REEs) in calcite and dolomite grain mounts.

We have applied IR–LA–ICP-MS to study the spatiotemporal variations of Mg, Mn, Sr, Ba and Pb in the calcite shell layer of Mytilus edulis (blue mussel) from the Scheldt estuary, in order to identify the biological and environmental factors that influence the accumulation of these elements in the shell (paper in preparation). We used 43Ca as internal standard and NIST 610 and 612 as calibration standards. In the present paper, we demonstrate the validity and limitations of these analyses. We verify the suitability of Ca as internal standard by determining its signal behaviour with respect to the ones of the analytes and by evaluating the influence of internal standardization on precision, for calcite substrates as well as for NIST 610/612. Furthermore we verify, by means of electron microprobe analysis, the homogeneity of the Ca distribution in the shell’s calcitic layer. Finally, we determine the precision and accuracy that are obtained when using NIST 610/612 as calibration standards for the analysis of calcite samples.
2. Experimental

2.1. Samples

Analyses were performed on the calcite layer of a *M. edulis* shell (*M. edulis* calcite), on a calcite monocrystal from Macuco, Brazil (MB calcite) and on the spiked silicate glass reference materials NIST 610 and 612 (Si, Ca, Na and Al glasses, spiked with 61 elements at nominal concentrations of 500 and 50 µg g⁻¹).

The mussel shell was sectioned along the axis of maximal growth using a diamond saw and the section was polished with silicon carbide paper of decreasing grain size to visualize the boundary between the calcite and aragonite layer. For electron microprobe analysis, the section was embedded in epoxy resin, polished with diamond paste and coated with carbon. Both LA–ICP-MS and electron microprobe analyses were made in the middle of the calcite layer, from the umbo towards the edge, with spacings of approximately 250 µm between successive analysis sites. That way, successively deposited layers were sampled (Fig. 1).

LA–ICP-MS screening showed that the MB calcite had a homogeneous distribution of Mg, Mn, Sr and Ba; variations on the blank-subtracted, Ca normalized signals of 37 analyses made randomly within the calcite grain varied between 2.3% and 8.5% relative standard deviation (RSD), falling into the same range as the variations on analyses made within the homogeneous NIST 610 (1.8–8.7% RSD). The LA–ICP-MS screening also showed that the MB calcite contained no detectable Pb. We assumed a uniform Ca distribution throughout the crystal. Analyte concentrations were determined by solution mode ICP-MS analysis after mineralization. The calcite grain was dissolved in 2.5 ml of Suprapur HCl (30%) (Merck) and the volume was adjusted to 25 ml with milliQ-grade water (Millipore). This sample was analysed five times.

Accepted concentrations for NIST 610 and NIST 612 were taken from Pearce et al. [12].

2.2. LA–ICP-MS instrumentation and data acquisition

A Fisons-VG PQII+ICP mass spectrometer and a Fisons-VG LaserProbe were used for this work. The LaserProbe is based on a Spectron 150 mJ Nd:YAG laser operating at 1064 nm (IR). The laser energy is attenuated to about 2–10 mJ per pulse by inserting circular apertures into the beam cavity. Experiments were performed at different instrument operating conditions, remaining within the range of routine settings shown in Table 1. Under these conditions the crater size is ≤60 µm.

Preablation time was set to 20 s, since this is the time necessary to obtain a stable signal for NIST 610/612. Acquisition time and number of repeats are described below for each experiment. Total ablation time never exceeded 100 s since this is the maximum.

![Fig. 1. Schematic view of a *M. edulis* shell valve, sectioned along the axis of maximal growth, showing the different calcium carbonate layers and the localisation of the LA–ICP-MS and the electron microprobe-analysis sites within the calcite layer (dots). Dashed lines indicate the direction in which the calcite grows.](image-url)

<table>
<thead>
<tr>
<th>LaserProbe</th>
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<tr>
<td>Flashlamp voltage (V)</td>
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<tr>
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<table>
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<td>Reflected power (W)</td>
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</tr>
<tr>
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<tr>
<td>Auxiliary gas (l min⁻¹)</td>
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<tr>
<td>Dwell time (ms)</td>
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<tr>
<td>Acquisition time (s)</td>
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possible time before ablation stops in the calcite substrates. During acquisition, signal intensities were recorded for $^{26}$Mg, $^{55}$Mn, $^{43}$Ca, $^{88}$Sr, $^{138}$Ba and $^{208}$Pb. In each experiment the mean signal intensity of the carrier gas was considered as the background. All signal intensities were background corrected before further treatment.

