

Introducing msFineAnalysis AI

Predictive Structural Analysis Software
Exclusively available on the GC-Alpha



Learn More

RESEARCH ARTICLE

Compositional and beam-size-dependent effects on pressure baseline in clumped isotope mass spectrometry

Ryan A. Venturelli  | Brad E. Rosenheim

College of Marine Science, University of South Florida, 140 7th Avenue South, St Petersburg, FL 33701, USA

Correspondence

R. A. Venturelli, College of Marine Science, University of South Florida, 140 7th Avenue South, St Petersburg, FL 33701, USA.
Email: raventurelli@mail.usf.edu

Rationale: The analysis of carbonate samples for the application of clumped isotopes to paleoclimate reconstruction necessitates smaller beam intensities. However, there is a relationship between beam intensity and pressure-dependent baseline (PBL), and therefore between beam intensity and the correction for PBL. Here we explain the relationship between PBL and beam intensity to develop a better correction protocol and an improved understanding of clumped isotope mass spectrometry.

Methods: We describe a beam size experiment using our Isoprime isotope ratio mass spectrometer in which samples of the carbonate standard IAEA-C1 were analyzed at 30, 50, and 70 nA to establish an optimal protocol and a new method to correct for PBL using the theoretical constraint of invariable Δ_{47} over a range of δ^{47} (bulk isotope composition) values. We also explore the effects of both over- and under-correction of PBL on equilibrated and heated gas samples to understand the effect of mis-correction of PBL.

Results: The results of our beam size experiments showed that a direct measurement of the baseline consistently introduced variability to measurements of the Δ_{47} of heated gases, equilibrated gases, and carbonate standards. These results necessitated a new protocol to account for PBL in our system. Our new approach flattens the reference frame line slope to 0 and, importantly, reduces the variability of data points about the heated gas line. We also describe, for the first time, an empirically derived description of the compositional effect of PBL.

Conclusions: A seemingly small change in our isotope ratio mass spectrometer resulted in a better understanding of PBL, for which we have developed an empirically based correction protocol to apply. Our new protocol has the potential to reduce analytical time for laboratories measuring PBL, and supports the need for carbonate mineral-based clumped isotope standards.

1 | INTRODUCTION

For nearly seven decades, stable oxygen isotope ratios ($\delta^{18}\text{O}$ values) have been utilized as a paleoclimate proxy.¹⁻³ Though oxygen isotopic compositions are dually dependent upon temperature and the composition of seawater (the $\delta^{18}\text{O}_w$ value), the use of $\delta^{18}\text{O}$ values in marine carbonates has been demonstrated as valuable for paleotemperature measurements when assumptions of seawater

chemistry are robust and variability through time is well constrained or minimal.⁴⁻⁷ For cases in which $\delta^{18}\text{O}_w$ is less constrained, independent temperature proxies (e.g. alkenones,⁸⁻¹⁰ coral Sr/Ca,¹¹ foraminifer Mg/Ca¹²⁻¹⁴) have been applied to separate the effects of the $\delta^{18}\text{O}_w$ value from temperature. Each independent temperature proxy has been applied in concert with $\delta^{18}\text{O}$ values with varying degrees of success due to discrepancies associated with vital effects and inter-laboratory comparability.¹⁵

The measurement of multiply substituted isotopologues (clumped isotopes), specifically the mass 47 variant (Δ_{47}) of CO_2 liberated by orthophosphoric acid from the lattice of carbonate minerals, has recently offered another opportunity to deconvolve paleotemperature and past seawater chemistry within a single measurement. As opposed to Urey's heterogeneous isotope exchange reaction,¹⁶ carbonate clumped isotope thermometry is based on a homogeneous isotope exchange reaction in which the ordering of ^{13}C and ^{18}O in bonds of CaCO_3 is inversely correlated with temperature.¹⁷ The mass 47 isotopologue ($^{13}\text{C}^{18}\text{O}^{16}\text{O} = 44.4$ ppm, $^{13}\text{C}^{17}\text{O}^{17}\text{O} = 1.6$ ppm, $^{12}\text{C}^{17}\text{O}^{18}\text{O} = 1.5$ ppm)¹⁸ of CO_2 derived from carbonate-bearing minerals is fundamentally difficult to measure due to the paucity of this particular multiply substituted isotopologue relative to isotopologues of masses 44–46. In order to make sufficiently precise measurements to differentiate variations in Earth surface temperatures, quite large samples (typically 3–15 mg) are often analyzed over a 2–4 h period.^{19–21}

The protocol to monitor the stability of an instrument for clumped isotope analyses involves the measurement of CO_2 with differing δ^{47} (ratio of masses 47/44 in a sample relative to that in a reference gas) compositions that have been driven to a stochastic distribution of isotopologues by heating to 1000°C for a minimum of 2 h. These data are used to construct a heated gas line of δ^{47} versus Δ_{47} values, used to correct for any “non-linearities” (e.g. a linear slope not equal to zero) present when δ^{47} compositions vary on the scale of 20–30% for any of the contributing isotope ratios.²² It has been demonstrated that observed slopes greater than zero in both heated and equilibrated gas lines^{23,24} can be a result of both analytical and mathematical discrepancies.^{25–28} The accuracy of clumped isotope analyses is complicated by the presence of a negative background effect (‘pressure baseline’, PBL) observed on Faraday cups when gas is admitted to the source of a mass spectrometer.^{25–27} Since the first account of PBL,²⁵ a number of correction attempts have been discussed. Whereas some utilize a linear regression from the correlation between the major beams and minor beams to remove the nonlinearity effects of the mass spectrometer,^{25,26} others have demonstrated the ability to perform a direct off-peak measurement of PBL, while gas is being ionized in the source but directed spaces between Faraday cups, to remove this effect.^{27,29–31} Our work sheds further light on PBL, allowing us to build upon the original definition as we demonstrate that PBL appears to be a function of beam size, source geometry, chemistry and stability, frequency of measurement, and characteristics of individual mass spectrometers.

