



Defining an absolute reference frame for ‘clumped’ isotope studies of CO₂

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Abstract

We present a revised approach for standardizing and reporting analyses of multiply substituted isotopologues of CO₂ (i.e., ‘clumped’ isotopic species, especially the mass-47 isotopologues). Our approach standardizes such data to an absolute reference frame based on theoretical predictions of the abundances of multiply-substituted isotopologues in gaseous CO₂ at thermodynamic equilibrium. This reference frame is preferred over an inter-laboratory calibration of carbonates because it enables all laboratories measuring mass 47 CO₂ to use a common scale that is tied directly to theoretical predictions of clumping in CO₂, regardless of the laboratory’s primary research field (carbonate thermometry or CO₂ biogeochemistry); it explicitly accounts for mass spectrometric artifacts rather than convolving (and potentially confusing) them with chemical fractionations associated with sample preparation; and it is based on a thermodynamic equilibrium that can be experimentally established in any suitably equipped laboratory using commonly available materials.

By analyzing CO₂ gases that have been subjected to established laboratory procedures known to promote isotopic equilibrium (i.e., heated gases and water-equilibrated CO₂), and by reference to thermodynamic predictions of equilibrium isotopic distributions, it is possible to construct an empirical transfer function that is applicable to data with unknown clumped isotope signatures. This transfer function empirically accounts for the fragmentation and recombination reactions that occur in electron impact ionization sources and other mass spectrometric artifacts. We describe the protocol necessary to construct such a reference frame, the method for converting gases with unknown clumped isotope compositions to this reference frame, and suggest a protocol for ensuring that all reported isotopic compositions (e.g., Δ_{47} values; Eiler and Schauble, 2004; Eiler, 2007) can be compared among different laboratories and instruments, independent of laboratory-specific analytical or methodological differences. We then discuss the use of intra-laboratory secondary reference frames (e.g., based on carbonate standards) that can be more easily used to track the evolution of each laboratory’s empirical transfer function. Finally, we show inter-laboratory reproducibility on the order of ± 0.010 (1σ) for four carbonate standards, and present revised paleotemperature scales that should be used to convert carbonate clumped isotope signatures to temperature when using the absolute reference frame described here. Even when using the reference frame, small discrepancies remain between two previously published synthetic carbonate calibrations. We discuss possible reasons for these discrepancies, and highlight the need for additional low temperature (<15 °C) synthetic carbonate experiments.

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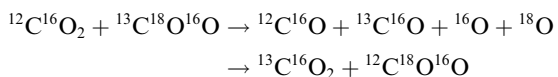
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1. INTRODUCTION

‘Clumped’ isotope geochemistry is the study of multiply-substituted isotopologues of naturally occurring molecules, and to date has been used primarily for analysis of mass 47 AMU CO₂ (predominantly ¹³C¹⁸O¹⁶O). The principles of quantum mechanical and statistical thermodynamics predict that multiply-substituted isotopologues of CO₂ have lower free energies than isotopologues with one or no heavy isotopes, principally because of their lower frequencies of intramolecular vibrations and consequent lower vibrational energies. As a result, multiply-substituted isotopologues of CO₂ are more stable than those with one or no heavy isotopes, and this thermodynamic preference for clumped isotopologues can be used to form the basis of a single-phase temperature proxy (i.e., a homogeneous equilibrium among isotopologues). Using gas source isotope ratio mass spectrometry, it is possible to measure deviations in abundances of multiply-substituted CO₂ from those expected if isotopologues were randomly distributed (the ‘stochastic’ distribution); such a deviation for mass 47 AMU isotopologues is defined as the Δ₄₇ value (Eiler and Schauble, 2004; Wang et al., 2004; Eiler, 2007). To date, the majority of work on CO₂ clumped isotopic species has focused on carbonate clumped isotope thermometry, in which acid digestion of a carbonate mineral yields CO₂ having a Δ₄₇ value dependent on the carbonate growth temperature (Ghosh et al., 2006; Schauble et al., 2006; Guo et al., 2009). Analyses of the Δ₄₇ value of atmospheric CO₂ have also been used to discriminate among contributions from combustion, respiration, and (in the stratosphere) photochemical reactions (Affek and Eiler, 2006; Affek et al., 2007; Yeung et al., 2009).