2.3. Electron microprobe instrumentation and data acquisition

A Camebax Microbeam SX 50 was used for this study. Ca was detected using a thallium phthalate crystal and a gas flux detector. A calcite crystal was used for standardization. Analysis time was set to 20 s and the beam diameter was 12.5 μm.

3. Analytical rationale

3.1. Evaluation of the use of Ca as internal standard

3.1.1. Signal behaviour of Ca relative to Mg, Mn, Sr, Ba and Pb

At fixed LA–ICP-MS operating conditions signal instability and drift result from fluctuations and gradual changes in e.g. laser energy, laser focus and condition of the ICP-MS interface. To improve reproducibility, the analyte yield (signal per concentration unit) is normalized to the yield of an internal standard. Longerich et al. [6] state that internal standardization is very effective when the analyte and the internal standard share similar ablation behaviours, i.e. they do not fractionate progressively during the ablation and transport process. This implies that the ratio between the analyte yield and the internal standard yield, which we refer to here as the element response factor (ERF), should remain constant during the ablation. According to the former view, we verified the suitability of Ca as internal standard for analysis for Mg, Mn, Sr, Ba and Pb in M.edulis calcite, MB calcite and NIST 610/612 by monitoring the evolution of the ERFs during the course of an ablation. Each sample was ablated 16 times, each ablation consisting of a preablation followed by four successive acquisitions of 20 s each, and each time the evolution of the ERFs relative to the first acquisition (i.e. the acquisition between 20 and 40 s of ablation) was calculated, assuming a uniform element distribution within the same crater. In the case of M. edulis calcite the latter assumption is justified by the fact that only simultaneously deposited carbonate is being sampled (Fig. 1). For each ERF-time profile (for each ablation) the time-averaged ERF-evolution was calculated as the average of the ERF's (absolute values; in % relative to the first acquisition) of the last three acquisitions.

3.1.2. Influence of internal standardisation with Ca on precision

In the following experiment we verify the effectiveness of Ca for improving precision. We compared precisions obtained with and without internal standardization with Ca for the analysis of Mg, Mn, Sr, Ba and Pb in MB calcite and in NIST 610/612. At fixed instrument conditions, four ablations were made as in experiment 1, in each of the three samples. Precisions, with and without Ca normalization of the analyte signals, were then calculated for the acquisitions between 20 and 40 s and between 60 and 80 s. This experiment was repeated at each of four different settings of laser energy and argon gas flows, and average precisions were calculated.

3.1.3. Analysis of the Ca distribution in the calcite layer of a M. edulis shell

In order to calculate absolute analyte concentrations, the concentration of the internal standard has to be known at each analysis site. We have determined the Ca distribution in the calcite layer of a two year old M. edulis shell, by means of electron microprobe analysis. This shell is representative for the samples analysed in our study of the spatiotemporal variation of shell chemistry. Furthermore, the electron microprobe analysis was done at discrete sites, proximal to those of the LA–ICP-MS study.

3.2. Evaluation of the use of NIST 610 and NIST 612 as external standards

The ERF is strongly influenced by LA–ICP-MS operating conditions. To correct for these day-to-day changes and to obtain (semi)quantitative results, a reference material with accurately known analyte and internal standard concentrations has to be used. A major requirement is that the ERF is the same in standard and samples, at all instrument settings:
in between the earlier craters. Data for each pair of second series of analyses was made with laser shots towards the instrument settings were changed and a Section 3.1.1) was made as described earlier. After-series of nine equally spaced analyses (ablation as in calibration standards. At the first instrument setting, a and the more matrix matched MB calcite alternately as instrument settings. We used NIST 610, NIST 612 M. edulis calcite layer of a by making two overlapping series of analyses in the after mineralization of the calcite. tions determined by solution mode ICP-MS analysis, the LA–ICP-MS results with the analyte concentrations obtained at the different operating conditions; accuracy was determined by comparing the standard and sample ERF to changes in instrument conditions; thus resulting in poor accuracy.