Isotope ratio mass spectrometers ionizing at 10 kV potentials are typically used to measure clumped isotopes, but smaller systems have demonstrated both the ability to achieve the same precision and the need for better monitoring of instrument stability.^{27,32–34} For instance, smaller instruments (the Elementar Isoprime²⁷ and the Thermo Fisher Delta series³⁴) have been shown to require accurate measurement of PBL for purposes of reproducibility of standards and samples because of the high, yet systematic, amount of variability in Δ_{47} over varying δ^{47} values (high “non-linearity”²⁷). Successful clumped isotope measurements on the Isoprime 5 kV isotope ratio mass spectrometer have quantified PBL through direct off-peak measurements at a static point, 41V less than the accelerating voltage at peak center.²⁴ This

practice has demonstrated an accurate assessment of PBL at a single, but large, beam intensity (100 nA), resulting in the use of this system for measurements and calibration of siderite³² and calcite.³³ These studies demonstrated that clumped isotope measurements could be reliably made and comparable reference frames could be produced from a system with an entirely different ion source, ion optics, and analytical technique from traditionally made measurements with a 10 kV MAT 253.³⁴

As the clumped isotope community continues to improve analytical precision while moving to smaller sample sizes,^{29,35,36} we have further modified the Isoprime mass spectrometer by the installation of higher ($10^{12} \Omega$) resistors on the Faraday cups configured to measure masses 47–49. These higher resistors facilitated increased sensitivity and decreased beam intensity, but also caused profound changes in the generation and, hence, the resultant measurement of PBL in our system. In this paper we present a new empirical, rather than measurement-based, PBL correction which we have preliminarily tested on an internal laboratory standard and gas reference frames spanning three separate analytical sessions. Furthermore, we apply what we have observed in our system to explore the effects of mis-correction of PBL on reference frames and transfer functions to the absolute reference frame. We share an improved understanding of compositional and pressure effects on PBL, which could become important as more laboratories using different mass spectrometer setups implement methods in which beam size is not held constant throughout a measurement.

2 | EXPERIMENTAL

2.1 | System

All isotopologue measurements were made on the compact 5 kV Isoprime isotope ratio mass spectrometer at the University of South Florida (USF, St Petersburg, FL, USA). The system was moved from the Stable Isotope Laboratory of Tulane University (SILTU, New Orleans, LA, USA) and is described in detail in Figure 2 of Rosenheim et al.²⁷ Measurements previously published from this system^{27,32,33} required large sample sizes to produce ion beams of 100 nA. In order to facilitate the measurement of smaller beams (30–50 nA), and ultimately reduce sample sizes for clumped isotope applications, a new head amplifier was installed with increased resistance on all cups (Table 1).

All gases (equilibrated, heated, and carbonate-derived) were passed twice through the glass vacuum separation line described in detail in Rosenheim et al.²⁷ Briefly, this consists of two independent water traps, four loops each, cooled to -70°C with isopropanol (cooled to liquid–solid phase transition by liquid nitrogen) followed by a U-shaped glass Porapak and a silver wool trap cooled to -15°C using ethylene glycol (cooled to liquid–solid phase transition with liquid nitrogen). Heated (1000°C) and equilibrated (25 and 50°C) gases were prepared within four analytical sessions between August 2015 and May 2017 to construct independent reference frames. Each equilibrated and heated gas line consisted of three points covering a range of different bulk isotopic composition (Table 2).

TABLE 1 Resistor values for CO₂ isotopologue cups on the Isoprime system. Previously published values for the SILTU system and values after installation of a new head amplifier at USF

<i>m/z</i>	Resistance at SILTU (Ω)	Resistance at USF (Ω)
44	5.0 × 10 ⁷	1.0 × 10 ⁹
45	5.0 × 10 ⁹	1.0 × 10 ¹¹
46	1.5 × 10 ¹⁰	1.0 × 10 ¹¹
47	2.0 × 10 ¹¹	1.0 × 10 ¹²
48	2.0 × 10 ¹¹	1.0 × 10 ¹²
49	2.0 × 10 ¹¹	1.0 × 10 ¹²

Each combination of CO₂ + H₂O was sealed in a borosilicate ampoule and placed in a water bath (set to 25 or 50°C) to equilibrate for at least 72 h before cryogenically separating CO₂ from H₂O. Each of the gases for heating was frozen into quartz ampoules and placed in a muffle furnace at 1000°C for at least 2 h to reach a stochastic distribution of isotopologues. The δ¹³C values ranged between -41.02‰ and 2.05‰ VPDB and the δ¹⁸O values ranged between 18.37‰ and 60.76‰ VSMOW.

An internal carbonate standard (IAEA-C1) was reacted under vacuum with 2–5 mL of concentrated H₃PO₄ (104.1%) and analyzed to verify our reference frame and test our method of PBL correction. All reactions were carried out at 100°C by simple immersion of a two-legged McCrea-type reaction vessel in boiling water and continuously trapped in the first four-loop trap with liquid nitrogen throughout the reaction in order to minimize re-equilibration with water.

2.2 | Mass spectrometry

Purified samples were introduced into the bellows of the Isoprime mass spectrometer by being frozen into an adjacent microvolume and then expanded at ambient temperature. Pressures were adjusted on both sample and reference side to produce ion beams of 50 nA for the majority of samples, with the exception of a beam size experiment in which additional ion beams of 30 and 70 nA were produced from the same aliquots of gas (described further in the next section). Before each analysis, an accelerating voltage scan with a width of 120 V was performed with a step size of 0.27 V (0.1 s each) in the accelerating voltage. The entire process, which we refer to herein as pre-acquisition peak scanning, lasts 45 s, and is only performed once at the start of each analysis (Figure 1). For measurements, the ion beams of masses 44–49 were monitored for 20 s on both the reference and the sample side, with 12 s of data omitted after changeover

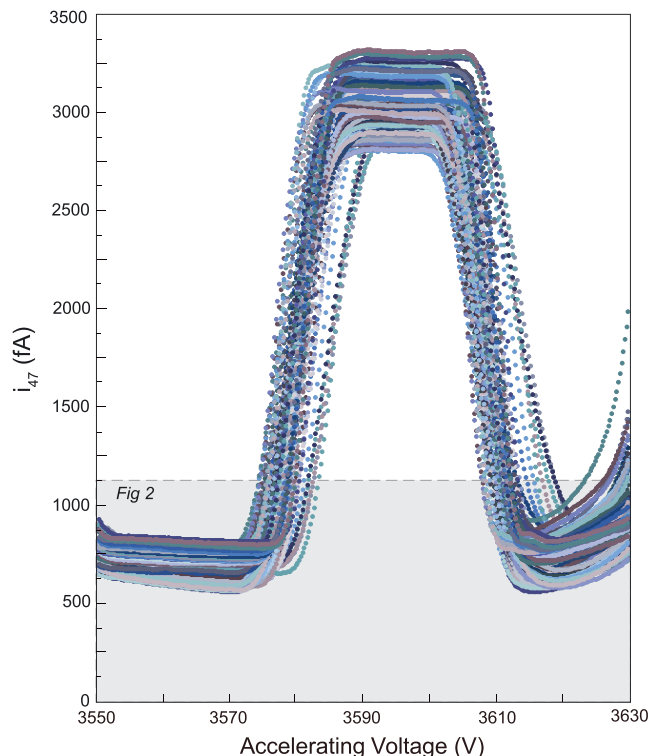


FIGURE 1 A compilation of pre-acquisition peak scans ($n = 130$), demonstrating the stability of our m/z 47 peak. The average peak center deviates by 3.1 V; however, the lowest point on the scan (peak scan center minimum) varied by as much as 14.68 V [Color figure can be viewed at wileyonlinelibrary.com]

