which should be cited to refer to this work.

In situ measurements of calcium isotopes by ion microprobe in carbonates and application to foraminifera

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Abstract

An analytical procedure has been developed for the in situ measurement of calcium isotope composition of carbonates with a spatial resolution of 15–20 μm on a Caméca IMS 1270 ion microprobe. By using two Faraday cup detectors, the ^{40}Ca and ^{44}Ca can be measured simultaneously, improving the internal reproducibility. Instrumental mass fractionation (IMF) of calcium isotopes was observed to be independent of primary ion beam intensity and of the Mg content of the carbonate, but can depend on vacuum conditions. Three calcite reference materials were used in this study (ENS 0, MEX and BRET 105E) and their δ^{44}Ca values relative to NIST915a were reproducible within a typical 1σ standard deviation of ≈ 0.15‰.

This analytical procedure was applied to planktonic foraminifera, *Globorotalia inflata*, dated at 2.8 Ma from Shatsky Rise (ODP leg 198). The range of measured δ^{44}Ca within a single test is 1.7‰. This intratest variation can be attributed to several processes such as temperature variation, ontogenic effects or differences between primary and secondary calcite (i.e. calcite precipitated by different biomineralization processes).

Despite this intratest variation, the averages δ^{44}Ca for each foraminifer are similar and are in agreement with published δ^{44}Ca values measured for this age. This study shows that in situ δ^{44}Ca measurements in tests of foraminifera are an appropriate tool for investigating biomineralization processes.

1. Introduction

Calcium plays a major role in many geological and biological processes. Ca has five naturally stable isotopes with abundances of: ^{40}Ca (96.941%), ^{42}Ca (0.647%), ^{43}Ca (0.135%), ^{44}Ca (2.086%) and ^{46}Ca (0.004%), and one more isotope, with such a long half-life (6×10^{18} years) that it can be considered as stable, ^{48}Ca (0.187%). Natural variations of Ca isotope ratios may be introduced by beta-decay of the potassium isotope ^{40}K (half-life: 1.277×10^9 years) increasing the relative abundance of ^{40}Ca (Marshall and DePaolo, 1982; Nägler and Villa, 2000). In carbonates, K abundance can be considered as negligible, preventing any in situ growth of ^{40}Ca. Ca isotopes can also fractionate during physico-chemical processes such as calcite precipitation with a fractionation $\Delta^{44/40}$Ca

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between 0 and -1.8‰ depending on the precipitation rate (Lemarchand et al., 2004; Marriott et al., 2004). δ^{44}Ca (with $\delta^{44}\mathrm{Ca} = \left(\frac{(^{44}\mathrm{Ca}/^{40}\mathrm{Ca})_{\mathrm{sample}}}{(^{44}\mathrm{Ca}/^{40}\mathrm{Ca})_{\mathrm{NISTSRM915a}}} - 1\right) \times 1000$) variations in biogenic carbonates can be interpreted in terms of geological, kinetic and/or biological processes. As a result of the different kinds of behaviour of δ^{44}Ca in biogenic carbonates, various authors have used Ca isotope ratios of biomaterials as tracers of (i) biomineralization by either using soft tissue and dietary δ^{44}Ca as an indicator of the calcium balance in living organisms (Skulan and DePaolo, 1999) or by using the fractionation of calcium isotopes during metabolic processes (Clementz et al., 2003), (ii) the global calcium cycle (De La Rocha and DePaolo, 2000; Fantle and DePaolo, 2005; Heuser et al., 2005; Farkas et al., 2007), and (iii) marine paleotemperatures (Zhu and MacDougall, 1998; Nägler et al., 2000; Gussone et al., 2004; Immenhauser et al., 2005; Hippler et al., 2006). The importance of Ca for foraminiferal biology suggests that the Ca isotope fractionation may be biologically mediated and hence may also contain information about the mechanisms of calcification in foraminifera and in other biological carbonates such as corals (Böhm et al., 2006) and coccolithophores (Gussone et al., 2006; Langer et al., 2007). In addition to changes in temperature and biological processes, variation in ancient foraminifera δ^{44}Ca may reflect changes in oceanic δ^{44}Ca values and observed changes have been attributed to differences in past weathering inputs (De La Rocha and DePaolo, 2000; Fantle and DePaolo, 2005). Thus, the δ^{44}Ca values in foraminifera could provide insight on paleotemperatures, biomineralization processes and also changes in weathering inputs, linked to the carbon cycle. Until now, Ca isotopes ratios in carbonates have been measured using TIMS and MC-ICPMS techniques on bulk samples. With the use of an ion probe, it should be possible to measure several spots in one single test and thus to explore possible intratest variability. This new aspect should allow the link between biomioneralization mechanism and Ca isotope fractionation to be studied from a different angle.

In many studies, the ion microprobe has proved to be an essential tool as a result of its ability to perform in situ measurements of isotopic/elemental compositions. Pioneering ion microprobe development measured Ca isotopes with an analytical precision around ± 5.4‰ ($2\sigma_{\mathrm{n}}$) for $\delta^{48/40}$Ca in CM and CV meteorites (Sahijpal et al., 2000), and around ± 3‰ (1σ) for $\delta^{42/40}$Ca, $\delta^{43/40}$Ca and $\delta^{48/40}$Ca in Ca–Al-rich inclusions of meteorites (Weber et al., 1995). For environmental applications, better precision is needed, and therefore we have systematically tested the factors that determine δ^{44}Ca precision by ion microprobe in carbonates.

