Research Article

Precision and long-term stability of clumped-isotope analysis of CO_2 using a small-sector isotope ratio mass spectrometer

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RATIONALE: The ratio of the measured abundance of ^{13}C–^{18}O bonding CO_2 to its stochastic abundance, prescribed by the $\delta^{13}C$ and $\delta^{18}O$ values from a carbonate mineral, is sensitive to its growth temperature. Recently, clumped-isotope thermometry, which uses this ratio, has been adopted as a new tool to elucidate paleotemperatures quantitatively.

METHODS: Clumped isotopes in CO_2 were measured with a small-sector isotope ratio mass spectrometer. CO_2 samples digested from several kinds of calcium carbonates by phosphoric acid at 25 °C were purified using both cryogenic and gas-chromatographic separations, and their isotopic composition ($\delta^{13}C$, $\delta^{18}O$, Δ_{47}, Δ_{48} and Δ_{49} values) were then determined using a dual-inlet Delta XP mass spectrometer.

RESULTS: The internal precisions of the single gas Δ_{47} measurements were 0.005 and 0.02 ‰ (1 SE) for the optimum and the routine analytical conditions, respectively, which are comparable with those obtained using a MAT 253 mass spectrometer. The long-term variations in the Δ_{47} values for the in-house working standard and the heated CO_2 gases since 2007 were close to the routine, single gas uncertainty while showing seasonal-like periodicities with a decreasing trend. Unlike the MAT 253, the Delta XP did not show any significant relationship between the Δ_{47} and δ^{47} values.

CONCLUSIONS: The Delta XP gave results that were approximately as precise as those of the MAT 253 for clumped-isotope analysis. The temporal stability of the Delta XP seemed to be lower, although an advantage of the Delta XP was that no dependency of δ^{47} on Δ_{47} was found. Copyright © 2012 John Wiley & Sons, Ltd.

The ratio of the measured abundance of $^{13}C^{18}O^{16}O$ digested from the carbonate mineral to its stochastic abundance, calculated from the $\delta^{13}C$ and $\delta^{18}O$ values, varies according to the formation temperature of the carbonate. Recently, clumped-isotope thermometry, which uses this ratio, has been adopted as a new tool to elucidate paleotemperatures quantitatively.[1] This novel tool requires information for neither carbon nor oxygen isotopic composition in the water where the carbonates have grown. Therefore, clumped-isotope thermometry has an advantage over conventional oxygen-isotope thermometry in cases where the isotopic composition of the water is unknown.

The theory of this thermometry is described elsewhere.[2,3] Briefly, carbonate minerals such as calcium carbonate are expected to contain ionic groups of $^{13}C^{18}O^{16}O_2$, $^{12}C^{18}O^{16}O_2$, $^{12}C^{16}O_3$, and $^{13}C^{16}O_3$ at thermodynamic equilibrium:[1]

$$K_{47} = \frac{\left[^{13}C^{18}O^{16}O_2^{2-} \right]\left[^{12}C^{16}O_3^{2-} \right]}{\left[^{12}C^{18}O^{16}O_2^{2-} \right]\left[^{13}C^{16}O_3^{2-} \right]} \quad (1)$$

The equilibrium constant K_{47} (Eqn. (1)) approaches the stochastic distribution at temperatures higher than ~1300 K and increases with decreasing temperature by excess formation of ^{13}C–^{18}O bonds. Consequently, the ratio K_{47}/K_{47}^*, where K_{47}^* is the stochastic distribution, is controlled only by temperature. One can obtain the temperature at which the carbonate was grown by measuring the $^{13}C/^{12}C$, $^{18}O/^{16}O$ and $(^{13}C^{18}O^{16}O)/(^{12}C^{16}O_2)$ ratios of the carbon dioxide generated from the carbonate sample, because the K_{47}/K_{47}^* ratio is preserved with an offset during acid digestion (predicted as 0.23‰ at 25 °C).[4] Dennis et al.[5] described a fourth-order polynomial fit for the case of CO_2 molecules, based on calculations for zero-point energy and normal mode wave numbers provided by Wang et al.,[2] as:

$$ln \frac{K_{47}}{K_{47}^*} = 0.003 \left(\frac{1000}{T} \right)^4 - 0.0438 \left(\frac{1000}{T} \right)^3$$
$$+ 0.2553 \left(\frac{1000}{T} \right)^2 - 0.2195 \left(\frac{1000}{T} \right) + 0.0616 \quad (2)$$

Therein, the left side of Eqn. (2) can be expressed from Eqn (1) as:

$$ln \frac{K_{47}}{K_{47}^*} = ln \frac{R_{47}}{R_{47}^*} - ln \frac{R_{46}}{R_{46}^*} - ln \frac{R_{45}}{R_{45}^*} \quad (3)$$

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where R_{47}, R_{46}, and R_{45}, respectively, represent $^{13}C^{18}O^{16}O/$ $^{12}C^{16}O_2$, $^{12}C^{18}O^{16}O/^{12}C^{16}O_2$ and $^{13}C^{16}O_2/^{12}C^{16}O_2$. The superscripts * correspond to the ratios for stochastic distributions.

The term $ln(K_{47}/K_{47}^*)$ is defined as Δ_{47} with a unit of permil (‰). Most previous studies have used the approximate term $(R_n/R_n^* - 1)$ instead of $ln(R_n/R_n^*)$ in Eqn. (3) because each of the R_n/R_n^* values is approximately unity.