valve switches. The sample gas isotope ratios were bracketed by measurements of reference gas (δ¹³C = -3.22‰ VPDB; δ¹⁸O = 25.46‰ VSMOW). In order to comparatively monitor PBL correction techniques, we employed the protocol outlined in Rosenheim et al.,²⁷ in which currents for all Faraday cups are measured when the m/z 44 beam is shifted 41 V lower in accelerating voltage for an off-peak measurement of PBL. This measurement occurs at the beginning of each sample and reference acquisition block, adding approximately 20 minutes to each measurement.

2.3 | Beam size experiment

Two large samples (25 mg each) of IAEA-C1 were measured repeatedly during two analytical sessions (in September 2015 and January 2016) to determine the precision and accuracy for clumped isotope analyses in our updated system at different beam intensities.

TABLE 2 Description of ingredients for reference frame gases. “Tank CO₂” refers to gas sourced from a bottle purchased from an AirGas vendor in New Orleans, LA, USA (δ¹³C = -3.22‰ VPDB; δ¹⁸O = 25.46‰ VSMOW) and “APICR” refers to Antarctic Peninsula Ice Core residual water

	Light	Middle	Heavy
Equilibrated	Tank CO ₂ + APICR water; δ ⁴⁷ ≈ -8 to -4‰	Tank CO ₂ + DI water; δ ⁴⁷ ≈ 8 to 12‰	Tank CO ₂ + DI water evaporated to 20% of the original volume; δ ⁴⁷ ≈ 30‰
Heated	Fisher Scientific CaCO ₃ (C-64, Lot #874601); δ ⁴⁷ ≈ -48 to -44‰	Tank CO ₂ ; δ ⁴⁷ ≈ -3 to -2‰	CO ₂ from headspace of the “heavy” equilibrated ampoule; δ ⁴⁷ ≈ 29 to 35‰

TABLE 3 PBL values (in fA) used to correct for each analysis of IAEA-C1 in our beam size experiment. The set value correction is the PBL value necessary to correct the standard to an accepted value. This point, at 50 nA, matches the intersection point of three heated gases of different bulk isotopic composition, with a forced varied background applied (described in detail in section 4)

	41 V correction (fA)	PSM correction (fA)	Set value correction (fA)
30 nA	752	770	822
50 nA	633	651	772
70 nA	493	539	739

Repeated measurements of large samples from a single reaction and vacuum-line preparation were performed to isolate beam size as the sole variable inciting change on δ^{47} and subsequently Δ_{47} results. By analyzing each of the prepared IAEA-C1 samples at a m/z 44 beam of 70 nA ($n = 3$), 50 nA ($n = 6$), and 30 nA ($n = 6$) we were able to investigate whether or not PBL could be measured at a set, off-peak, point, as well as the stability of this set point through time. During the September 2015 analytical session, the previously established protocol²⁷ was employed, in which on-peak signal measurements were alternated with 41 V off-peak PBL measurements. During the January 2016 analytical session, however, the off-peak PBL measurement location was adjusted to the lowest point on a pre-acquisition peak scan, similar to previously published methods from a different laboratory.^{30,31} This point will be referred to as the peak scan minimum (PSM) herein. The PSM remained stable at each beam size for the entirety of this experiment. Heated and equilibrated gases were regularly analyzed during the duration of these experiments. When previously accepted values of IAEA-C1 were not achieved with any of the PBL values from either the PSM or the 41 V monitoring points, an arbitrary set value for PBL was used to correct raw voltages to the accepted value. This practice was only performed on the IAEA-C1 samples in the beam size experiment to assess the degree of mismeasurement and failure to consistently achieve accuracy and precision for a known standard value. Eventually this value for the 50 nA samples (see Table 3) was able to be tied to an empirically derived multi-compositional intersection (MCI) point described in detail in section 4 and Figure 6.

2.4 | Compositional PBL sensitivity test

Gas reference frame data were used to test the sensitivity of equilibrated and heated gas lines, as well as the subsequent transfer function, to an improper PBL correction. Observed differences (as much as 120 fA) between PBL correction methods in our beam size experiment were used to simulate extreme cases of over-correction and under-correction. Using the maximum observed differences of 120 fA, we applied the same offset to the entire range of reference frame samples in order to empirically derive the compositional effect^{30,31} of PBL (Figure 5).

3 | RESULTS

Observation of pre-acquisition peak scans from over 100 analyses revealed that the PSM varies at beam intensities lower than 100 nA. At a large beam size (100 nA), the assumed PSM was probably stable ($n > 400$) at 41 V less than the accelerating voltage at peak center based on the achieved reference frame lines with slopes = 0 over broad compositional differences.²⁷ This is only assumed because, with the success of the simple PBL measurements at that ion beam intensity, there was no impetus to measure pre-integration peak scans with every sample. We subsequently observed that this point changes as a function of m/z 44 beam size (Figure 2). Consistent with previous observations,^{30,31} an inverse relationship exists between the PSM and beam size; i.e. PSM was driven lower by increasing the beam intensity. Consequently, the PSM can be found 7–11 V nearer the peak center than when a m/z 44 beam of 100 nA is used (Figure 2). From our beam size experiment and previous analyses performed at 100 nA ($n > 400$), we have established a relationship between PSM and beam size best described by a second-degree polynomial (Figure 3), although we have no theoretical basis for such a functional form and the number of points tested is not significant enough to constrain such a relationship. Nonetheless, the parabolic fit changes insignificantly between ~50 nA and 0 nA, with a minimum offset of 28.7 V at 25.1 nA (where $dV/dnA = 0$). This modelled variability (approximately 3 V) over a large range of beam intensities is smaller than the variability recorded at 50 nA using the direct measurements of PSM.