Instrumental mass fractionation (IMF) during calcium isotope measurement, which causes the measured isotope ratios to be shifted towards values usually depleted in the heavy isotope compared to the true abundances (Shimizu and Hart, 1982), is defined as:

$$\alpha_{\mathrm{inst}} = \frac{(^{44}\mathrm{Ca}/^{40}\mathrm{Ca})_{\mathrm{meas}}}{(^{44}\mathrm{Ca}/^{40}\mathrm{Ca})_{\mathrm{true}}},$$

where $(^{44}\mathrm{Ca}/^{40}\mathrm{Ca})_{\mathrm{meas}}$ is the calcium isotope ratio measured by ion microprobe with no correction for instrumental fractionation and $(^{44}\mathrm{Ca}/^{40}\mathrm{Ca})_{\mathrm{true}}$ is the calcium isotope ratio measured by a conventional method. The IMF can also be reported in permil units (Δ_{inst}), calculated using:

$$\Delta_{\mathrm{inst}} = 1000 \times \ln(\alpha_{\mathrm{inst}}).$$

The IMF variability and its dependence on instrumental conditions are known to be the main limitation for the precision and accuracy of ion microprobe analyses (Shimizu and Hart, 1982). Values of α_{inst} are usually determined on reference materials of known isotopic composition, which are analysed under the same conditions as the samples. The most commonly applied correction scheme consists of determination of the IMF by analysing reference materials of chemical and physical properties identical to the sample. This standard procedure has successfully been applied to δ^{11}B, δ^{13}C, δ^{18}O and δ^{7}Li measurements in pure matrices like calcite or aragonite (e.g. Rollion-Bard et al., 2003; Vigier et al., 2007).

This study tests the main instrumental conditions that are likely to vary from analysis to analysis and their influence on the IMF. This development is applied to δ^{44}Ca measurements in calcites and, we present, for the first time, in situ δ^{44}Ca measurements, with an average precision of ± 0.15‰ (1σ). This analytical precision allows us to quantify the intra-shell variability of δ^{44}Ca in tests of planktonic foraminifera (*Globorotalia inflata*).

2. Reference materials and samples

Three calcite reference materials were used: A calcite experimentally precipitated from NIST SRM 915a powder and two natural calcites, MEX and BRET 105E. The method of precipitation is as follows. A calcite standard (ENS 0) was synthesized hydrothermally (under high temperature and pressure). A powder of NIST SRM 915a

Table 1
Ion microprobe Caméca IMS 1270 settings

Primary source ions	$^{16}O^-$
Primary beam intensity	10–15 nA
Field aperture	5000 μm
Entrance slit	80 μm
Exit slit	Multicollection no. 1: 500 μm
Mass resolution power (MRP)	≈5000

calcite was enclosed, together with LiOH and de-ionized water, in a gold capsule, which was welded shut. The sample was run at 550 °C and 150 MPa for 1 week in an externally heated pressure vessel at the ENS (Ecole Nationale Supérieure, Paris). The run product is composed of sub-millimetre calcite grains (as confirmed by Raman micro-spectrometry). The calcite produced was rinsed with de-ionized water before sample preparation and analyses. $\delta^{44}Ca$ values determined by TIMS, relative to NIST SRM 915a, for these reference materials are 0.94 ± 0.1‰ (MEX) and 0.74 ± 0.11‰ (BRET 105E) (A. Eisenhauer, pers. com.). By definition, the $\delta^{44}Ca$ of NIST SRM 915a is 0‰.

The studied specimens of *G. inflata* (planktonic foraminifera) were picked from sediments retrieved during ODP leg 198 (Shatsky Rise) at hole 1209A (32°39 N, 158°30E). Site 1209 is located at 2387-m water depth (middle bathyal) close to the most elevated, central part of the Southern High of Shatsky Rise. ODP Hole 1209A was drilled to 259.6 m below sea floor (mbsf) into a Paleogene section. Studied *G. inflata* are from 1209A-4H-CC (Zone N21). They are dated at 2.8 Ma.

Contamination from clay minerals, iron and manganese oxide coatings, barite crystals, calcareous nanofossils, and organic material is often a major problem in trace element and isotopic analyses performed using a batch of bulk tests. Most contamination is acquired after the death of the organism onto the outer surface of the tests or accumulated in pores. The pre-cleaning techniques that are classically used aim therefore at eliminating the contaminant phases located on the exterior of the test and within chamber cavities (e.g. Martin and Lea, 2002; Pena et al., 2005). Using the 1270 ion microprobe, cleaning procedures are not required because measurements can be done on small spots located in the centre of calcite walls. Before analyses, reference materials and samples were embedded in epoxy sections, polished using a diamond paste down to 1 μm and then coated with gold.

3. Ion microprobe measurements

The ion microprobe calcium isotope compositions reported in this study were determined using the Caméca IMS 1270 ion microprobe at CRPG-CNRS, Nancy, France.

3.1. Basic settings

A liquid nitrogen cold-trap was used to remove water from the residual gases and to lower the gas pressure in the specimen chamber. Samples were sputtered with a primary beam of $^{16}O^-$ ions of 10–15-nA intensity. The primary beam was focused through the primary diaphragm to produce an ellipsoid, flat-bottomed crater of around 10–15-μm length and 5–10-μm width. Secondary positive Ca ions were accelerated at 10 kV and transfer optics of 150 μm and field aperture of 5000 μm were used. The analyses were performed without energy filtering and the energy window was fully opened. The circular focusing mode was used. The mass spectrometer entrance and exit slits were closed to achieve a mass resolution ($M/\Delta M$) of ≈5000. Secondary $^{40}Ca^+$ and $^{44}Ca^+$ ions were simultaneously measured in multicollection mode using two off-axis faraday cups (L′2 and H1). The relative gains of faraday cups and amplifiers were calibrated at the beginning of the analytical session using the Caméca built-in amplifier calibration routine (De Chambost, 1997). Count rates were typically in the 8×10^6–1×10^7-cps (counts per second) range for the $^{44}Ca^+$ on calcite reference materials and on foraminifera. All these analytical settings are summarized in Table 1. One analysis consists of 25 cycles of 3 s each.