This equilibrium constant has also been validated experimentally through the analysis of the sets of CO_2 gases digested from synthetic calcites precipitated at particular temperatures[6–8] and from assemblages of various biogenic carbonates.[1,9] It has been widely applied to paleotemperature reconstruction, especially for terrestrial carbonates and those formed during the early Cenozoic or earlier.[8–16]

Similarly to Δ_{47}, we can define Δ_{48} and Δ_{49} as:

$$\Delta_{48} = ln\frac{K_{48}}{K_{48}^*} = ln\frac{R_{48}}{R_{48}^*} - 2ln\frac{R_{46}}{R_{46}^*} \qquad (4)$$

$$\Delta_{49} = ln\frac{K_{49}}{K_{49}^*} = ln\frac{R_{49}}{R_{49}^*} - 2ln\frac{R_{46}}{R_{46}^*} - ln\frac{R_{45}}{R_{45}^*} \qquad (5)$$

Since the foundation of the theory and methodology by Eiler and Schauble,[17] and the establishment of the isotope-temperature relation by Ghosh *et al.*,[6] there has been considerable progress in clumped-isotope thermometry. Huntington *et al.*[18] considered the theoretical and practical limits of precision and methods of standardization with regard to instrumental linearity, and demonstrated a typical precision of 0.01‰. With a specific type of isotope ratio mass spectrometer, the MAT 253 (ThermoFinnigan, Bremen, Germany), the calculated Δ_{47} values showed a positive linear relationship with measured δ^{47} ($R_{47,sample}/R_{47,WG} - 1$) values. They applied a correction to this relationship, based on results from heated gases because these should, in principle, have identical Δ_{47} values, irrespective of the δ^{47} values. Subsequently, automated systems with a Kiel-type carbonate device were developed at two different laboratories.[19,20] Schmid and Bernasconi[19] achieved a reduction in the sample size to less than 2 mg for calcium carbonate, and obtained result that were comparable in precision with those from conventional amounts of samples (8 mg). Zaarur *et al.*[23] reduced the sample size to 3–4 mg without a carbonate device, by modification of CO_2 flow rates at the dual inlet of the mass spectrometer. In addition, Dennis *et al.*[5] defined a reference frame to calibrate the measured isotopic composition that might be affected by scale contraction because of a recombination reaction (scrambling) in the ionization chamber of the mass spectrometer.

All the described studies were conducted with a MAT 253 mass spectrometer, which can achieve an external precision (1 SE) broadly better than 0.03‰. The performance of any other type of isotope ratio mass spectrometer has never been validated.[1] Here we demonstrate both internal and external precision and conduct a comparison with data obtained in earlier studies, using a smaller sector isotope ratio mass spectrometer: the Delta XP (ThermoFinnigan). This report describes long-term results obtained for an international standard material (NBS-19), in-house reference materials, and heated gases for the last 5 years, along with calibration results

according to Dennis *et al.*[5] Differences between the performance of these two instruments and several specific features of the Delta XP are also presented.

EXPERIMENTAL

Isotope ratio mass spectrometer and carbonate materials used for this study

This study used a 3 kV acceleration voltage isotope ratio mass spectromter, the Delta XP, with a dual inlet configured to collect ion beams corresponding to *m/z* 44, 45, and 46 (read through 10^8 to 10^{11} ohm resistors), as well as *m/z* 47, 48, and 49 (read through 10^{12} ohm resistors) simultaneouly. For our purposes, the original triple collector configuration was modified to nine collectors for rare isotopologue analyses on CO_2. The sensitivity, as defined by the manufacturer, is that 1500 molecules of CO_2 are required to produce single ion of *m/z* 44. The ion source linearity is 0.02‰ per 1 nA of mass-44 ion current. All the measurements were made in dual-inlet mode with respect to the reference CO_2 gas (Oztech Trading Corp., Safford, AZ, USA), whose $\delta^{13}C$ and $\delta^{18}O$ values are −10.97 (‰ PDB) and +31.31 (‰VSMOW), respectively, while maintaining a typical source (bellow) pressure of 80 hPa to obtain the mass-44 ion beam at a voltage of 10 V. Most analyses involved 100 cycles of sample-to-reference combinations. Each cycle involved a signal integration time of 8 s.

Our programme of clumped-isotope analyses started in 2007. The total number of acquisitions exceeded 8000 by November 2011. During this period, we occasionally used this instrument in the GC-C-IRMS mode for the analysis of volatile organic compounds, by connecting a gas chromatograph-combustion unit. It is noteworthy that no significant difference was found in the clumped-isotope results between those obtained before and after GC-C-IRMS operations.

This study describes results obtained for the international carbonate standard NBS-19 and synthetic calcium carbonate (Wako Pure Chemical Industries Ltd., Osaka, Japan; hereinafter Wako) as an in-house reference. This synthetic carbonate was produced by bubbling the mixed gas CO_2/N_2 into $CaCl_2$ solution. When the initial $CaCl_2$ concentration was set to 0.3 mol/L, only rhombic calcite could be formed in the final product (Wako, personal communication). The typical grain size of these carbonate powders was 40–50 μm. In addition, results for another in-house reference (Carrara marble provided by Dr Hagit Affek, Yale University, New Haven, CT, USA; hereinafter CM2) are shown for comparison with previous studies.