Despite an improvement in accuracy and precision when switching from a 41 V to a PSM correction for PBL, the overall accuracy did not reach acceptable levels (Figure 4). Our findings were

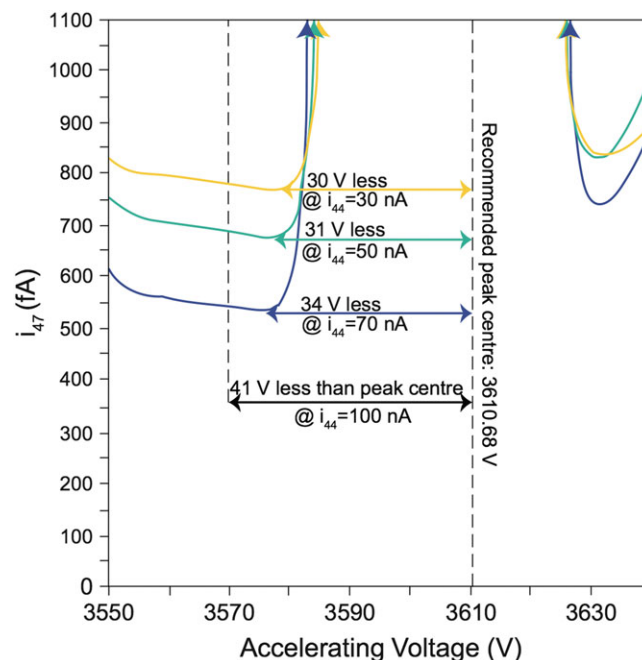


FIGURE 2 Close-up view of mass 47 peaks in the Isoprime system with major CO_2 beams (m/z 44) of 30, 50, and 70 nA. Traditional measurements in this system were performed at 100 nA and PBL was monitored at an AV adjusted to 41 V left of the peak center. Here we show that with a changing beam intensity, PSM shifts closer to the peak center [Color figure can be viewed at wileyonlinelibrary.com]

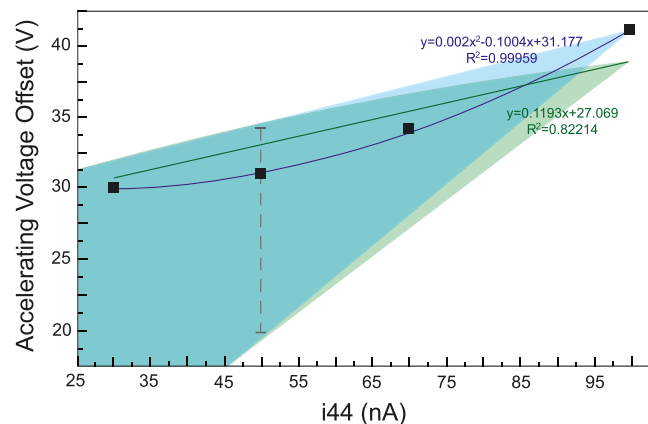


FIGURE 3 The relationship between major beam intensity and the offset in PSM from peak center. Lower beam intensities require lower offsets from the m/z 45 peak center (the mass to which the instrument is tuned). We have empirically fitted a polynomial (blue line) to these data, as it provided the best fit, from our beam size experiment (30 nA, $n = 6$; 50 nA, $n = 6$; 70 nA, $n = 3$) and the established methods of Rosenheim et al²⁷ (100 nA, $n > 400$). Because we have no theoretical basis for such a functional form, we also provide a linear fit (green line) through these data. With the addition of 120 analyses at 50 nA, error bars have been added to encompass the full range of accelerating voltage offsets between peak center and PSM. The shading indicates the full range of uncertainty based on a linear fit through the endpoint of each line (100 nA) and the high and low accelerating voltage offsets at 50 nA. The green shading encompasses the cloud of error associated with the linear fit, while the blue encompasses that associated with the polynomial fit [Color figure can be viewed at wileyonlinelibrary.com]

consistent with those of Rosenheim et al,²⁷ in which precision between measurements, regardless of beam size, was improved upon by applying a PBL correction. When PBL was monitored at the PSM, the signal on the empty Faraday cups was consistently and systematically greater than when PBL was monitored at 41 V off of the center. These two measurements differed by 18 fA for 30 and 50 nA measurements, and 46 fA for the 70 nA measurements. The improvement in precision of these measurements between PBL correction techniques was not linear for each beam size (Figure 4), further implying that both the 41 V and the PSM set PBL correction points do not sufficiently eliminate PBL effects. The objective of this experiment was to constrain the position at which PBL is monitored and determine which beam size would reduce both sample size and instrumental variability. The outcome of this experiment did not allow us to assess this objective; however, it did raise a question of whether or not the correction for PBL must come from a direct measurement.

4 | DISCUSSION

Improving inter-laboratory comparability of measurements remains one of the most important tasks for the growing clumped isotope community to accomplish. Conventional practice prescribes measurement of CO_2 with different bulk isotope compositions (δ^{47} values) that have been equilibrated at a set temperature ($\sim 25^\circ$, and $\sim 50^\circ\text{C}$ in our laboratory) or driven to a stochastic

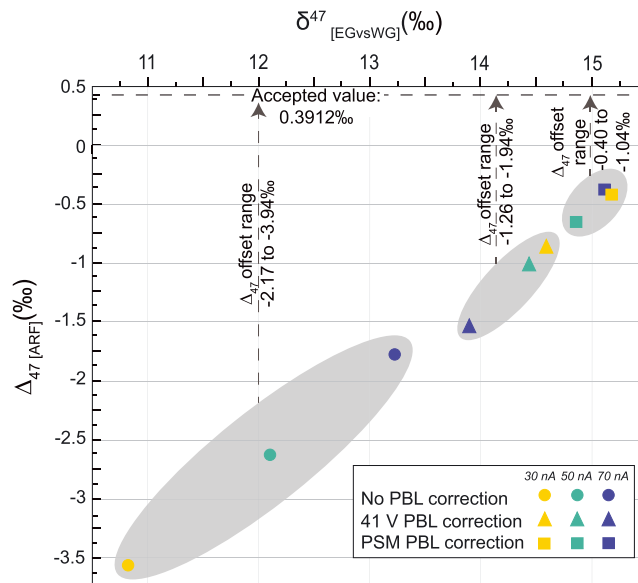


FIGURE 4 Mean Δ_{47} values obtained with different PBL corrections in the beam size experiment. An amount of 25 mg each of IAEA-C1 was measured in September 2015 and January 2016 at 30, 50, and 70 nA. Samples denoted by the 41 V symbol were corrected with the protocol described in Rosenheim et al,²⁷ while the PSM correction refers to those points corrected with a value for PBL measured at the lowest point on a pre-analysis peak scan. It should be noted that, despite a lack of accuracy, a PBL correction improves measurement precision. Previously published values from Rosenheim et al²⁷ for IAEA-C1: $\Delta_{47} = 0.3912\text{‰}$ [Color figure can be viewed at wileyonlinelibrary.com]