3.2.1. Precision and accuracy in MB calcite

We determined precision and accuracy for the analysis for Mg, Mn, Sr and Ba in MB calcite using NIST 610/612 as calibration standards. At each of four different settings of laser energy and argon gas flows, four analyses (each ablation consisting of a preablation followed by a 20 s acquisition) were made in each of the three substrates. At each setting, the average signals of the standards were used for the calculation of the sample analyte concentrations (Eq. (1)). Precision was calculated using the four average analyte concentrations obtained at the different operating conditions; accuracy was determined by comparing the LA–ICP-MS results with the analyte concentrations determined by solution mode ICP-MS analysis, after mineralization of the calcite.

\[
\begin{align*}
ERF_s &= ERF_{\text{ref}} \\
\frac{S(E)_s / C(E)_s}{S(IS)_s / C(IS)_s} &= \frac{S(E)_{\text{ref}} / C(E)_{\text{ref}}}{S(IS)_{\text{ref}} / C(IS)_{\text{ref}}} \\
C(E)_s &= \frac{S(IS)_{\text{ref}} / C(IS)_{\text{ref}}}{S(E)_{\text{ref}} / C(E)_{\text{ref}}} \times \frac{S(E)_s}{S(IS)_s / C(IS)_s},
\end{align*}
\]

where \(S(E)_s\) and \(S(IS)_s\) are the analyte and internal standard signals, \(C(E)_s\) and \(C(IS)_s\) are the analyte and internal standard concentrations and the subscripts \(s\) and \(\text{ref}\) denote the sample and external standard, respectively.

Matrix differences between standard and samples could result in different ablation characteristics. This could in turn lead to a non-similar response of the standard and sample ERF to changes in instrument operating conditions, thus resulting in poor accuracy.

3.2.2. Precision and accuracy in M. edulis calcite

For the heterogeneous \(M.\ edulis\) calcite we measured precision of analysis for Mg, Mn, Sr, Ba and Pb by making two overlapping series of analyses in the calcite layer of a \(M.\ edulis\) shell at two different instrument settings. We used NIST 610, NIST 612 and the more matrix matched MB calcite alternately as calibration standards. At the first instrument setting, a series of nine equally spaced analyses (ablation as in Section 3.1.1) was made as described earlier. Afterwards the instrument settings were changed and a second series of analyses was made with laser shots in between the earlier craters. Data for each pair of successive craters from the first series were averaged to match the second series. At each instrument setting each standard was analysed four times and the average signals were used for the calculation of the analyte concentrations (Eq. (1)). This experiment was performed twice: the first time the argon gas flows were kept constant while the laser energy was changed; the second time the laser energy was left unchanged while the torch position and the carrier and auxiliary gas flows were varied.