Sample preparation

The digestion of carbonate samples involved reacting its powder with anhydrous phosphoric acid, following the methods described by McCrea[21] and Swart *et al.*[22] The extraction procedure used McCrea-type reaction vessels for the carbonate reaction. The reaction temperature of 25 °C in a water bath was maintained with a precision of better than 0.1 °C. This was followed by the vacuum-based cryogenic separation protocol described by Ghosh *et al.*[6] to trap the produced CO_2 and purify it from any water contamination. Figure 1 shows the design of a stainless steel vacuum extraction unit fabricated for these experiments. Most of the other contaminants apart from water,
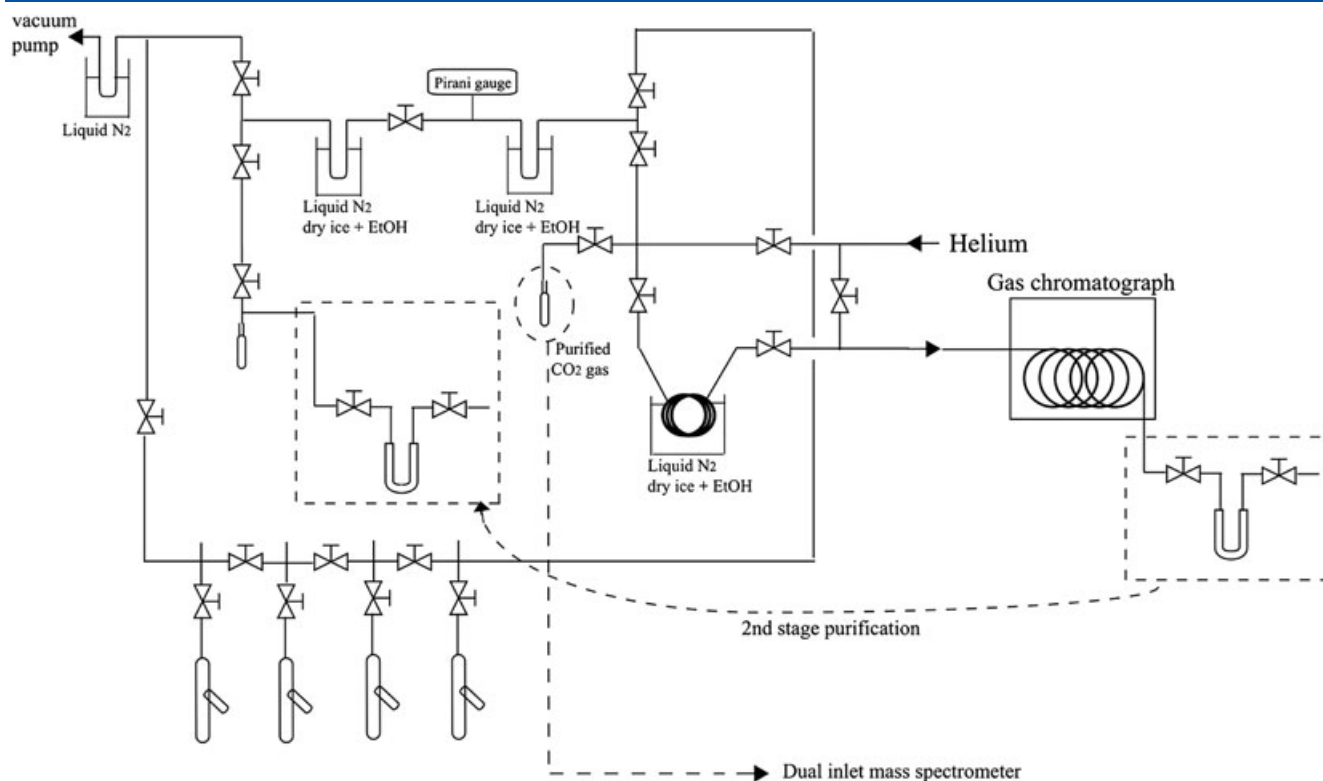
Figure 1. Schematic diagram of the CO_2 purification line.

such as hydrocarbons and halocarbons, were difficult to remove efficiently using a cryo-trapping protocol.[17] These could, however, be easily removed by gas chromatography, and could be monitored in all samples using simultaneous analysis of masses 47, 48 and 49. These contaminants typically contributed nearly equally to all three of these masses, thereby producing distinctive and highly correlated relations between smaller changes in δ^{47} values (tenths of permil) and proportionately greater changes in the δ^{48} and δ^{49} values of several permil and tens of percent, respectively.[17] Therefore, each CO_2 sample analyzed in this study was entrained in a helium stream controlled using a mass flow controller with a flowing velocity of 5 mL min^{-1} and passed through a capillary column (PoraPLOTQ, 25 m × 0.32 mm i.d.; Varian Inc., Palo Alto, CA, USA) held at −10 °C, and re-collected in a U-shaped glass trap immersed in a liquid nitrogen dewar. The column was held in a gas chromatograph (HP-6890; Agilent Technologies Inc., Santa Clara, CA, USA), fitted with a cryo-valve for purging with the boil-off gas from a tank of liquid nitrogen. For our study, a typical sample size of 10–15 mg (ca. 100–150 mmol $CaCO_3$) was maintained. The elution time to complete the CO_2 purification was typically 40 min, with a collection efficiency of better than 95%. Finally, the cleaned CO_2 was condensed back into the small glass ampoule and transferred into the dual-inlet system of the Delta XP. The GC column was baked at 200 °C at a helium flow rate of 5 mL min^{-1} for more than 30 min after each sample treatment.