3.2. Interferences

For the measurement of $\delta^{44}Ca$, a MRP (mass resolution power) of at least 4500 is required to resolve most common isobaric interferences, as shown in Table 2. We performed the measurement in multicollection mode with a MRP around ≈5000 (Fig. 1). The peaks of $^{40}Ca^+$

Table 2
Interferences and mass resolutions needed to resolve them from $^{40}Ca^+$ and $^{44}Ca^+$ peaks

Elements	MRP	Elements	MRP
Mass 40		Mass 44	
^{40}Ca	–	$^{88}Sr^{2+}$	−18 000
^{40}K	+28 000	^{44}Ca	–
^{39}KH	+4470	^{43}CaH	+3960
$^{24}Mg^{16}O$	+2301	$^{26}Mg^{18}O$	+1674
		$^{12}C^{16}O_2$	+1280

MRP: Mass resolution power needed to separate the peaks.
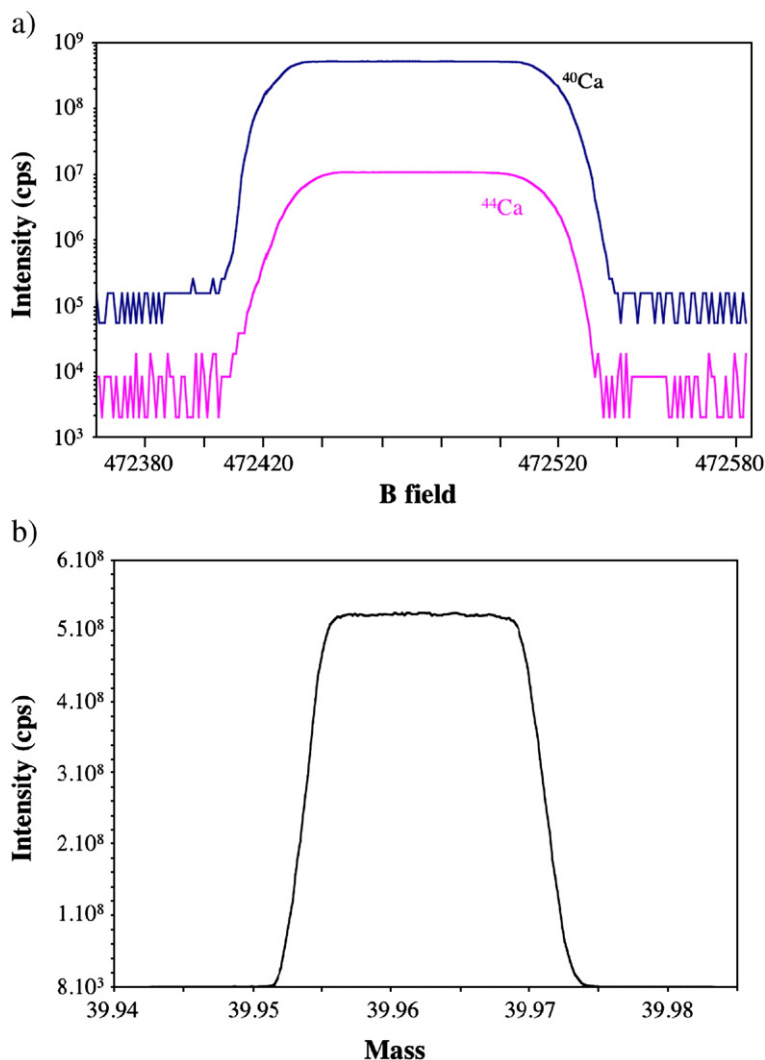
Fig. 1. High resolution mass spectra showing in a) the $^{40}Ca^+$ and $^{44}Ca^+$ peaks in multicollection and on a log scale, and in b) at mass 40, the $^{40}Ca^+$ peak on a linear scale.

and $^{44}Ca^+$ have a flatness dI/I (I = intensity) better than 0.1‰ for a range of 20 ppm. However, two interferences cannot be resolved at this MRP: the $^{40}K^+$ peak on $^{40}Ca^+$ and the $^{88}Sr^{2+}$ peak on the $^{44}Ca^+$ peak (needing +28 000 and −18 000 of MRP, respectively). To check for the possible interference of $^{40}K^+$ on $^{40}Ca^+$, we monitored mass 41 ($^{41}K/^{40}K \approx 576$) after each in situ analysis. The isobaric interference on mass 40 was found to be negligible in carbonates. To check if interference by $^{88}Sr^{2+}$ significantly effects our measurements, we also systematically measured mass 43.5 ($^{87}Sr^{2+}$). The counts were no more than 20–30 cps, representing around 230–350 cps of $^{88}Sr^{2+}$ (^{87}Sr has an abundance of 7.001% and ^{88}Sr 82.581%), negligible compared to the counts of $^{44}Ca^+$ ($8-10 \times 10^6$ cps).

3.3. Data notation and correction

The standard normally used as a reference material is NIST SRM 915a (Coplen et al., 2002; Carignan et al., 2004; Eisenhauer et al., 2004). However, other reference materials are used in the literature such as reagent calcium fluoride CaF_2, natural CaF_2, a *Tridacna* shell, seawater or an ultrapure $CaCO_3$ (e.g. Fletcher et al., 1997; Skulan et al., 1997; Zhu and MacDougall, 1998; Nägler et al., 2000; De La Rocha and DePaolo, 2000; Schmitt et al., 2003).