distribution of isotopologues by heating the samples to 1000°C .³⁷ Measurements of Δ_{47} at different δ^{47} values are used to construct a reference frame of Δ_{47} versus δ^{47} , from which intercepts are plotted against theoretical values to construct an empirical transfer function to the absolute reference frame, on which all clumped isotope measurements can be compared.²⁴ It has been suggested that slopes > 0 for heated and equilibrated gas lines (Δ_{47} versus δ^{47}) can be attributed to differences in correction for the influence of ^{17}O .^{28,38} and improper/absent correction for PBL.²⁵⁻²⁷ To resolve these issues, the clumped isotope community has moved from a set of correction parameters used in the calculation of Δ_{47} (R^{13}_{VPDB} , R^{17}_{VSMOW} , R^{18}_{VSMOW} , λ) from Gonfiantini/Santrock parameters (0.112372, 0.0003799, 0.0020052, 0.5164)³⁹⁻⁴¹ to Brand/IUPAC parameters (0.0118, 0.038475, 0.0020052, 0.528)^{28,38,42} in an effort to improve inter-laboratory comparability mathematically. Where non-linearities cannot be resolved mathematically, they can be attributed to poor understanding of, and thus correction for, PBL.²⁵⁻²⁷ Although not all laboratories apply a PBL correction, it has been demonstrated that both direct²⁵⁻³¹ corrections can be made and indirect²³ (heated gas lines) offsets can be observed.

Pre-acquisition peak scans, as well as a beam size experiment, described herein have revealed that despite an apparent relationship between major beam size and PSM (Figure 3), we are unable to tie the point in which PBL should be directly measured to a single physical location.^{27,32,33} Our inability to directly measure PBL at smaller beam intensities, however, allows us to shed light on the effect of incorrectly accounting for PBL in clumped isotope

measurements of a carbonate standard, reference frames, and transfer functions. When a 41 V PBL correction was applied in our beam size experiment, the Δ_{47-ARF} values of IAEA-C1 varied by as much as 0.52‰ from our laboratory accepted value, translating to an offset of 20.5°C between 30 nA and 50 nA analyses and 43.6°C between 50 nA and 70 nA analyses.²⁴ Using a correction tied to PSM^{30,31} in our beam size experiment, the Δ_{47-ARF} variability of IAEA-C1 reduced to 0.22‰, although the 70 and 30 nA analyses yielded Δ_{47} values within error of one another. Despite an improvement in the precision of these measurements with a change in correction for PBL from 41 V to PSM, the measurements remain inaccurate, yielding Δ_{47-ARF} values as much as 1.04‰ lower than the published Δ_{47-ARF} value of 0.3912‰ for IAEA-C1 from our laboratory²⁷ (see Figure 4).

Given that heated gas lines should be invariant in Δ_{47} regardless of bulk isotopic composition, it is possible to determine PBL in a system without direct measurement. If measured at a single beam intensity, on-peak signals for three different points (different bulk isotopic composition) on a heated gas line are corrected for a linear range of possible PBL values, the point at which the three samples intersect should theoretically correspond both with the Δ_{47} value for the temperature to which they were heated or equilibrated³⁷ and with the true PBL of the system during the period in which those gases were analyzed. We refer to this intersection point as the MCI point. In the case of our laboratory, we have applied this test by forcing a PBL correction ranging from 100 to 1200 fA, in a linear, stepwise fashion, on our raw m/z 47 signals. These values were well in excess of previously observed ranges of PBL values with different direct PBL measurement techniques. We illustrate this practice in Figure 6 with three heated gases each having a different bulk isotope composition (described in Table 3). We demonstrate that the calculated Δ_{47} values using this range of PBL values will intersect at a single MCI point, of which the abscissa value is indicative of the actual PBL while the ordinate value at the MCI point corresponds to the Δ_{47} for the temperature at which samples were heated.

We suggest that the practice of varying background approach can be tested in systems outside of our laboratory by analyzing internal carbonate standards (such as IAEA-C1 in our laboratory) or potentially external carbonate standards (such as ETH 1-4^{29-31,43}) bolstered by heated and/or equilibrated gas analyses to find an instrument's or analytical session's individual MCI point. By performing a simple check with the varied background approach on heated gases analyzed at 50 nA surrounding the beam size experiment, we were able to confirm that the MCI point-derived PBL value (Figure 6) in fact matched the PBL necessary to correct the carbonate standard to our laboratory accepted value (50 nA set value in Table 3). By confirming that these values are in fact the same, for analyses performed at 50 nA, we can safely assume that the same would also be true at other beam sizes.

PBL was always the lowest for the 70 nA analyses and highest for the 30 nA analyses, further supporting the idea that PBL is a function of beam size (Figure 4), regardless of which PBL measurement or correction technique was used. Differences between the PBL as measured at the PSM and MCI point used to correct each sample to an accepted value exist, but are not systematic. The greatest difference between PBL values for both approaches and set values exists for analyses performed at 70 nA, whereas the smallest difference exists for analyses performed at 30 nA. Furthermore, the values in our beam size experiment (Figure 4) yielded a consistent (but not systematic) negative offset from the previously published²⁷ value for IAEA-C1, indicating that the negative signal of PBL was not being fully eliminated and that we were effectively under-correcting for PBL. Furthermore, the order in which each PBL correction changed δ^{47}/Δ_{47} values was apparently random, suggesting that monitoring for PBL at a static point, whether it be 41 V or PSM, does not adequately eliminate PBL at lower beam intensities than 100 nA in our modified Isoprime system. The set correction for PBL applied to find the accepted value of our carbonate standard was always greater than both the 41 V and the PSM corrections. However, it matched the MCI point correction at 50 nA. This confirms that the PBL correction that sufficiently removes the negative effects of pressure-dependent