Some CO_2 gases were frozen back into the ultrapure synthetic quartz tube (6 mm o.d. provided by Shin-Etsu Chemicals Co. Ltd., Tokyo, Japan) and sealed after isotopic determination;

then heated in a muffle furnace at 1000 °C for 1 h. These heated CO_2 gases were quickly quenched to room temperature, and then purified according to the protocol described above.

RESULTS AND DISCUSSION

Internal precision obtained with the Delta XP

Repeated analyses of the reference CO_2 gas were conducted to confirm the internal precision immediately after installation of the analytical system (April–May 2007) and it was checked again recently (November 2011; presented in Table 1) for comparison with previous investigations. Intensities of signals for m/z 44 and number of cycles for sample-to-reference combination were obtained for 12, 8 and 16 V, and 400, 500 and 500 cycles, respectively, in April, May 2007, and November 2011. The signal integration time in each cycle was 8 s for all the analyses. The one-standard errors of Δ_{47} were 7, 7 and 5 ppm, respectively, for each case mentioned above. The value of 5 ppm was consistent with that obtained using a MAT 253 mass spectrometer involving 400 cycles at 16 V (MS-1 in Huntington et al.[18]). It should be noted that if our recent analysis had stopped at up to 400 cycles, the standard error value would have been 6 ppm.

For routine analyses, the signal on m/z 44 and cycles were set to 10 V and 100 cycles, respectively. Those average standard errors of internal precisions obtained during May 2007 to June 2011 were 17–21 ppm (Table 2). A range of 13–16 ppm was obtained using MAT 253 analysis, for which the signal and

Table 1. Optimum precisions obtained by the Delta XP with single CO_2 gas analysis

Measured date	Signal at *m/z* 44 (V)	Number of cycles	Total integration time (s)	Standard error[a] (ppm)				
				$\delta^{13}C$	$\delta^{18}O$	Δ_{47}	Δ_{48}	Δ_{49} (‰)
03 Apr 2007	12	400	3200	0.4	0.8	7	34	2
02 May 2007	8	500	4000	0.5	0.8	7	37	3
10 Nov 2011	16	500	4000	0.5	0.6	5	26	2
MAT253[b]	*16*	*400*	*3200*			*5*		

[a]Standard errors are calculated by dividing standard deviations by the square root of cycles. All values are expressed in ppm except for Δ_{49} (in ‰).
[b]Data from Huntington *et al.*[18]

Table 2. Average internal precisions for routine analyses. Numbers in parentheses represent standard deviations for each standard error distribution

	Average standard error (ppm)				
	$\delta^{13}C$	$\delta^{18}O$	Δ_{47}	Δ_{48}	Δ_{49} (‰)
NBS-19	2 (0.6)	2 (1.1)	18 (5)	106 (46)	18 (8)
Wako	2 (0.8)	3 (1.9)	17 (5)	119 (40)	20 (9)
Heated gas	3 (3.3)	3 (2.6)	21 (8)	138 (67)	31 (45)

cycles were 16 V and 60–90 cycles.[18] Comparison of the results suggested that that the Delta XP yielded similar results to those obtained using the MAT 253.

Determining the isotopic composition of the in-house working standard and its long-term variation

The Δ_{47} value of the in-house working standard material (Wako) was calibrated with respect to the three-set results of NBS-19 and various heated gases (Table 3). In May, October 2007 and February 2010, all three materials were measured within 3 weeks. Except for NBS-19 in February 2010, independently replicated analyses were conducted for each material for each period. The standard errors shown in Table 3 were treated as short-term external precisions. It was particularly interesting that these showed a range of 9–21 ppm, indicating similarity to those achieved by single-gas analyses (Table 2), implying that the uncertainty of the Δ_{47} values attributable to preparation processes (acid digestion, vapor, and/or hydrocarbon removal) was apparently negligible in our system.

Table 3. Δ_{47} values of NBS-19, in-house Wako calcium carbonate and various heated gases. Values in the upper panel are uncalibrated and expressed relative to the Oztech reference CO_2 gas. Values in the lower panel are calibrated with respect to an assigned value of NBS-19 by Dennis *et al.*[5] and a calculated value at 1000 °C by Wang *et al.*[2]

	NBS-19 (‰)	SE (ppm)	*n*	Wako (‰)	SE (ppm)	*n*	Heated gas (‰)	SE (ppm)	*n*
wrt reference gas									
May–Jun 2007	−0.736	11	6	−0.424	21	2	−1.069	14	9
Oct–Nov 2007	−0.739	18	5	−0.441	17	4	−1.089	18	3
Feb–Mar 2010	−0.744		1	−0.429	9	6	−1.051	15	9
average	−0.740			−0.431			−1.070		
SD	0.004			0.009			0.019		
calibrated with NBS-19 and heated gas									
May–Jun 2007	0.392[a]			0.704			0.027[b]		
Oct–Nov 2007	0.392[a]			0.690			0.027[b]		
Feb–Mar 2010	0.392[a]			0.708			0.027[b]		
average				**0.701**					
SD				0.009					