In the present study, calcium isotope ratios are expressed as a delta notation, $\delta^{44}Ca$, relative to NIST SRM 915a. All carbonate samples analysed by ion microprobe were first standardized with MEX. Then,
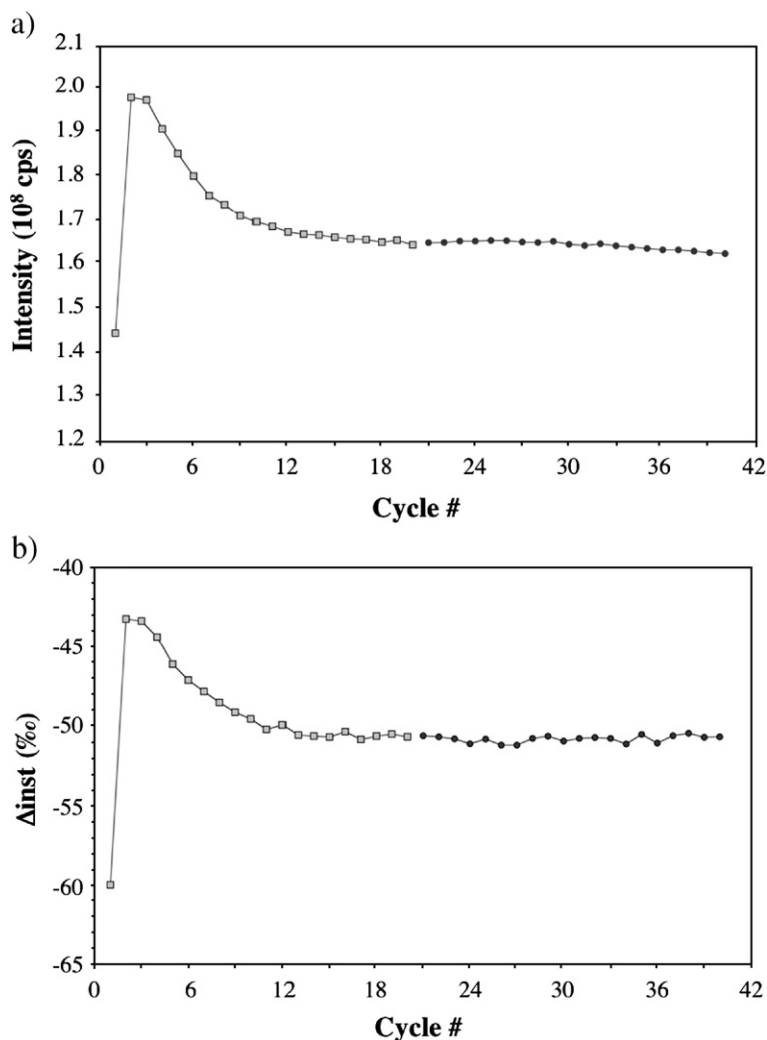
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Fig. 2. Intensity of $^{40}Ca^+$ (a) and Δ_{inst} (b) as a function of pre-sputtering time on MEX standard. Intensity and Δ_{inst} increase and reach a plateau after cycle #12. After this cycle, intensity and Δ_{inst} can be considered to be constant. Repeated measurement in the same spot (black circles) indicates no degradation of the $^{40}Ca^+$ intensity and of the Δ_{inst}.

each $\delta^{44}Ca$ measurement is calculated relative to NIST 915a according to the following relationship:

$$\delta^{44}Ca_{(sample,NIST915a)}$$
$$= \left(\delta^{44}Ca_{(MEX,NIST915a)} + 1000\right)$$
$$\times \left[\frac{\left(\frac{^{40}Ca}{^{44}Ca}\right)_{(sample,meas)}}{\left(\frac{^{44}Ca}{^{40}Ca}\right)_{(MEX,meas)}}\right] - 1000$$

$\left(^{44}Ca/^{40}Ca\right)_{(MEX,meas)}$ being the average of calcium isotope ratios measured in MEX calcite during the same analytical session as the sample measurements. Samples are analysed only if the drift is negligible for

MEX analyses and if the external reproducibility, given by $\delta^{44}Ca$ data for MEX, is better than $\pm 0.25‰$ (1σ).

3.4. Testing instrumental conditions

3.4.1. Pre-sputtering time

The sputtering time is a parameter likely to influence the sputter-ionization process (Benninghoven et al., 1987; Janssens et al., 2003) due to implantation of primary ions into the sample, and thus the IMF during $\delta^{44}Ca$ measurements in carbonates. We note a trend in both $^{40}Ca^+$ count rates and $\delta^{44}Ca$ values from cycle to cycle over a period of around 150 s. After this period, no consistent trend is observed, indicating that sputtering equilibrium has been reached (Fig. 2). However, to test
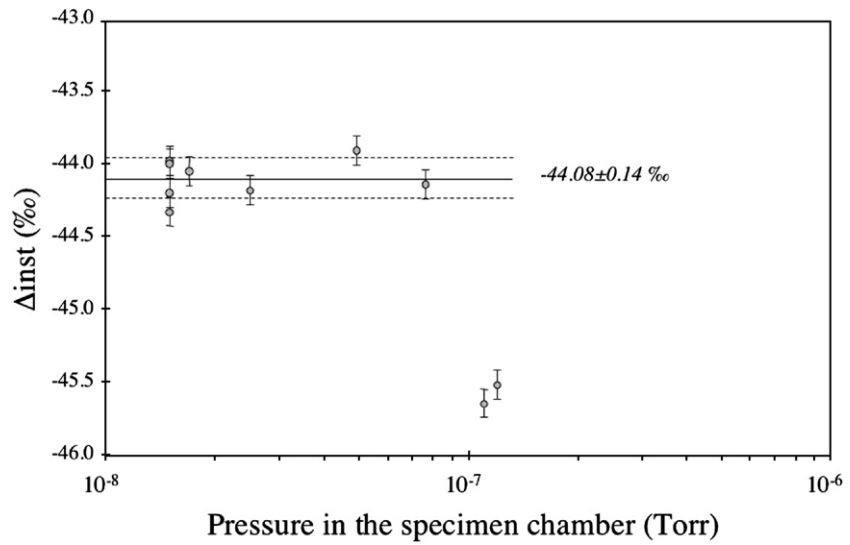
Fig. 3. Δ_{inst} measured on the MEX standard during the drop of the pressure in the specimen chamber immediately following introduction of the sample. The magnitude of Δ_{inst} decreases by $\approx 2.5‰$ over the 15-min pumping time. The solid line indicates the mean Δ_{inst} ($\overline{\Delta}_{inst}$) value calculated over measurements performed at pressures below 8×10^{-8} Torr. The dashed lines indicate $\overline{\Delta}_{inst} + 1\sigma$ and $\overline{\Delta}_{inst} - 1\sigma$ values, where σ is the standard deviation obtained for these measurements. Below a pressure of 8×10^{-8} Torr, Δ_{inst} values can be considered constant within a standard deviation of $\pm 0.14‰$, which does not exceed the 1σ external precision of measurements.