4. Results and discussion

4.1. Evaluation of the use of Ca as an internal standard

4.1.1. Signal behaviour of Ca relative to Mg, Mn, Sr, Ba and Pb

The mean ERF-time profiles for Sr and Ba obtained during ablation are shown in Fig. 2(a) and (b); data for Mg, Mn and Pb are not shown here but will be discussed. For most analytes and samples the ERFs progressively increase during the ablation. However, in the case of NIST 612 the increase is limited to the last acquisitions (after 60–80 s) for Mg, Mn and Pb; the earlier acquisitions may show a decrease of maximally 5% relative to the first acquisition. For \(M.\ edulis\) calcite the mean ERF shows a slight decrease for Sr, Ba and Pb during the last 20 s of ablation (maximally 6% relative to the preceding acquisition). For Sr, the ERF remains constant during the ablation for each of the studied samples (Fig. 2(a)); consequently, the mean TA–ERF evolutions do not exceed 3.6% (Fig. 3). For the other analytes the ERF evolution is clearly larger for the MB calcite and \(M.\ edulis\) calcite than for NIST 610/612 (e.g. Fig. 2(b) for Ba), which is reflected by the TA–ERF evolutions (Fig. 3). \(t\)-tests (two samples; unequal variances) show that in the case of Mg, Mn, Ba and Pb, the TA–ERF evolutions for the calcite substrates are significantly different (\(\alpha \leq 0.02\)) from those for the silicate glasses, while there are no significant differences for substrates of the same matrix type (0.2\(\leq \alpha \leq 0.8\)). From this experiment we can conclude that, for ablation times \(\leq 100\) s, Ca and Sr show similar ablation behaviours in all of the tested sample types, while Mg, Mn, Ba and Pb show an ablation behaviour that is quite similar to the one of Ca.
Longerich et al. [6] made an extensive study of the signal behaviour of 59 elements during a 4 min ablation of NIST 612 with UV–LA–ICP-MS and demonstrated that this system also suffers from fractionation: in terms of ablation behaviour, elements fell into several distinct clusters, with the elements within the same cluster correlating well with each other. It is suggested that during ablation, elements in the sample may distribute themselves into several phases which are then differentially transported into the plasma and spectrometer. Since no cluster analysis was performed in our study, it is difficult to compare our results for NIST 612 with the ones from Longerich et al. [6]. Nevertheless, there seems to be a good agreement: in the UV–LA–ICP-MS study Ca correlated strongly with 37 elements, including Sr, Ba, Mn and Mg; this corroborates our results. Pb, on the contrary, did not seem to correlate well with Ca, while in our work, results were comparable for all analytes in the case of NIST 612. Since we showed that fractionation generally increases with ablation time, this apparent discrepancy could be explained by the different ablation times used in both studies, more specifically 4 min in the work of Longerich et al. [6] and 100 s in our work. The composition of the clusters in the work of Longerich et al. [6] is said to be similar for different minerals, which implies that the fractionation process

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**Fig. 2.** Evolution of the element response factors (ERFs) for Sr (a) and Ba (b) relative to Ca versus ablation time for the different samples. Each ERF-time profile represents the average of 16 ablations; error bars represent ±1 standard deviation.
is probably independent of the sample matrix. This is in contrast with the results of our study where a clear matrix effect is demonstrated. This discrepancy may be explained by the different ablation mechanisms of infrared and ultraviolet lasers: according to Geertsen et al. [13], direct ablation caused by laser energy absorption is the main mechanism controlling removal of sample material in ultraviolet laser ablation; in contrast, removal of sample material using an infrared laser is in part caused by plasma erosion, rather than by direct ablation. Jeffries et al. [14] have observed differences in the ablation characteristics of different sample types using IR–LA–ICP-MS and have explained this in terms of differences in the proportion of plasma erosion and direct ablation. They suggest that the relative importance of both mechanisms is determined by sample properties, with plasma erosion probably being more important for light coloured minerals such as calcite, which have a poor absorption in the near-infrared region of the electromagnetic spectrum. Consequently, the composition of the aerosol, in terms of particle size and element distribution, is likely to be different from one sample type to another. This could explain the matrix effect observed in our study.

4.1.2. Influence of internal standardization with Ca on precision

At fixed instrument settings Ca normalization improves precision, although not always significantly, for all analytes, for both studied time intervals (Fig. 4(a) and (b)). For acquisitions between 20 and 40 s precision is improved to values better than 7.3% RSD, for acquisitions between 60 and 80 s precisions are generally, but not systematically poorer, but still are ≤9.5% RSD. In contrast with experiment 1, statistical analysis did not reveal a systematic significant difference between the precisions obtained for NIST 610/612 on the one hand and for MB calcite on the other. Consequently, we can conclude that, although the ablation behaviour of Ca relative to the ones of the analytes is dependent on the ablation time and substrate matrix (calcite versus NIST 610/612, experiment 1), internal standardization with Ca improves precision to <10% RSD for all tested substrates, for ablation times ≤80 s. Since the signal behaviours of Ca and the analytes in M.edulis calcite were shown to be similar to the ones in MB calcite, a similar precision can probably be assumed for M. edulis calcite.