[a]Data from Dennis *et al.*[5]
[b]Data from Wang *et al.*[2]

For the calibration of the Wako CaCO$_3$, two published values were used for NBS-19 and heated CO$_2$ at 1000 °C, according to the calculation reported by Dennis *et al.*[5] The Δ_{47} value of 0.392 for NBS-19 used here was an average value calibrated from four different laboratories, as compiled by Dennis *et al.*[5] For heated CO$_2$, the value of 0.0266 was used, as calculated by Wang *et al.*[2] The measured Wako Δ_{47} value for each period was calibrated by extrapolating the linear relation of these values (Eqn. (6)):

$$\Delta_{wako\_SCR} = \left(\Delta_{wako\_SCR} + 1\right)$$
$$\times \frac{\left(\Delta^*_{NBS\_HG} + 1\right)}{(\Delta_{NBS\_WG} + 1)/(\Delta_{HG\_WG} + 1)} - 1 \quad (6)$$

where the subscripts 'wako_SCR', 'NBS_HG', 'NBS_WG' and 'HG_WG' represent the Δ_{47} value of Wako with respect to the stochastic distribution, the NBS-19 value with respect to heated CO$_2$, the NBS-19 value with respect to Oztech reference CO$_2$ and the heated CO$_2$ value with respect to Oztech reference CO$_2$, respectively. Superscript * represents the calibrated Δ_{47} value. Using this equation, an average value of 0.701 was obtained for Wako. It should be noted that the standard deviations of the Wako Δ_{47} values were equal (at 9 ppm)

for the measured and the calibrated results, whereas the uncertainty in the calibrated values had been expected to be smaller than in the measured values if the latter were affected by long-term fluctuations, as described below. For these periods, however, the effects caused by scale contraction and those related to mass spectrometric conditions might have been similar.

The long-term variations in the measured Δ_{47} values of Wako and various heated gases during May 2007 to June 2011 are depicted in Fig. 2. Because the $\delta^{13}C$ and $\delta^{18}O$ values of the heated gases showed large variations depending on source materials, these values are not plotted in Fig. 2. The standard deviations, defined as long-term external precisions, of the Wako $\delta^{13}C$, $\delta^{18}O$, Δ_{47}, Δ_{48} and Δ_{49} values were 0.048, 0.138, 0.033, 0.3, and 52‰, respectively. Those of the heated gases were 0.043, 1.1 and 183, respectively, for the Δ_{47}, Δ_{48} and Δ_{49} values. The short-term standard deviations of the Δ_{47} values ranged between 0.021 and 0.044‰, averaging 0.033‰ (from Table 3), which was consistent with its 4-year average value. This consistency implied that longer-term variation of Δ_{47} relative to the short term of weeks was sufficiently small for sufficient precision to be obtained. In other words, the short-term variation in Δ_{47} was dominant in controlling the uncertainty. It was also interesting to find that the standard deviations of the Δ_{47} values of both Wako and
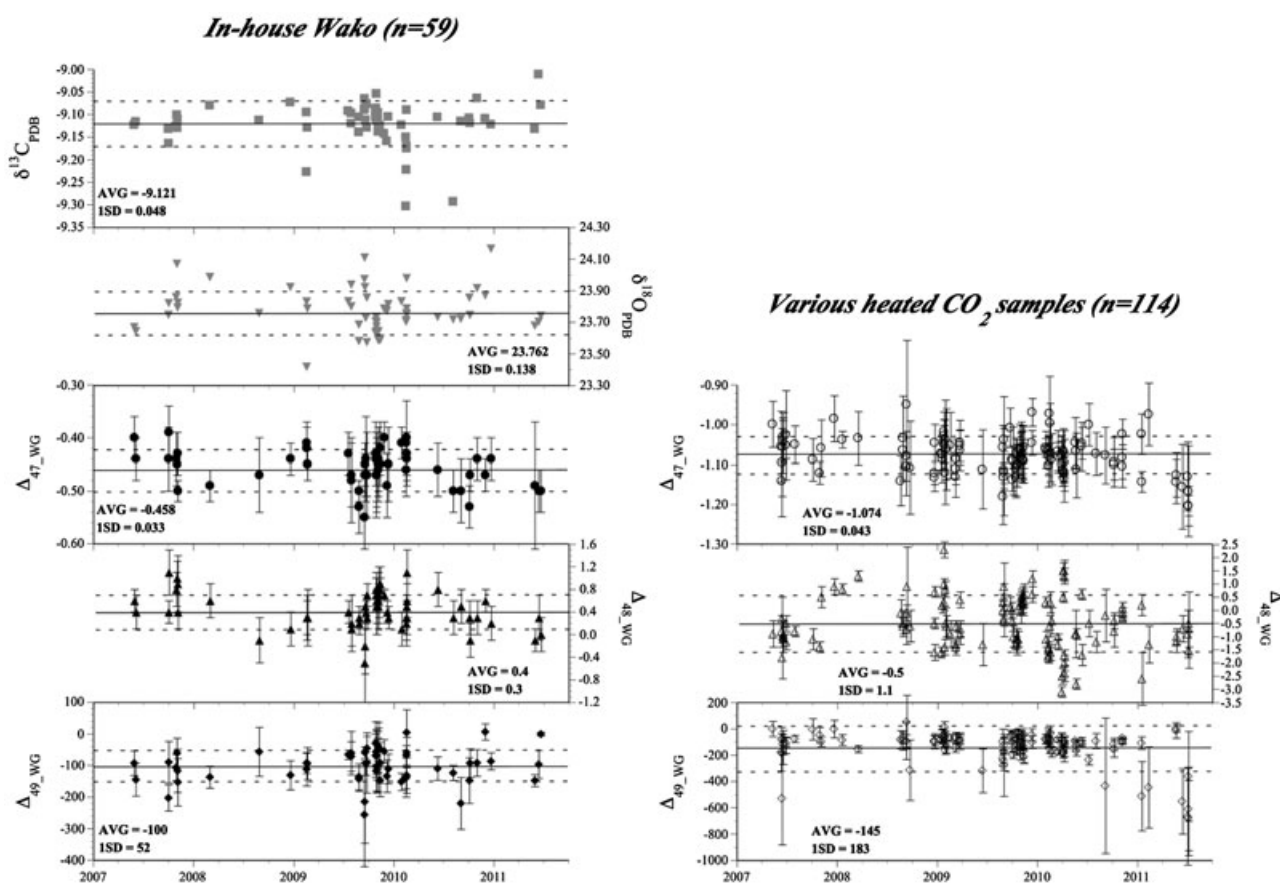