this observation, repeated measurements on the same spots were performed and no degradation of Δ_{inst} ($\Delta_{inst} = \delta^{44}Ca_{(MEX,meas)} - \delta^{44}Ca_{(MEX,true)}$) values was observed (Fig. 2). Consequently, before all the analyses, we applied a pre-sputtering time between 150 s and 180 s. A primary beam of 10–15 nA produces a crater of less than 1 µm into the calcite.

3.4.2. Pressure in the specimen chamber

Vacuum conditions are also likely to influence the sputtering-ionization process (e.g. Sangely et al., 2005). When the vacuum is poor, the probability that secondary ions strike particles present in the specimen chamber is increased and thus disturb the ion yield. When a certain vacuum is reached, this probability becomes very low. In
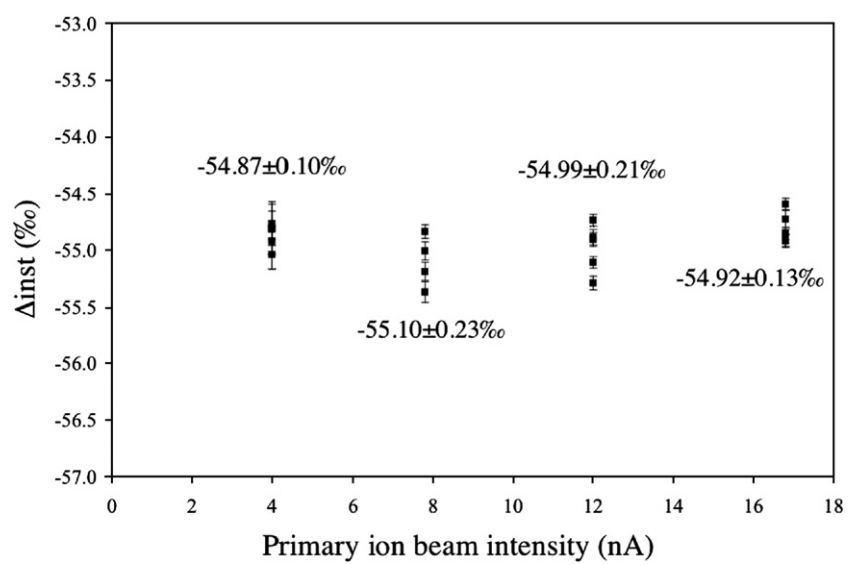

Fig. 4. Δ_{inst} values as a function of primary ion beam intensity for BRET 105E reference material measurements. Δ_{inst} was calculated using an arbitrary $^{44}Ca/^{40}Ca$ of 0.212116 (no absolute ratio is certified yet for carbonate reference material). We obtained average $2\sigma_n$ values of $\pm 0.2‰$ indicating that Δ_{inst} values can be considered as constant over a primary beam intensity ranging from 4 to 16.8 nA.
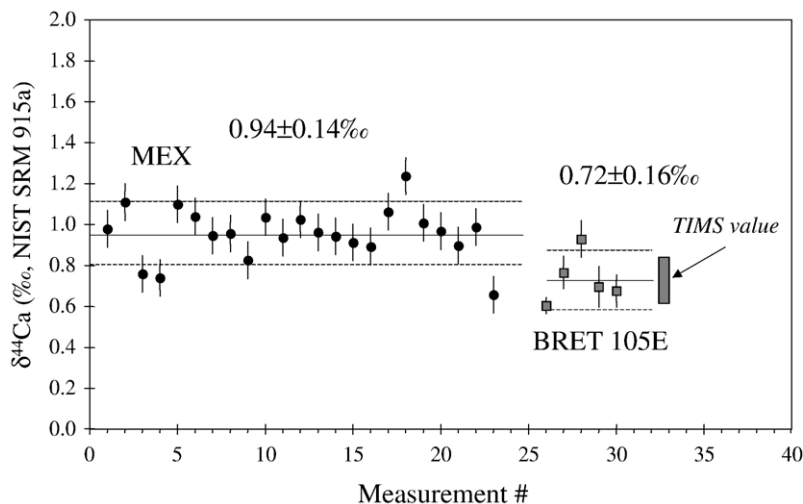
Fig. 5. Point-to-point reproducibility of $\delta^{44}Ca$ on MEX reference material obtained during a one-day session (black circles). Solid line indicates the mean $\delta^{44}Ca$ ($\bar{\delta}^{44}Ca$) obtained over the 23-repeated analyses and dashed lines indicate $\bar{\delta}^{44}Ca+1\sigma$ and $\bar{\delta}^{44}Ca-1\sigma$ values, where σ is the standard deviation from the mean $\delta^{44}Ca$ values. For $n=20$–25, we obtained typical 1σ values of ±0.13‰ for the standards. Values for BRET 105E (grey squares) were obtained using the $\varDelta_{inst}$ determined with MEX standard. Within instrumental errors, no difference is observed between $\delta^{44}Ca$ measured on BRET 105E by ion microprobe and the value obtained by TIMS (grey rectangle).

this section, we examine whether variations in the specimen chamber pressure affect the IMF. $\varDelta_{inst}$ values were monitored during the 15-min pumping following the introduction of the sample in the specimen chamber, when the pressure drops from 1.2×10^{-7} to 1.5×10^{-8} Torr. An increase of 1.4‰ was observed over this pressure range (Fig. 3). However, when the pressure reached a threshold of about 8×10^{-8} Torr, $\varDelta_{inst}$ can be considered as constant within a 1σ standard deviation of ±0.14‰, corresponding to the external reproducibility of measurements.