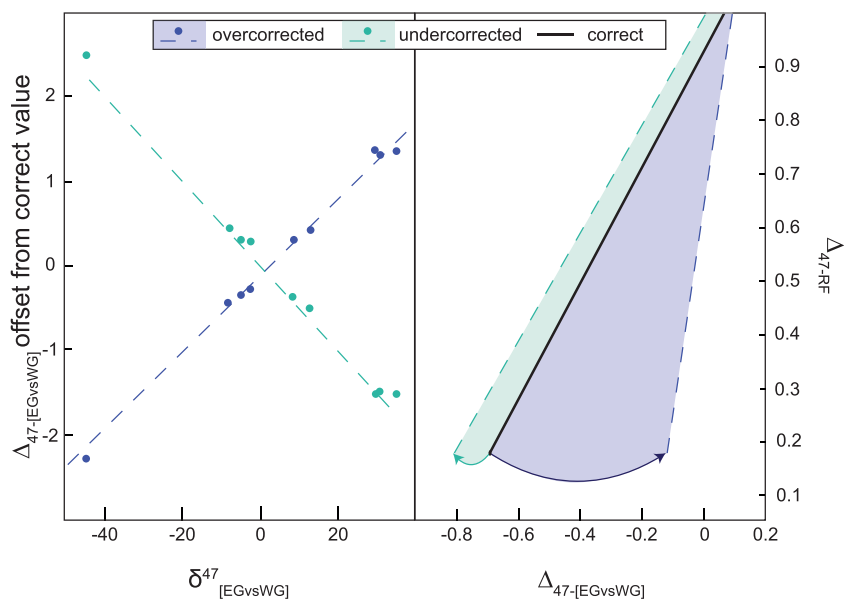


FIGURE 5 The effect of PBL mis-correction on reference frame samples. In the left panel the bulk isotopic (δ^{47}) value is plotted against the offset of the mis-corrected value from the known value of each equilibrated gas and heated gas from our reference frame. Because samples nearest the origin (δ^{47} value near 0) are less sensitive to changes in PBL than those further away, there is a compositional effect. In the right panel, the mis-corrected samples are plotted with a published reference frame to demonstrate that under-correcting for PBL results in a shallower slope, while over-correcting yields a steeper transfer function slope [Color figure can be viewed at wileyonlinelibrary.com]

baseline comes from the varied background approach that we have presented herein, whereas all other approaches tested were consistently under-correcting for this effect.

The femtoamp-magnitude offset in PBL between the correction methods was applied to existing data in order to gain an understanding of how mis-correction would affect reference frames, and ultimately the translation of clumped isotope measurements to temperature (Figure 5). When the same PBL offset was applied to every reference frame sample (three different points each on 25, 50, and 1000°C lines), we observed that samples nearest the composition of our working gas (i.e. a δ^{47} near 0) were affected less than those further away from the origin (δ^{47} near +40 or -40), consistent with previous findings about the compositional PBL effect³¹ (Figure 5). We calculate, for the first time, a compositional effect for PBL expressed as:

$$\Delta_{47} = 0.0456 \times (\delta^{47}) \text{ when an over-correction is applied, and}$$

$$\Delta_{47} = -0.0503 \times (\delta^{47}) \text{ when an under-correction is applied (Figure 5).}$$

The compositional effect also affects the sensitivity of an empirical transfer function (ETF) to mis-correction for PBL. Where many laboratories have published ETF equations with a slope of

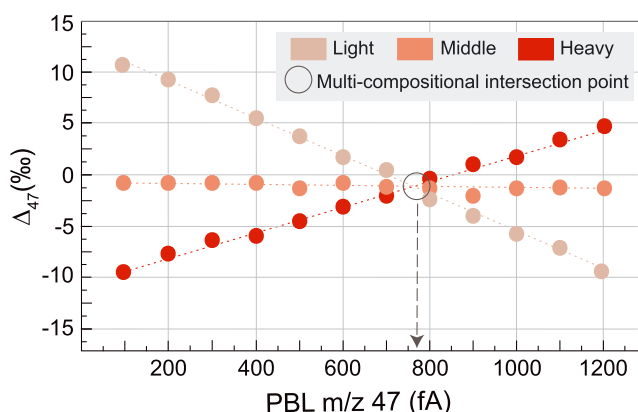


FIGURE 6 Using a set of samples with different bulk isotopic composition from a heated gas line, we correct for varied PBL values from 100 to 1200 fA. By plotting these heated gases together with varied background, we are able to determine the PBL in our system at the point of intersection [Color figure can be viewed at wileyonlinelibrary.com]

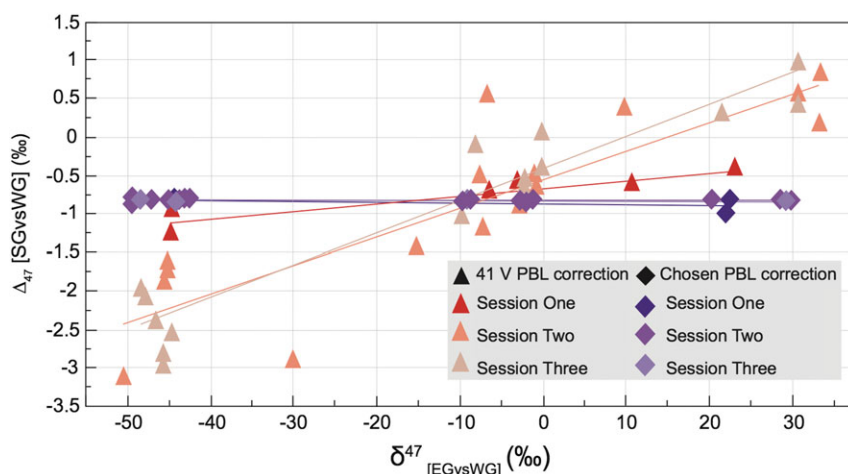


FIGURE 7 Heated gas lines corrected with the traditional 41 V PBL measurements and our newly established correction method. Using our new method, we are able to reduce the slope from 0.0295 ± 0.017 to -0.0004 ± 0.0007 and reduce the variability of the δ^{47} and Δ_{47} values of each of the categories of heated gases (light, mid, heavy) [Color figure can be viewed at wileyonlinelibrary.com]

around one,²⁴ an under-correction for PBL will yield a shallower slope and over-correction will yield a steeper slope. This observed change in slope has an effect on the sensitivity of Δ_{47} measurements, meaning that laboratories with an ETF of shallower slope are making less sensitive measurements, and are able to measure a potentially larger range of temperatures than those with a steeper slope. This is of particular importance when Δ_{47} measurements are being translated to temperature, and could possibly have an effect on clumped isotope-temperature calibrations.