Figure 2. Left: Measurement results of $\delta^{13}C$, $\delta^{18}O$, Δ_{47}, Δ_{48}, Δ_{49} values for Wako since 2007 to 2011. Values for $\delta^{13}C$ and $\delta^{18}O$ are calibrated relative to the PDB scale and the values for Δ_{47}, Δ_{48}, and Δ_{49} are relative to the Oztech Working gas. The average and standard deviation of each isotope ratio are expressed as solid and dashed lines, respectively. The corresponding standard deviations for single measuremnts of $\delta^{13}C$ and $\delta^{18}O$ values are small enough within those symbols. Right: Measurement results of Δ_{47}, Δ_{48}, and Δ_{49} for heated gas since 2007 to 2011.

the heated gases were slightly better than those of the Wako $\delta^{13}C$ and $\delta^{18}O$ values, although these uncertainties of single-gas analysis were 10–20 times smaller than that of Δ_{47}. This was also found to be the case in the short-term precisions (Table 2), and might be attributable to errors in the $\delta^{13}C$ and $\delta^{18}O$ values being sensitive to kinetic isotope fractionation as a result of incomplete CO_2 recovery during preparation, although the error in Δ_{47} was not influenced greatly relative to them. If kinetic isotope fractionation were constrained by Knudsen-type gas diffusion, the changes in the $\delta^{13}C$ and $\delta^{18}O$ values during the gas diffusion would have been −11.2 and −22.2‰, respectively. In this case, the change in Δ_{47} would have been +0.5‰, less than one-twentieth of that in the $\delta^{13}C$ value.[17] Huntington *et al.*[18] indicated that δ^{47} values in chemically pure CO_2 were not affected significantly by analytical artifacts. Our results support their observations.

To clarify the long-term variations in Δ_{47}, although sufficiently small, the monthly average values were calculated from the data presented in Fig. 2; these 3-month running averages are depicted in Fig. 3. The differences in Δ_{47} between Wako and heated gases are also indicated in Fig. 3. Three distinct features are apparent in the upper panel. First, significant annual variations were found in both Wako and heated gases, with ranges of 0.05–0.07 and 0.06‰, respectively. Second, the Δ_{47} values of both Wako and the heated gases decreased substancially from 2007 to 2011. Their annual decrements are 8 and 9 ppm, respectively, without a significant difference between them. Consequently, no monotonic trend was evident in the difference between Wako and heated gas (Fig. 3, bottom). The reasons for these decreasing trends were unclear, but they might be attributed to the isotopic enrichment of our reference gas (Oztech) introduced to the reference side of the mass spectrometer, because of consecutive operation for these 4 years. The annual decrements for the $\delta^{13}C$ and $\delta^{18}O$ values were 3 and 10 ppm, respectively, which corresponded to an apparent decrease in Δ_{47} of 13 ppm. Third, an approximate 1-year periodicity was found

in the Wako variation. Although it was a 1.3-year cycle in 2007/8, other years showed clear seasonal variation, for which the maximum and minimum values were found, respectively, in winter and summer. The laboratory room temperature was controlled to a constant 24 ± 1 °C so the temperature in the atmosphere surrounding the instrument could not have been a direct factor controlling the isotopic composition. The electric power consumed in the building and/or the entire university campus is expected to show a synchronous seasonal variation with temperature. The power consumption or related variation in supplied voltage might, therefore, induce ionization efficiency fluctuation. However, the temporal variation in the Δ_{47} values for heated CO_2 showed no clear seasonal variation, suggesting that there must be an additional source of uncertainty relating to the CO_2 heating process.