3.4.3. Primary beam intensity

In some cases, changes in the intensity of the primary ion beam could not be avoided during the course of an analytical session, due to slight change in pressure in the duoplasmatron source, or to the erosion of the primary diaphragm, for instance. The second lens of the primary column optical system (L2) was used to change the intensity of the primary ion beam. $\delta^{44}Ca$ measurements were repeated on BRET 105E reference material using primary ion beam intensities ranging from 4 to 16.8 nA. The $\varDelta_{inst}$ was found to be constant within an average 1σ standard deviation of ±0.2‰ over the whole intensity range (Fig. 4). The range of the primary beam intensity during all in situ analyses performed in this study was 10 to 15 nA.

3.4.4. Matrix effect

As the Mg content in inorganic calcites and also within the same test and between tests of foraminifera

can vary (e.g. Erez, 2003; Eggins et al., 2004), the influence of Mg content on the IMF has to be tested. The calcite reference materials used in this study have different MgO contents (0% for ENS 0, 0.012 wt.% for BRET 105E and 0.42 wt.% for MEX). This is completely within the range of MgO contents for Globorotaliids, which have less than 0.12 wt.% MgO (Blackmon and Todd, 1959). For the three calcite reference materials, we obtained the same IMF, within the analytical uncertainty, strongly suggesting that the Mg content does not affect the IMF at this level. The
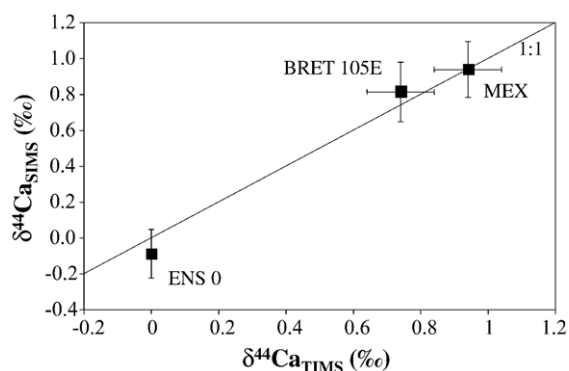

Fig. 6. $\delta^{44}Ca$ measured by SIMS relative to $\delta^{44}Ca$ measured by TIMS. Since MEX was used to determine the instrumental fractionation, its value is, by definition, exactly on the 1:1 line. Note that the two other reference materials are in good agreement with TIMS data, demonstrating the reliability of the ion microprobe $\delta^{44}Ca$ analysis.
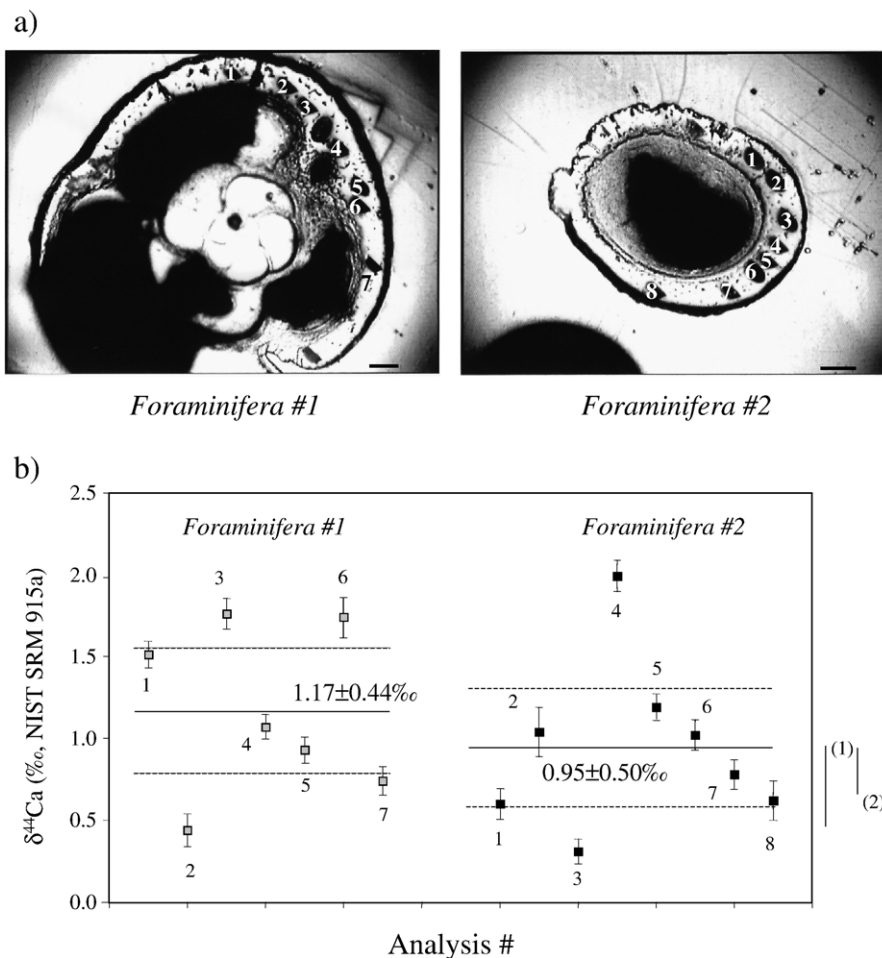
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Fig. 7. a) Pictures of the two specimen of *G. inflata* measured in this study. The position of the analytical points is indicated for each foraminifera. Scale bars are 15 μm. b) δ^{44}Ca intratest variability in the 2.8-Ma old *G. inflata* tests. Solid lines indicate the mean δ^{44}Ca ($\overline{\delta}^{44}$Ca) obtained over the two foraminifera analysis and dashed lines indicate $\overline{\delta}^{44}$Ca$+1\sigma$ and $\overline{\delta}^{44}$Ca-1σ values, where 1σ is the standard deviation. Despite significant intratest variability, mean δ^{44}Ca are similar for both tests, within external error. The ion microprobe δ^{44}Ca values are also compared with literature data: (1) Fantle and DePaolo (2005) for bulk carbonates with ages between 2.55 and 3.19 Ma, and (2) Sime et al. (2005) for *G. inflata* of different box-core locations in North Atlantic and Indian Ocean.