In order to demonstrate that applying a PBL correction derived from the MCI point abscissa value would not only correct for the proper PBL in our system on a single composition carbonate standard, but also prove effective over a range of samples with a large range of bulk isotopic values, we have corrected samples from three heated gas lines during three separate analytical sessions (Figure 7). Using the abscissa MCI point for every three reference frame samples (Figure 6) as the value of PBL in our system during that time, we are able to reduce the slope from 0.0295 ± 0.017 to -0.0004 ± 0.0007 . More notable, however, is a reduction in variability about the heated gas lines (Figure 7), demonstrating that this method of empirically deriving PBL improves both accuracy and precision better than directly measuring PBL at any set point tied to our peak scans. The standard deviation of the intercept throughout the three sessions is also reduced from 0.6700 to 0.0262, which will ultimately produce a more stable reference frame through time. To implement this approach, all unknowns in our laboratory are preceded and followed by an equilibrated or heated gas. Once three gases equilibrated to the same temperature have been analyzed at one beam size, the MCI point can be applied to correct for a set PBL value for all unknowns analyzed between the gases. The same may also be true for carbonate standards, in which a set PBL value can be used to correct a standard to its accepted value, and thus the unknowns analyzed adjacent to each standard. Although this is different from the use of carbonate standards proposed by Bernasconi et al,⁴³ this method adds additional support to the necessity of there being well-established carbonate standards for clumped isotope analyses. The pre- and post-run standards, whether carbonate-derived or equilibrated/heated gas, if run within the same session, have never demonstrated differences greater than 7 fA in PBL voltages to achieve the previously published value of the given standard, regardless of

composition. Differences of the order of 100s of femtoamps are, however, observed when analytical sessions are punctuated by major mass spectrometric changes (discontinuous filament or source cleaning and rebuild), lending insight to our claim that PBL may also be sensitive to changes in source geometry. The apparent success of this newly developed protocol in place of directly measuring off-peak has the potential to reduce instrument time by eliminating the off-peak measurement step and thus the sample size without making additional modifications to the instrument. It would require, though, that a laboratory analyze a potentially high frequency of equilibrated/heated gases and carbonate standards if they are not already doing so.

5 | CONCLUSIONS

1. The desire to operate our instrument at smaller beam sizes to facilitate smaller sample sizes prompted a new understanding of PBL in the Isoprime system.
2. Our observations of the changes in PBL with respect to changes in major beam intensity support the ideas previously suggested by Meckler et al.³⁰ and Müller et al.³¹ that both a compositional and a pressure dependence are present.
3. Whereas fixing a point of PBL monitor to pre-acquisition peak scans has proven useful in the past, we have found that an empirical approach to account for PBL better serves our system at major beam intensities lower than 100 nA. This approach has the potential to reduce analytical time and ultimately further reduce sample size; however, it may increase the frequency at which a laboratory analyzes standards.
4. Our new approach to PBL correction helps us to reduce reference frame slopes and achieve similar transfer functions to previous studies from the Isoprime.^{27,32,33} We have demonstrated that this approach works both with gases and with our laboratory's internal carbonate standard, but it could easily be modified with standards used more widely in the clumped isotope community (ETH 1-4^{30-32,43}), supporting work to establish carbonate clumped isotope standards with community-wide accepted Δ_{47} values.⁴³
5. Although many laboratories still operate a 'stable' instrument and do not monitor for PBL or apply any PBL correction to their measurements, at the very least, our method may serve as a useful check on a longer time scale.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the helpful reviews from Cedric John, Inigo Muller, and two anonymous reviewers whose contribution and insight helped to improve this manuscript.

ORCID

Ryan A. Venturelli  <https://orcid.org/0000-0001-9548-0382>

REFERENCES

1. McCrea JM. On the isotopic chemistry of carbonates and a paleotemperature scale. *J Chem Phys.* 1950;18(6):849-857.
2. Urey HC, Lowenstam HA, Epstein S, McKinney CR. Measurement of paleotemperatures and temperatures of the Upper Cretaceous of England, Denmark, and the southeastern United States. *Geol Soc Am Bull.* 1951;62(4):399-416.
3. Emiliani C. Pleistocene temperatures. *J Geol.* 1955;63(6):538-578.
4. Cole JE, Fairbanks RG, Shen GT. Recent variability in the Southern Oscillation: isotopic results from a Tarawa Atoll coral. *Science.* 1993;260(5115):1790-1793.
5. Linsley BK, Dunbar RB, Wellington GM, Mucciarone DA. A coral-based reconstruction of Intertropical Convergence Zone variability over Central America since 1707. *J Geophys Res Oceans.* 1994;99(C5):9977-9994.
6. Quinn TM, Crowley TJ, Taylor FW, Henin C, Joannot P, Join Y. A multicentury stable isotope record from a New Caledonia coral: interannual and decadal sea surface temperature variability in the southwest Pacific since 1657 AD. *Paleoceanogr Paleoclimatol.* 1998;13(4):412-426.
7. Felis T, Pätzold J, Loya Y, Fine M, Nawar AH, Wefer G. A coral oxygen isotope record from the northern Red Sea documenting NAO, ENSO, and North Pacific teleconnections on Middle East climate variability since the year 1750. *Paleoceanogr Paleoclimatol.* 2000;15(6):679-694.
8. Müller P, Kirst G, Ruhland G, Von Storch I, Rosell-Meleh A. Calibration of the alkenone paleotemperature index U_k^{37} based on core-tops from the eastern South Atlantic and global ocean (603N-603S). *Geochim Cosmochim Acta.* 1998;62(10):1757-1772.
9. Sachs JP, Schneider RR, Eglinton TI, et al. Alkenones as paleoceanographic proxies. *Geochem Geophys Geosyst.* 2000;1(11):2000GC000059.
10. Volkman JK. Ecological and environmental factors affecting alkenone distributions in seawater and sediments. *Geochem Geophys Geosyst.* 2000;1(9):2000GC000061.
11. Gagan MK, Ayliffe LK, Hopley D, et al. Temperature and surface-ocean water balance of the mid-Holocene tropical western Pacific. *Science.* 1998;279(5353):1014-1018.
12. Delaney ML, Bé AW, Boyle EA. Li, Sr, Mg, and Na in foraminiferal calcite shells from laboratory culture, sediment traps, and sediment cores. *Geochim Cosmochim Acta.* 1985;49(6):1327-1341.
13. Anand P, Elderfield H, Conte MH. Calibration of Mg/Ca thermometry in planktonic foraminifera from a sediment trap time series. *Paleoceanography.* 2003;18(2):1050.
14. Elderfield H, Yu J, Anand P, Kiefer T, Nyland B. Calibrations for benthic foraminiferal Mg/Ca paleothermometry and the carbonate ion hypothesis. *Earth Planet Sci Lett.* 2006;250(3-4):633-649.
15. Lea D. Elemental and isotopic proxies of past ocean temperatures. *Treatise Geochem.* 2003;6:365-390.
16. Urey HC. The thermodynamic properties of isotopic substances. *J Chem Soc.* 1947;562-581.
17. Schauble EA, Ghosh P, Eiler JM. Preferential formation of ^{13}C - ^{18}O bonds in carbonate minerals, estimated using first-principles lattice dynamics. *Geochim Cosmochim Acta.* 2006;70(10):2510-2529.
18. Eiler JM. "Clumped-isotope" geochemistry—the study of naturally-occurring, multiply-substituted isotopologues. *Earth Planet Sci Lett.* 2007;262(3-4):309-327.
19. Ghosh P, Adkins J, Affek H, et al. ^{13}C - ^{18}O bonds in carbonate minerals: a new kind of paleothermometer. *Geochim Cosmochim Acta.* 2006;70(6):1439-1456.
20. Passey BH, Levin NE, Cerling TE, Brown FH, Eiler JM. High-temperature environments of human evolution in East Africa based on bond ordering in paleosol carbonates. *Proc Natl Acad Sci USA.* 2010;201001824.
21. Zaarur S, Affek HP, Brandon MT. A revised calibration of the clumped isotope thermometer. *Earth Planet Sci Lett.* 2013;382:47-57.
22. Eiler JM, Schauble E. ^{18}O ^{13}C ^{16}O in Earth's atmosphere. *Geochim Cosmochim Acta.* 2004;68(23):4767-4777.
23. Huntington K, Eiler J, Affek H, et al. Methods and limitations of 'clumped' CO_2 isotope (Δ_{47}) analysis by gas-source isotope ratio mass spectrometry. *J Mass Spectrom.* 2009;44(9):1318-1329.