Although the 'true' difference in the Δ_{47} values between Wako and heated gases is 0.674, as shown in Table 2, Fig. 3 (bottom) shows the long-term variation in the difference that reflects the process of scale contraction. These differences mainly reduced from mid-2007 to early-2008, and in mid-2010. Except for these periods, rather small variation, within 0.05‰, was observed. These large falls and other smaller fluctuations were compared with changes in instrumental conditions such as filament replacements, switching to GC-C, and so on; however, none of these were found to be related. Dennis *et al.*[5] reported that partial randomization caused by fragmentation and recombination in the ionization chamber of the mass spectrometer might modify the measured Δ_{47} values. Our results showed clearly that the range of the variation during the entire period was ca. 0.1‰ and that the differences were always less than 0.674 in any given period. The average ratio of this true value of 0.674 to measured values for the entire period was 1.100 ($\pm$0.061; 1σ). Another approach to calibrate measured Δ_{47} values was proposed by Dennis *et al.*,[5] which is based on the measurements of CO_2 gases equilibrated with H_2O at several known temperatures and heated CO_2 gas at 1000 °C. Their slopes obtained using four different IRMS instruments were 1.011–1.155. Our result was consistent with them.

In principle, it is possible to calibrate Δ_{47} values by making the difference between Wako and heated gases uniform, at a value of 0.674, as in Eqn. (6). The open squares in Fig. 3 represent average Δ_{47} values of NBS-19 (also shown in Table 3). In addition, data from another reference material, called ZSTD, whose isotopic composition is almost equal to that of NBS-20 (Solnhofen Limestone), are also plotted for verification of this calibration. Unfortunately, no significant improvement was found in the standard deviation of Δ_{47} values of ZSTD (from 37 ppm before calibration to 39 ppm after calibration). In particular, two ZSTD values measured within a week in March 2008 distinctly differed by 0.1‰. ZSTD is a long-term (since 1993) laboratory working standard in Nagoya University, and its $\delta^{13}C$ and $\delta^{18}O$ values are much more constant than those of NBS-19. Dennis *et al.*[5] reported Δ_{47} values as being sufficiently stable for weeks. However, based on the assumption that the Δ_{47} value of ZSTD might be conserved as well as the $\delta^{13}C$ or $\delta^{18}O$ values, we conclude that the period of time for stable analysis seems to be much shorter in the Delta XP mass spectrometer, and thus daily measurements for Wako/ZSTD and heated gas, and calibration with them, might be important.
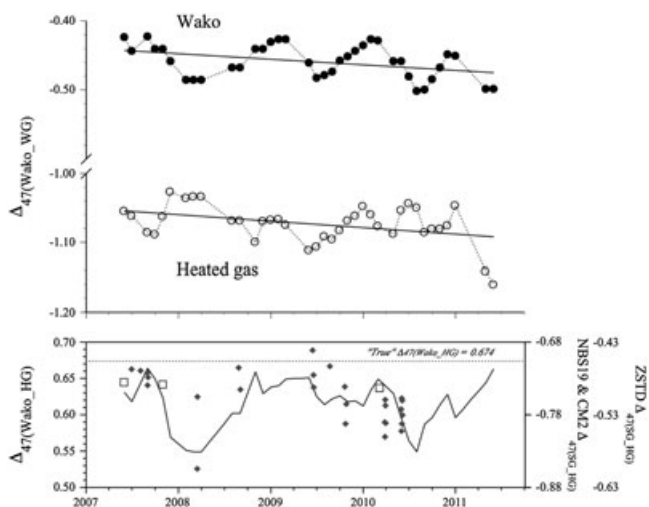

Figure 3. Top: Moving averages of 3 months for Δ_{47} values in Wako and Heated gas shown as measurement data. Bottom: The difference between Δ_{47} values in Wako and Heated gas shown as measurement data. The solid line is the regression line for the least-squares method.

Confirmation of nonlinear effect on δ^{47}-Δ_{47} relationship

A striking feature of clumped-isotope analysis using the MAT 253 was a strong δ^{47}-dependency of the Δ_{47} values (Huntington et al.[18]; Dennis et al.[5]). According to the definition of Δ_{47} (Eqn. (1)) and generally wider variation in δ^{45} and δ^{46} values relative to the Δ_{47} value, the δ^{47} value is approximately equal to ($\delta^{45} + \delta^{46}$). It is therefore related to the value of ($\delta^{13}C + \delta^{18}O$).[18]

Because the Δ_{47} value depends only on the formation temperature, CO_2 heated at 1000°C was expected to have a uniform Δ_{47} value irrespective of the original $\delta^{13}C$ and $\delta^{18}O$ values of the samples. The values measured using the MAT 253, however, generally show postive relationships between the δ^{47} and Δ_{47} values. Huntington et al.[18] showed that the observed relationship between the Δ_{47} and δ^{47} values for heated gases reflected a subtle nonlinearity in the relationship between the actual R_{47} values and the measured intensity ratio between the mass-47 and mass-44 ion beams. They also argued that it remained unclear whether the ultimate cause of this nonlinearity derived from the performance of the detectors, the resistors through which ion currents are measured, or some component of the source or analyzer. It was proposed recently that a negative shift in the baseline due to secondary electron production was proportional to the extent of the Δ_{47} bias (He, personal communication).