MgO content in the studied foraminifera was measured by electron microprobe and found to be systematically lower than the detection limit (≈ 0.05 wt.%). Therefore, the same IMF as for reference materials is expected.

3.4.5. Drift of instrumental mass fractionation with time

In some sessions, a slight drift in the IMF may be observed with time. This drift rarely exceeded 1‰ during a one-day analysis period (≈ 15 h). To check if there was a drift during the day, we applied the "standard bracketing" technique with measurements of MEX reference material every ≈ 40 points. If a drift lower than 1‰ was observed during one session, we applied a linear regression with time for the IMF drift

and we corrected each sample measurement accordingly. If the drift was too significant, the data were discarded.

4. Results

4.1. Reference material analyses

The point-to-point reproducibility (or external precision) was estimated by repeated analyses of three reference materials under strictly identical analytical conditions. The δ^{44}Ca values of the reference materials were constant within a typical 1σ standard deviation between ± 0.13 and 0.25‰ (Fig. 5). Consequently, the

calcium isotope composition of the reference materials can be considered homogeneous at the spatial resolution of the technique and within this uncertainty. The internal precision over the 20 cycles of a single measurement is less than 0.1‰.

To check the accuracy of ion probe technique, we used MEX as the reference material and BRET 105E and ENS 0 were considered as unknown samples. We calculated the $\delta^{44}Ca$ values of BRET and ENS 0 with the instrumental mass fractionation determined by the measurements of MEX. The ion probe value for BRET 105E was $0.72\pm0.16‰$ (1σ), which is in excellent agreement with the TIMS value $(\delta^{44}Ca=0.74\pm0.11‰)$, and the ion probe value for ENS 0 was $-0.09\pm0.14‰$, which is again indistinguishable from the theoretical value of 0‰ (Fig. 6).

4.2. Foraminifera tests

We applied the in situ Ca isotopes measurement technique developed on calcite reference materials and described above, on specimen of the planktonic foraminifera, *G. inflata*, dated at 2.8 Ma. The aim was to check the variability of $\delta^{44}Ca$ in natural foraminiferal tests.

We used the intensities of contaminant elements (Si, Al) to filter the calcium isotope data set and to remove any analyses that could be significantly affected by sample contamination (Allison and Austin, 2003; Vigier et al., 2007).

For this, we performed several in situ $\delta^{44}Ca$ analyses in two different tests of *G. inflata*. $\delta^{44}Ca$ values range from 0.44 to $1.96\pm0.2‰$ and 0.31 to $1.99\pm0.2‰$ (Fig. 7). The averages obtained for each foraminifera are very close: 1.17 ± 0.44 $(1\sigma)‰$ and 0.95 ± 0.5 $(1\sigma)‰$, respectively. These $\delta^{44}Ca$ values are also similar to $\delta^{44}Ca$ measurements of Sime et al. (2005) on *G. inflata* of different box-core locations in North Atlantic and Indian Ocean ($\delta^{44}Ca$ ranging from 0.67 to 0.9‰), suggesting that seawater $\delta^{44}Ca$ has not varied significantly since 2.8 Ma. Our $\delta^{44}Ca$ measurements are also similar to calcium isotopic values obtained on bulk carbonates of similar ages (2.55 and 3.19 Ma: $0.87\pm0.1‰$ and $0.67\pm0.4‰$, respectively, Fantle and DePaolo, 2005).

5. Discussion

$^{44}Ca/^{40}Ca$ ratios measured by ion microprobe are constant within analytical uncertainty in inorganic calcite reference materials, but are variable in foraminifera tests. This kind of variability has already been observed for boron and oxygen isotopes in corals. The $\delta^{11}B$ and $\delta^{18}O$ variations have been shown to be independent of analytical artefacts and have been related to pH variations and to kinetic effects during calcite precipitation (Rollion-Bard et al., 2003). The scale of sampling of our study should be able to detect $\delta^{44}Ca$ heterogeneities, which would not be apparent when analysing the entire foraminiferal test chambers. A significant intratest variability has already been observed by Allison and Austin (2003) using SIMS for Sr/Ca and Mg/Ca ratios in benthic foraminifera *Ammonia batavus* and by Eggins et al. (2004) for Mg/Ca ratio measured in the planktonic foraminifera *Orbulina universa*. Allison and Austin (2003) interpreted these variations as the juxtaposition of calcite of different ages as the test is formed. Eggins et al. (2004) suggest that these variations are due to diurnal changes in the biological activity of algal symbionts. Erez (2003) also observed Mg variation in benthic foraminifera *Amphistegina lobifera* and linked it to differences in the biomineralization processes of primary and secondary calcites (Bentov and Erez, 2006). Several factors could affect the $\delta^{44}Ca$ values of the foraminifera chambers i.e. variations in precipitation rates, temperature, possible ontogenic effects or differences between primary and secondary calcite.