24. Dennis KJ, Affek HP, Passey BH, Schrag DP, Eiler JM. Defining an absolute reference frame for 'clumped' isotope studies of CO₂. *Geochim Cosmochim Acta*. 2011;75(22):7117-7131.
25. He B, Olack GA, Colman AS. Pressure baseline correction and high-precision CO₂ clumped-isotope (Δ_{47}) measurements in bellows and micro-volume modes. *Rapid Commun Mass Spectrom*. 2012;26(24):2837-2853.
26. Bernasconi SM, Hu B, Wacker U, Fiebig J, Breitenbach SF, Rutz T. Background effects on Faraday collectors in gas-source mass spectrometry and implications for clumped isotope measurements. *Rapid Commun Mass Spectrom*. 2013;27(5):603-612.
27. Rosenheim BE, Tang J, Fernandez A. Measurement of multiply substituted isotopologues ('clumped isotopes') of CO₂ using a 5 kV compact isotope ratio mass spectrometer: performance, reference frame, and carbonate paleothermometry. *Rapid Commun Mass Spectrom*. 2013;27(16):1847-1857.
28. Schauer AJ, Kelson J, Saenger C, Huntington KW. Choice of ¹⁷O correction affects clumped isotope (Δ_{47}) values of CO₂ measured with mass spectrometry. *Rapid Commun Mass Spectrom*. 2016;30(24):2607-2616.
29. Müller IA, Fernandez A, Radke J, et al. Carbonate clumped isotope analyses with the long-integration dual-inlet (LIDI) workflow: scratching at the lower sample weight boundaries. *Rapid Commun Mass Spectrom*. 2017;31(12):1057-1066.
30. Meckler AN, Ziegler M, Millán MI, Breitenbach SF, Bernasconi SM. Long-term performance of the Kiel carbonate device with a new correction scheme for clumped isotope measurements. *Rapid Commun Mass Spectrom*. 2014;28(15):1705-1715.
31. Müller IA, Violay ME, Storck J-C, et al. Clumped isotope fractionation during phosphoric acid digestion of carbonates at 70 C. *Chem Geol*. 2017;449:1-14.
32. Fernandez A, Tang J, Rosenheim BE. Siderite 'clumped' isotope thermometry: a new paleoclimate proxy for humid continental environments. *Geochim Cosmochim Acta*. 2014;126:411-421.
33. Tang J, Dietzel M, Fernandez A, Tripathi AK, Rosenheim BE. Evaluation of kinetic effects on clumped isotope fractionation (Δ_{47}) during inorganic calcite precipitation. *Geochim Cosmochim Acta*. 2014;134:120-136.
34. Yoshida N, Vasilev M, Ghosh P, Abe O, Yamada K, Morimoto M. Precision and long-term stability of clumped-isotope analysis of CO₂ using a small-sector isotope ratio mass spectrometer. *Rapid Commun Mass Spectrom*. 2013;27(1):207-215.
35. Schmid TW, Bernasconi SM. An automated method for 'clumped-isotope' measurements on small carbonate samples. *Rapid Commun Mass Spectrom*. 2010;24(14):1955-1963.
36. Hu B, Radke J, Schlüter HJ, Heine FT, Zhou L, Bernasconi SM. A modified procedure for gas-source isotope ratio mass spectrometry: the long-integration dual-inlet (LIDI) methodology and implications for clumped isotope measurements. *Rapid Commun Mass Spectrom*. 2014;28(13):1413-1425.
37. Wang Z, Schauble EA, Eiler JM. Equilibrium thermodynamics of multiply substituted isotopologues of molecular gases. *Geochim Cosmochim Acta*. 2004;68(23):4779-4797.
38. Daëron M, Blamart D, Peral M, Affek H. Absolute isotopic abundance ratios and the accuracy of Δ_{47} measurements. *Chem Geol*. 2016;442:83-96.
39. Nier AO. A redetermination of the relative abundances of the isotopes of carbon, nitrogen, oxygen, argon, and potassium. *Phys Rev*. 1950;77(6):789-793.
40. Santrock J, Studley SA, Hayes J. Isotopic analyses based on the mass spectra of carbon dioxide. *Anal Chem*. 1985;57(7):1444-1448.
41. Gonfiantini R, Stichler W, Rozanski K. Standards and intercomparison materials distributed by the International Atomic Energy Agency for stable isotope measurements. 1995.
42. Brand WA, Assonov SS, Coplen TB. Correction for the ¹⁷O interference in $\delta^{13}\text{C}$ measurements when analyzing CO₂ with stable isotope mass spectrometry (IUPAC Technical Report). *Pure Appl Chem*. 2010;82. <https://doi.org/10.1351/PAC-REP-09-01-05>
43. Bernasconi SM, Müller IA, Bergmann KD, et al. Reducing uncertainties in carbonate clumped isotope analysis through consistent carbonate-based standardization. *Geochem Geophys Geosyst*. 2018. in press. <https://doi.org/10.1029/2017GC007385>

How to cite this article: Venturelli RA, Rosenheim BE. Compositional and beam-size-dependent effects on pressure baseline in clumped isotope mass spectrometry. *Rapid Commun Mass Spectrom*. 2019;33:140-148. <https://doi.org/10.1002/rcm.8303>