Delta XP analysis yields different results for the Δ_{47} values (Table 4 and Fig. 4). Although the ranges of both the Δ_{47} values, as depicted in Fig. 3, and the δ^{47} values were similar, no significant linear relationship was found in the entire period or in each short-term period between the Δ_{47} and δ^{47} values ($p > 0.1$). In addition, the filament in the ionization chamber had been replaced three times in the 4 years (August 2007, March 2008, and August 2009). Nevertheless, these processes did not affect either the Δ_{47} values or the δ^{47} vs. Δ_{47} relationship. On the other hand, the correlation coefficients of the linear regression between Δ_{48} and δ^{48}, and Δ_{49} and δ^{49}, showed significant negative and positive values, respectively (Table 4). If the negative shift of baseline were a cause of the Δ-δ relationship, they should all have shown positive values. It would, however, be worth testing whether the negative baseline could be found in the Delta XP. The major difference between the Delta XP and the MAT 253, rather than sector size and accelerating voltage, is the geometry of the ionization chamber which affects the beam width, and causes the Delta XP to have a longer residence time of
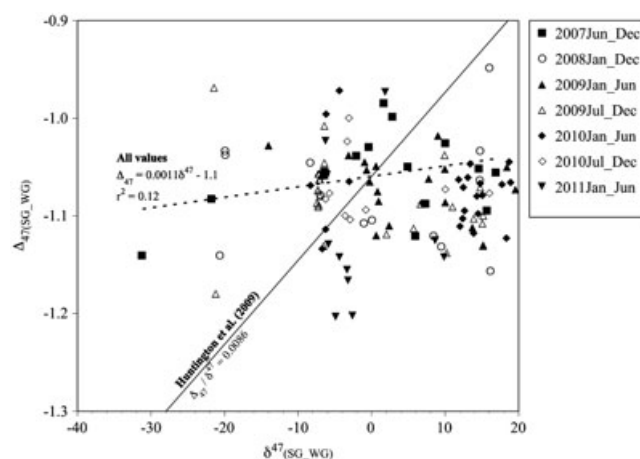

Figure 4. Relationship between Δ_{47} values and δ^{47} values.

gas molecules and related lower ionization efficiency than the MAT 253. However, other effects might also have to be taken into consideration.

The performance of the Delta XP mass spectrometer on clumped-isotope analysis and comparison with that of the MAT 253 mass spectrometer are summarized in Table 5. Although the short-term stability of the measured Δ_{47} values with the Delta XP might be less than that measured using the MAT 253, it is advantageous that clumped-isotope analysis with the Delta XP is not influenced by any variation in δ^{47}.

CONCLUSIONS

Clumped isotopes in CO_2 were measured using a small-sector isotope ratio mass spectrometer, the Delta XP. The average internal precisions of the single-gas Δ_{47} measurements were 0.006 and 0.02‰ (1 SE), respectively, for the optimum condition (m/z 44 signal of 16 V and integration time of 3200 s) and the routine condition (signal of 10 V and integration time of 800 s), which were close to the instrumental limit and were approximately equal to those obtained with the MAT 253. Both short-term and long-term external precision obtained from replicate analyses of identical sample powders were similar to those of single-gas analysis with the routine condition, which implied that most analytical uncertainties derive from short-term

Table 4. Correlation matrix of measured and clumped values for various heated CO_2 samples (n = 116). Italic numbers represent insignificant relationship ($p > 0.01$)

	δ^{45}	δ^{46}	δ^{47}	δ^{48}	δ^{49}	Δ_{47}	Δ_{48}
δ^{46}	0.471**						
δ^{47}	0.867**	0.848**					
δ^{48}	0.467**	0.998**	0.844**				
δ^{49}	*0.025*	*0.150*	*0.101*	*0.184*			
Δ_{47}	*−0.006*	*−0.056*	*−0.031*	*−0.034*	0.352**		
Δ_{48}	−0.374**	−0.732**	−0.637**	−0.689**	0.277*	0.297*	
Δ_{49}	*−0.027*	*0.085*	*0.033*	*0.120*	0.997**	0.361**	0.327**

**$p < 0.001$ *$p < 0.005$

Table 5. Summary of the performance for clumped-isotope analysis by the Delta XP isotope ratio mass spectrometer and comparison with the MAT 253

Instrument	Typical accelerating voltage (V)[a]	Sector radius (mm)[a]	Resolution (m Δm^{-1})[a]	Sensitivity (hPa V^{-1})[b]	internal precision for Δ_{47} (1SE; ppm)[c]	external precision for Δ_{47} (1SE; ppm)[d]	δ^{47}-Δ_{47} relation	δ^{48}-Δ_{48} relation	δ^{49}-Δ_{49} relation
Delta XP	3000	180	95	8	6	15	insignificant	negative	positive
MAT 253	10000	460	200	4.3	5	10	positive	n.a.	n.a.

[a]Data from brochures provided by the manufacturer.
[b]This value is dependent on the aperture of the dual-inlet capillary. The value for the Delta XP is observed in our instrument used in this study, and the value for the MAT 253 is calculated with the difference of absolute sensitivity provided by the manufacturer
[c]Based on the measurement with m/z 44 intensity of 16 V and integration time of 3200 s. The value for the MAT 253 is referred from Huntington et al.[18]
[d]Average values of standard errors shown in Table 3. The value for the MAT 253 is also referred from Huntington et al.[18]

fluctuations. Therefore, daily measurements of the reference material and heated CO_2 gas might be necessary to achieve higher reproducibility when using the Delta XP. Nevertheless, it is advantageous for Delta XP-based analysis that the strong dependence of δ^{47} values on Δ_{47} values commonly found in the MAT 253 were not found in this study.

Acknowledgements

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