A recent study by Lemarchand et al. (2004) of calcium isotopes in synthetic calcites suggests that $\delta^{44}Ca$ is strongly dependent on the rate of crystal growth. This relationship was also observed in sclerosponges (*Ceratoporella nicholsoni* and *Acanthochaetetes wellsi* (Gussone et al., 2005)). The relationship between Ca isotope fractionation of calcite (α_{cc}) and the precipitation rate R is the following:

$$1000 \times \ln(\alpha_{cc}) = -1.91 + 0.37 \times \log(R)$$

with R in $\mu mol\ m^{-2}\ h^{-1}$ (Gussone et al., 2005).

Using this formula, our measured $\delta^{44}Ca$ range would correspond to a R variation of 500 000 $\mu mol\ m^{-2}\ h^{-1}$. In foraminifera, the precipitation rate has been estimated to range between 1–4 $\mu mol\ m^{-2}\ h^{-1}$ (Carpenter and Lohman, 1992; Lea et al., 1995) and $\approx 40\ \mu mol\ m^{-2}\ h^{-1}$ (Erez, 1983). So, if the whole $\delta^{44}Ca$ variation measured in the two specimen of *G. inflata* was due to variations in precipitation rate, this would require unrealistic changes in growth rate. Therefore variation in R alone can hardly explain our $\delta^{44}Ca$ range.

For some species (e.g. *G. sacculifer*), some authors have found that temperature variation can affect the calcium isotope fractionation by up to 0.2‰ per °C (Nägler et al., 2000). *G. inflata*, like all globorotaliids, produce calcite both in surface waters where they live and

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in deeper water where they sink for their reproduction (Hemleben et al., 1989). The δ^{44}Ca range measured in each foraminifera would correspond to a temperature variation of 7.6 and 8.4±0.2 °C, respectively. This temperature variation is compatible with the mode of calcification of *G. inflata*. However, Sime et al. (2005) found no relationship between δ^{44}Ca and temperature for this species in a temperature range of 2.4 °C (from 9.4 to 11.8 °C). Without further constraints on the behaviour of δ^{44}Ca in *G. inflata* as a function of temperature, it is difficult to attribute δ^{44}Ca variations entirely to temperature changes.

Some ontogenic (development stage) effects on proxy incorporation have been identified in benthic (Wefer et al., 1981; Boyle, 1995; Hintz et al., 2006) and in planktonic foraminifera (Bijma et al., 1998). The latter found δ^{18}O and δ^{13}C variation in planktonic foraminifera *Globigerinella siphonifera* of up to 0.4‰ for oxygen isotopes and 1.5‰ for carbon isotopes due to ontogenic effects. However, such changes over the lifespan of individuals are not fully understood yet. Hintz et al. (2006) proposed that this ontogenic effect could be due to biomineralization processes or precipitation of an unstable phase of Mg-rich calcium carbonate. As there are some ontogenic effects on δ^{18}O and δ^{13}C detected in foraminifera tests, these effects may also change the δ^{44}Ca values.

In perforate foraminifera (planktonic and benthic), the test consists of successive laminations of primary and secondary calcite (Erez, 2003). The primary calcite precipitates over newly formed organic matrix. This calcite is enriched in Mg, Na and S. The secondary calcite consists of a low-Mg calcite. This calcite is formed from the vacuolization of seawater after a modification within the cytoplasm (decrease of Mg/Ca ratio, increase of pH…). It was recently observed that the primary calcite is at least 2‰ lower in δ^{18}O than the secondary calcite (Rollion-Bard et al., 2006). Even if the exact cause of this depletion in the light isotope is not yet fully understood, it is probably linked to the two different processes of biomineralization involved in the formation of primary and secondary calcite. This difference could also have some effect on the δ^{44}Ca, although no relationship was found between our δ^{44}Ca values and Mg/Ca ratios measured in the same spot (not shown here).

The natural variations of *G. inflata* δ^{44}Ca cannot be explained by temperature change only and/or by growth variation only. Rather, differences between primary/secondary calcite and ontogenic effects are inferred. In fact, δ^{44}Ca variations are probably the result of the combination of several varying parameters. In order to deconvolve the effect of each parameter on δ^{44}Ca internal variations measured by in situ methods, studies of experimentally grown foraminifera are required.

6. Conclusions

An ion microprobe technique has been developed for δ^{44}Ca measurements in carbonates with a spatial resolution of about 20 μm. Under suitable vacuum conditions and after 3 min of pre-sputtering, it has been shown that the instrumental fractionation, Δ_{inst}, was constant with a standard deviation ≈ 0.15‰, in spite of variations in analytical conditions including primary ion beam intensity and sputtering time. Under these analytical conditions, stability of Δ_{inst} values over the duration of a session can be expected.

We applied this technique to two planktonic foraminifera, *G. inflata*, dated at 2.8 Ma. The overall variation of δ^{44}Ca measured in a single test is 1.7‰, about 10 times the uncertainty of the measurement performed on calcite reference materials. This intratest variation can be explained by several processes such as temperature variation, precipitation rate variation, ontogenic effects or differences between primary and secondary calcite. It is unlikely that only one of these processes is responsible for the total variation, more probably the measured δ^{44}Ca range results from a combination of several mechanisms.

Despite this intratest variation, the average δ^{44}Ca for each foraminifer test is constant and is in agreement with previous δ^{44}Ca data for this age. This strongly suggests that in situ δ^{44}Ca measurements in foraminifera test are significant when ≈ 5 measurements are performed on a single test.

Measurements of Ca isotopes by ion microprobe on foraminifera grown under controlled conditions are clearly needed in order to better understand the role of the different possible causes of the variations highlighted in this study.

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