The measurement technique employed in current clumped isotope studies utilizes electron impact (EI) gas source isotope ratio mass spectrometry, with ionization energies of approximately 70 eV and accelerating voltages approaching 10 kV (to date published measurements have all used a Thermo Finnegan MAT 253 configured to collect masses 44 through 48 or 49). The ion sources of such mass spectrometers promote fragmentation and recombination of CO₂⁺ ions in the source, which can lead to the redistribution of isotopes among the analyzed isotopologues. A possible reaction pathways is:



Other mechanisms involving exchange with H₂O or other background species in the source are also possible. The amount of fragmentation and recombination will vary with the mass spectrometer used, source conditions (e.g., source pressure, filament temperature, ionization energy and ion residence time), and minor adjustments in source geometry and surface composition following tuning and cleaning. Generally, fragmentation and recombination in EI sources leads to isotopic fractionation, but this can be ignored when measuring bulk C and O isotopic compositions because it can be safely assumed that the fractionations equally affect the standard and sample and so are

accounted for by common standardization schemes. However, clumped isotope measurements aim to measure the difference in isotopic ordering between sample and standard, and therefore rely on both gases preserving the integrity of their isotopic ordering, or correcting for a known amount of change in isotopic ordering. We suggest fragmentation and recombination reactions result in partial randomization of isotopic ordering (because the energies of electron-molecule interaction are much greater than C–O bond energies) and therefore their effects will depend on how far a given sample or standard is from a stochastic distribution (i.e., samples that are already stochastic will undergo no change in distribution, whereas those that are far from stochastic will undergo large changes). As such, the standard and sample may not be equally affected.

Previously, measurements of mixtures of isotopic end member gases with known Δ₄₇ values suggested that fragmentation and recombination reactions in the source were negligible and could be ignored for clumped isotope studies (Eiler and Schauble, 2004). Since then, improvements in measurement technique and the continued collection of data have illustrated that fragmentation and recombination reactions are plausibly responsible for variations on the order of 10%, relative, in measured Δ₄₇ values of CO₂ gases (Affek et al., 2009; Huntington et al., 2009; Passey et al., 2010). If Δ₄₇ values are to be measured consistently and with absolute accuracy as good as the external precisions of the best measurements (0.005–0.010‰), we must directly account for these source ‘scrambling’ effects. More generally, an increasing number of laboratories are measuring clumped isotope compositions of CO₂. Inter-comparison of data from these laboratories will require an absolute reference frame that corrects for source scrambling and other analytical and methodological artifacts, permitting comparison of data from different times and laboratory conditions.

In this paper, we describe a new approach to data analysis that empirically accounts for fragmentation and recombination reactions in ion sources and other analytical artifacts. The empirical transfer function described here can be used to convert data to a common absolute reference frame that is based on the theoretical temperature dependence of equilibrium clumping in CO₂ (Wang et al., 2004). The advantage of such a reference frame is fourfold. First, it enables all laboratories to report their data in a common reference frame that is based on calculable and experimentally verifiable values for clumping in gas-phase CO₂ (Wang et al., 2004). This is preferred to tying the reference frame to carbonates, which can exhibit non-equilibrium isotope effects (e.g., Affek et al., 2008; Daeron et al., 2011) and must be analyzed using acid digestion procedures that introduce an additional source of interlaboratory bias. Calculating the theoretical temperature dependence of clumping in carbonate minerals is also less constrained than clumping in gas-phase CO₂ due to more complicated quantum mechanical and thermodynamic theory, and uncertainties associated with calculating phonon frequency differences between isotopically substituted carbonates (Schauble et al., 2006). Second, it is applicable to all laboratories measuring multiply substituted isotopologues of CO₂, regardless of whether the CO₂ is derived from carbonate minerals or is naturally found in gaseous form

(e.g., stratospheric CO₂). Third, it empirically accounts for source effects associated with electron impact ionization and any analytical artifacts resulting from methodological differences between laboratories, thereby eliminating a source of inter-laboratory uncertainty. Likewise, for studies of gaseous CO₂, use of the reference frame enables interpretations to be made relative to theoretical predictions. Fourth, it provides an example of approaches to standardization for other clumped isotope systems as they are developed (e.g., multiply substituted CH₄).