4.1.3. Ca-distribution in the calcite layer of a M. edulis shell

Electron microprobe analysis has shown that Ca is uniformly distributed over the analysed M. edulis calcite section, with a mean concentration of 398 000 μg g⁻¹ ±5600 SD. A variation of 16 800 μg g⁻¹ (3SD) on the mean Ca concentration results in a variation of 4.2% on the analyte concentration, which is comparable to the precision of <10%
RSD. This implies that we can assume a constant Ca concentration.

4.2. Evaluation of the use of NIST 610 and NIST 612 as external standard

4.2.1. Precision and accuracy in MB calcite

The precisions obtained at varying instrument settings (Table 2) are comparable to the ones obtained at fixed instrument settings for acquisitions between 20 and 40 s (in both cases ≤7.3% RSD). Accuracy is generally better than 10%, even for Mn, whose concentration in MB calcite is as low as 4 μg g⁻¹ (Table 2). The only exception is the accuracy of 36% obtained for the determination of Mg using NIST 612 as standard. This poor accuracy is probably caused by an error in either the Mg concentration in MB calcite, as determined by solution mode ICP-MS, or in the accepted Mg concentration in NIST 612, the latter being more likely, since better accuracy (10%) is obtained when using NIST 610 as standard. These results corroborate the work of Feng [11] who demonstrated that an accuracy better than 10% and a precision ≤5% RSD (analyte concentrations ≥1 μg g⁻¹) can be obtained for the IR–LA–ICP-MS analysis of Sr, Y, Ba and REEs in calcite and dolomite.

![Graph showing precision for different samples with NIST 610, NIST 612, and MB calcite.](image-url)
grain mounts, using NIST 612 as standard. In addition, our work shows that an equally good accuracy and precision can be obtained for the analysis for Mn and Mg in a calcite grain, using NIST 610/612 as standards in the case of Mn, and NIST 610 in the case of Mg.

### Table 2

Analyte concentrations in MB calcite determined by LA–ICP-MS, using NIST 610 or NIST 612 as external standard, and by solution mode ICP-MS

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Standard</th>
<th>LA–ICP-MS concentration ($\mu$g g$^{-1}$)</th>
<th>Precision (RSD %)</th>
<th>Solution mode ICP-MS concentration ($\mu$g g$^{-1}$)</th>
<th>Precision (RSD %)</th>
<th>Accuracy (difference %)</th>
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<tr>
<td>Mg</td>
<td>NIST 610</td>
<td>2830</td>
<td>4.5</td>
<td>2565</td>
<td>0.4</td>
<td>10</td>
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<tr>
<td></td>
<td>NIST 612</td>
<td>3489</td>
<td>7.3</td>
<td>36</td>
<td></td>
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<tr>
<td>Mn</td>
<td>NIST 610</td>
<td>3.7</td>
<td>5.6</td>
<td>3.6</td>
<td>0.4</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>NIST 612</td>
<td>3.9</td>
<td>6.6</td>
<td>8</td>
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<td>NIST 610</td>
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<td>1876</td>
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<td>2.9</td>
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<tr>
<td>Ba</td>
<td>NIST 610</td>
<td>40</td>
<td>4.8</td>
<td>40</td>
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<td>0.1</td>
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<tr>
<td></td>
<td>NIST 612</td>
<td>42</td>
<td>4.5</td>
<td>6</td>
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</table>

For LA–ICP-MS, four analyses were made at each of four instrument settings; for solution mode ICP-MS one sample was digested and an aliquot was analysed five times.

4.2.2. Precision and accuracy in *M. edulis* calcite

When making two overlapping analysis series in *M. edulis* calcite at different laser energy settings, the average differences between these series, calculated with the same standard, are <10.6% (e.g. Fig. 5(a) for