We test this reference frame by comparing data from four different laboratories for CO₂ equilibrated at various temperatures, demonstrating that differences among these laboratories can be corrected for with a high level of accuracy and precision (of order 0.01‰). We then demonstrate the use of this empirical transfer function by comparing results for CO₂ extracted from carbonate standards, showing that our approach can be applied to carbonate clumped isotope thermometry – the principal application of clumped isotope analyses at present. Finally, we present revised calibrations of the carbonate clumped isotope thermometer that convert previously published calibration data to be consistent with this new reference frame. These revised paleotemperature scales can be used to convert Δ_{47} values of natural carbonates to temperature, and enable inter-laboratory standardization of the carbonate clumped isotope thermometer.

2. DEFINING AN ABSOLUTE REFERENCE FRAME FOR CLUMPED ISOTOPE MEASUREMENTS

2.1. Constructing the empirical transfer function

We suggest the following approach for converting raw measurements of the clumped isotope composition of CO₂ samples to a common, and absolute, reference frame: first, the reference frame has been defined by calculations of the expected abundances of isotopologues in a thermodynamically equilibrated population of CO₂, based on application of quantum mechanical and statistical thermodynamic principles to spectroscopic data for CO₂ (Wang et al., 2004). We chose this reference frame, rather than isotopic fractionations associated with mixing, diffusion or gravitational setting, for several reasons: the spectroscopy of CO₂ vapor at low and moderate pressure has been studied extensively and is confidently known (e.g., Chedin and Teffo, 1984; Rothman et al., 1992); the thermodynamic theory used to translate those spectroscopic data into reduced partition coefficient ratios for the various relevant isotopologues is well understood and applies well to small, simple molecules like CO₂ (see discussion in the Appendix of Wang et al. (2004)); and there are well established protocols for driving CO₂ gas to isotopic equilibrium, by isotope exchange with liquid water (see Assonov and de Groot (2009) for a review of CO₂-water exchange methods), so it should be possible to experimentally reproduce this reference frame in multiple laboratories. If, in the future, the community is able to improve upon estimates of the expected abundances of isotopologues in a thermodynamically equilibrated population of CO₂, the reference frame can be easily adjusted.

Improvements in the theoretical calculations could arise from utilization of more complete spectroscopic data and/or improvement in the models of CO₂ clumping by incorporation of higher-order theory. Likewise, although the absolute errors stemming from uncertainties associated with the quantum mechanical and statistical thermodynamic predictions should not be disregarded, they are independent of the mass spectrometric effects we are accounting for, and as such, are not correlated with the errors associated with measuring clumped isotope species.

Next, a suite of CO₂ gases that differ from one another in bulk composition (i.e., in $\delta^{18}\text{O}$ and/or $\delta^{13}\text{C}$ value) and that were equilibrated to a common temperature (i.e., so they are all nominally identical in Δ_{47} value) are analyzed. The linear trend defined by these data in a plot of Δ_{47} vs. δ^{47} (both variables measured vs. a common working reference gas of constant isotopic composition) is used to define the non-linearity of the mass spectrometer being calibrated (see Huntington et al. (2009) for a discussion of non-linearity artifacts in Δ_{47} measurements). This is typically done using CO₂ heated to 1000 °C for approximately 2 h, but can be done using CO₂ that is isotopically equilibrated at any controlled temperature. The intercept of this trend indicates the difference in Δ_{47} between the equilibrated gas ('EG') and the working reference gas ('WG') when the two are identical in bulk isotopic composition; this difference is defined as $\Delta_{47-[\text{EGvsWG}]_0}$. Assuming a constant working gas for all measurements on a particular instrument, this value is characteristic of the temperature at which the gas was equilibrated.

Values of $\Delta_{47-[\text{EGvsWG}]_0}$ are determined for suites of equilibrated gases produced at two or preferably more temperatures. Because the absolute Δ_{47} value of equilibrated CO₂ is known at each temperature (based on quantum mechanical and statistical thermodynamic theory), one now has a data set consisting of 'true' Δ_{47} values and $\Delta_{47-[\text{EGvsWG}]_0}$ values for each temperature. We have made such determinations at four temperatures in each of the four laboratories participating in this study: one at high temperature (1000 °C) and three at lower temperatures (approximately 10, 25 and 50 °C, though these were not identical in all laboratories) (Fig. 1). The lower temperature equilibrations were performed by exposing CO₂ of variable bulk isotopic composition to water of variable oxygen isotopic composition for at least 1 day (typically 1–3 days). CO₂ is then extracted from the water-equilibration vessel through –70 to –80 °C ethyl-alcohol traps to ensure isolation from water, and cleaned via the same method as unknown carbonates and CO₂ gases (for details on cleaning procedures, see Ghosh et al. (2006), Dennis and Schrag (2010) and Passey et al. (2010)). The high temperature equilibration is prepared by sealing CO₂ gases of variable bulk isotopic compositions into quartz tubes and heating them to 1000 °C for at least 2 h (Eiler and Schauble, 2004; Huntington et al., 2009). The heated CO₂ is then quickly quenched to room temperature to prevent isotopic exchange during cooling. Again, prior to analysis, the CO₂ gas is cleaned using the same procedure.

Once values for $\Delta_{47-[\text{EGvsWG}]_0}$ at multiple temperatures have been obtained (Fig. 1a), we plot these values against

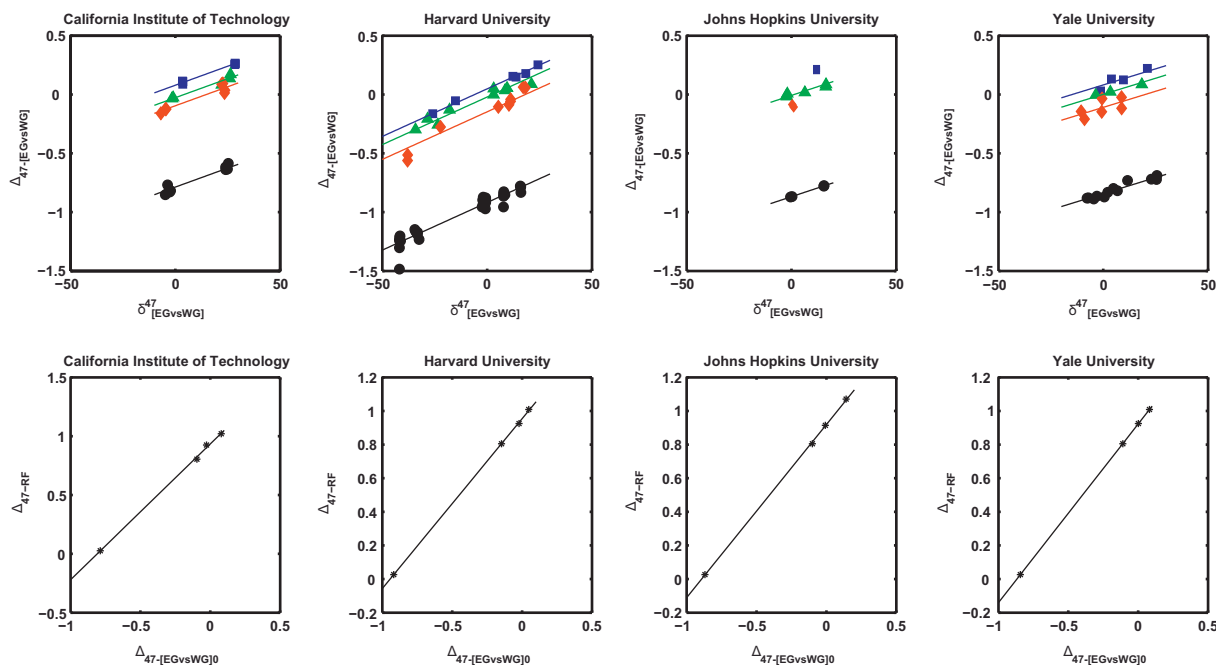


Fig. 1. Water-equilibrated and heated gas line regressions from the four laboratories participating in this study (a; first row). The data shown cover a range of equilibration temperatures that are not identical between laboratories. The coldest equilibration temperatures (between 0 and 10 °C) are shown in blue squares; room temperature equilibrations (between 25 and 27 °C) are shown in green triangles; higher temperature equilibrations (50 °C) are shown in red diamonds; and heated gas data (1000 °C) are shown in black circles. (b; second row) Plots the intercepts of the equilibration and heated gas lines ($\Delta_{47-[\text{EGvsWG}]0}$) against the theoretical value ($\Delta_{47-\text{RF}}$), and the linear regression shown defines the empirical transfer function. The corresponding data used to generate these plots is found in Table 1. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

the ‘true’ (theoretical equilibrium) values for Δ_{47} at the corresponding equilibration temperatures (Wang et al., 2004) (Fig. 1b). The linear regression defines the empirical transfer function (Table 1 and Fig. 1b) for each mass spectrometer (and associated purification and preparation devices) on which the $\Delta_{47-[\text{EGvsWG}]0}$ values were measured. Strictly speaking, this transfer function applies only for the specific reservoir of working reference gas used during these analyses and for a narrow window of time when all of the measurements were made. However, in practice we find these transfer functions are sufficiently stable that they can be assumed to apply for periods of weeks – long enough for calibration of secondary intralaboratory standards that can be more conveniently used to perform daily standardizations of unknowns. Notwithstanding, rapid and unexpected changes in a mass spectrometer’s non-linearity and source scrambling have also been observed, therefore it is advisable to analyze a smaller subset of equilibrium CO_2 on a regular basis to identify and address such changes.