![Sr-concentration profiles determined by LA–ICP-MS for two sets of overlapping analysis series made in the calcitic layer of a *M. edulis* shell: (a) the laser energy was different from one series to another, and (b) the torch position and argon gas flows were different from one series to another. The two sets were acquired in different zones of the bivalve shell.](image)
Sr, using NIST 610 as standard). Paired \( t \)-tests show that there is no significant difference between both series for any of the analytes (\( 0.08 \leq \alpha \leq 0.9 \)), neither when using MB calcite, nor when using NIST 610/612 as a standard. These average differences are comparable to the precision of <8% RSD obtained in Section 4.2.1 for the analysis of MB calcite with NIST 610/612 as standards. However, when making two overlapping analysis series in *M. edulis* calcite at different conditions of torch position and carrier and auxiliary gas flows, these series are found to be significantly different from each other (\( \alpha \leq 0.05 \)), even when using the more matrix matched MB calcite as standard. The maximal mean difference between both series falls into the same range as when changing the laser energy (resp. 10% and 10.6%), but the deviation between two corresponding data points always tends to be in the same direction, i.e. either generally positive or generally negative (e.g. Fig. 5(b) for Sr, using NIST 610 as standard). As is described earlier

![Graphs of Mg, Mn, and Sr concentrations](image)

Fig. 6. Concentration profiles determined by LA–ICP-MS in the calcitic shell layer of a *M. edulis* specimen collected in the intertidal zone of the Western Scheldt esuary (the Netherlands); error bars represent ±10%.
(Section 3.1.1), the properties of the aerosol that is formed using IR–LA–ICP-MS are likely to vary from one sample type to another. Consequently, for certain conditions of torch position and argon gas flows, the sample ERFs may be different from the standard ERFs due to differences in transport and/or ionization efficiencies for the respective aerosols. This hypothesis could explain the observed differences in reproducibility: a minor variation in laser energy probably has no significant influence on the aerosol properties, neither for the sample nor for the standards, and consequently has no effect on the transport and ionization efficiencies. Changing the torch position and argon gas flows, however, may influence the latter factors to a different extent for respectively the sample and standard aerosols.

The matrix difference between *M. edulis* calcite and NIST 610/612 is obvious; however, standardization with MB calcite does not lead to a better fit between the overlapping concentration profiles. This is possibly due to the fact that MB calcite is a large calcium carbonate monocrystal while *M. edulis* calcite is composed of microcrystals of calcium carbonate embedded in an organic matrix. If this explanation is correct, it implies that one should use perfectly matrix matched standards to obtain accurate results. Since, as far as we know, biogenic calcite reference materials that are homogeneous at the level of 60 μm do not exist, we decided to use NIST 610/612 as calibration standards in our study of bivalve shells, and we assured intercomparability of our data by normalizing all data with respect to one selected shell. The analyte concentration profiles in this common heterogeneous shell were determined by LA–ICP-MS at a given instrument setting. Whenever the operating conditions were changed an overlapping analysis series of 10 craters was made as described earlier and, if necessary, results were recalculated to match the original concentration profile. However, the true analyte concentrations of this “reference” shell are unknown. Indeed, in view of the heterogeneity of the *M. edulis* calcite it would be very difficult, if not impossible, to verify the accuracy of the results by determining the concentration profiles of our “reference” shell via manual subsampling followed by mineralization and solution analysis. Recently,
ICP-MS analyses of the calcium carbonate skeletons of sclerosponges were made in our laboratory. The results indicate that the latter substrates contain zones that show a homogeneous distribution of some of the analytes that were discussed in the present study. Further research will show if these sclerosponge fragments can be used as calibration standards for the LA–ICP-MS analysis of bivalve shells.

5. Conclusions

In this study we evaluated the possibilities and limitations of the IR–LA–ICP-MS analysis of Mg, Mn, Sr, Ba and Pb in the calcitic layer of *M. edulis* shells. Major problems are the matrix dependent fractionation of elements during ablation and the influence of matrix differences between standards and samples on accuracy. Still, we demonstrated that for the analysis of inorganic calcite, using $^{43}$Ca as internal standard and the non-matrix matched NIST 610/612 as calibration standards, an accuracy better than 10% and a precision <8% RSD can be obtained, provided that ablation time is sufficiently short ($\leq 40$ s). For *M. edulis* calcite a comparable precision is obtained (<11% average difference between overlapping analysis series) but since no shell reference material is available accuracy cannot be determined. To assure at least intercomparability of our results, all data were normalized with respect to one selected shell. In spite of these limitations, the precision that is obtained with IR–LA–ICP-MS is quite sufficient to study the spatiotemporal variations of Mg, Mn, Sr, Ba and Pb in *M. edulis* shells (Fig. 6).

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