Fig. 1a presents data for equilibrated gases generated by the four laboratories participating in this study. Linear regressions, with one common slope for each laboratory and based on data from all temperatures, have been fitted to raw measured δ^{47} and Δ_{47} values of equilibrated gases vs. a common working gas. The single slope regressions are shown in Table 1, along with the corresponding correlation coefficients (r^2). We have chosen to fit each laboratory’s data with a single slope as non-linearity effects are

expected to be equivalent at all Δ_{47} values at any given time, and the data are approximately consistent with this expectation. We infer that the small variations in slope among gases of different Δ_{47} values are likely an artifact of the fact that we cannot simultaneously measure more than one gas (i.e., the slope is weakly time dependent), as well as limits to measurement precision and sample preparation errors. In general, we suspect that CO_2 -water equilibrations at temperatures further from room temperature (i.e., 0, 8, 10 and 50 °C equilibrated gases) will be prone to larger errors associated with extracting the CO_2 gas at room temperature. Such errors do not apply to heated gases because the CO_2 gas is dry, and we see no evidence for equilibration back to room temperature for heated gases that have been stored in their original quartz tubes over a period of months. The parallel linear regressions for Johns Hopkins University have been calculated at two (1000 and 27 °C), rather than four, temperatures because only one bulk δ^{47} composition was measured at low temperature (0 °C) and high temperature (50 °C). Thus, the 10 and 50 °C equilibrations are corrected for linearity effects assuming a slope fit to the data at 1000 and 27 °C before being used to construct the empirical transfer function between measured and ‘true’ Δ_{47} values.

Fig. 1b plots the intercepts of these four lines (i.e., the respective $\Delta_{47-[\text{EGvsWG}]0}$) vs. the ‘true’ (theoretical) value for CO_2 equilibrium at the given temperature ($\Delta_{47-\text{RF}}$). Regression of each laboratory’s data yield:

Table 1

Water-equilibrated gas and heated gas line regressions used to generate the empirical transfer function for four different Thermo Finnegan MAT 253s, housed at the California Institute of Technology, Harvard University, Johns Hopkins University and Yale University. The theoretical calculations of equilibrium CO₂ clumping are also shown.

Theoretical	Temperature	Δ_{47-RF} ^a					
Wang et al. (2004)	0	1.0705					
	8	1.0208					
	10	1.0089					
	25	0.9252					
	27	0.9147					
	50	0.8050					
	1000	0.0266					
Laboratory	Temperature	n ^b	Slope ^c	Intercept ($\Delta_{47-[EGvsWG]0}$)	r^2	Empirical transfer function	r^2
California Institute of Technology	8	6	0.0065	0.0788	0.9859	$\Delta_{47-RF} = 1.1548\Delta_{47-[EGvsWG]0} + 0.9343$	0.9986
	25	5		-0.0271	0.9417		
	50	7		-0.0957	0.9133		
	1000	9		-0.7869	0.9386		
Harvard University	10	6	0.0081	0.0480	0.9916	$\Delta_{47-RF} = 1.0105\Delta_{47-[EGvsWG]0} + 0.9539$	0.9999
	25	9		-0.0216	0.9367		
	50	9		-0.1473	0.9375		
	1000	32		-0.9182	0.9167		
Johns Hopkins University ^d	0	2	0.0059	0.1412		$\Delta_{47-RF} = 1.0303\Delta_{47-[EGvsWG]0} + 0.9194$	0.9997
	27	6		-0.0077	0.8316		
	50	2		-0.1002			
	1000	4		-0.8686	0.9988		
Yale University	10	4	0.0055	0.0812	0.8086	$\Delta_{47-RF} = 1.0630\Delta_{47-[EGvsWG]0} + 0.9227$	0.9999
	25	3		0.0013	0.8886		
	50	6		-0.1094	0.4155		
	1000	12		-0.8431	0.9206		

^a Based on a fourth order polynomial fit to data from Wang et al. (2004) Table 4(I), which calculated equilibrium clumped isotope compositions using zero point energies and normal mode wave numbers presented in Wang et al. (2004) (Table 3). See Appendix A and Supplementary Data.

^b Number of samples used to define the equilibrated or heated gas line.

^c The slope used to define non-linearity is calculated based on data from all temperatures, not just the heated gases.

^d Only two samples were measured at 0 and 50 °C, therefore the determination of the slope of equilibrated and heated gas lines has been calculated using two sets of data (27 °C equilibrated gas and 1000 °C heated gas).

$$\Delta_{47\text{-RF}} = 1.1548\Delta_{47\text{-[EGvsWG]0}} + 0.9343$$

$$\Delta_{47\text{-RF}} = 1.0105\Delta_{47\text{-[EGvsWG]0}} + 0.9539$$

$$\Delta_{47\text{-RF}} = 1.0303\Delta_{47\text{-[EGvsWG]0}} + 0.9194$$

$$\Delta_{47\text{-RF}} = 1.0630\Delta_{47\text{-[EGvsWG]0}} + 0.9227$$

$$\text{California Institute of Technology} \quad (1)$$

$$\text{Harvard University} \quad (2)$$

$$\text{Johns Hopkins University} \quad (3)$$

$$\text{Yale University} \quad (4)$$

These linear regressions and their associated r^2 values are also shown in Table 1, and are referred to as the ‘empirical transfer functions’. Once a laboratory’s empirical transfer function (ETF) has been obtained, it can be used to convert measurements of unknown gases to the absolute reference frame by a two step procedure: first, the raw measured value of $\Delta_{47\text{-[SGvsWG]}}$, where SG denotes sample gas, is corrected for non-linearity to yield the respective $\Delta_{47\text{-[SGvsWG]0}}$ value using the slope of the equilibrated (and heated) gas lines (EGL) (Eq. (5)); also see [Huntington et al. \(2009\)](#)). This is then followed by application of the empirical transfer function, where $\Delta_{47\text{-RF}}$ represents Δ_{47} data projected in the absolute reference frame (Eq. (6)), and is considered the ‘true’ Δ_{47} value of the gas sample:

$$\Delta_{47\text{-[SGvsWG]0}} = \Delta_{47\text{-[SGvsWG]}} - \delta_{\text{[SGvsWG]}}^{47} \times \text{Slope}_{\text{EGL}} \quad (5)$$

$$\Delta_{47\text{-RF}} = \Delta_{47\text{-[SGvsWG]0}} \times \text{Slope}_{\text{ETF}} + \text{Intercept}_{\text{ETF}} \quad (6)$$

Each empirical transfer function can now be used to project raw sample gas data into a common and absolute reference frame. A schematic illustration of the correction procedure is presented in Fig. 2. This diagram shows how a sample’s Δ_{47} changes as one applies the linearity correction (step 1–2; Eq. (5)), followed by the empirical transfer function (step 2–3; Eq. (6)); and finally the acid correction for carbonates reacted at temperatures other than 25 °C (step 3–4; see Section 3.1 for details).

2.2. Secondary reference frame

Source scrambling and non-linearity evolve over time and, as a result, the empirical transfer function is technically only valid for a short period of time. In practice, one can continually evaluate and modify the empirical transfer function by routinely analyzing equilibrated gases of disparate equilibrium temperatures (e.g., 1000 and 25 °C) and with different bulk isotopic compositions. But as a practical measure, we recognize that laboratories may choose to monitor the empirical transfer function indirectly by constructing a secondary reference frame (i.e., some set of intra- or interlaboratory standards) that is monitored and used to adjust the empirical transfer function over time. In this section, we suggest a secondary reference frame that may help streamline clumped isotope analysis and enable laboratories to focus on standards that are prepared in identical methodology to samples (e.g., a carbonate standard for a laboratory focused on carbonate paleothermometry; or a CO₂ standard for a laboratory focused on environmental CO₂), while still reporting data relative to the absolute, interlaboratory reference frame we suggest.

The suggested approach to defining a secondary reference frame is to use accepted values for carbonate (or CO₂) standards and heated gases to define a second transfer function that is based on a plot of ‘observed’ vs. ‘accepted’ values. This approach is analogous to that taken for

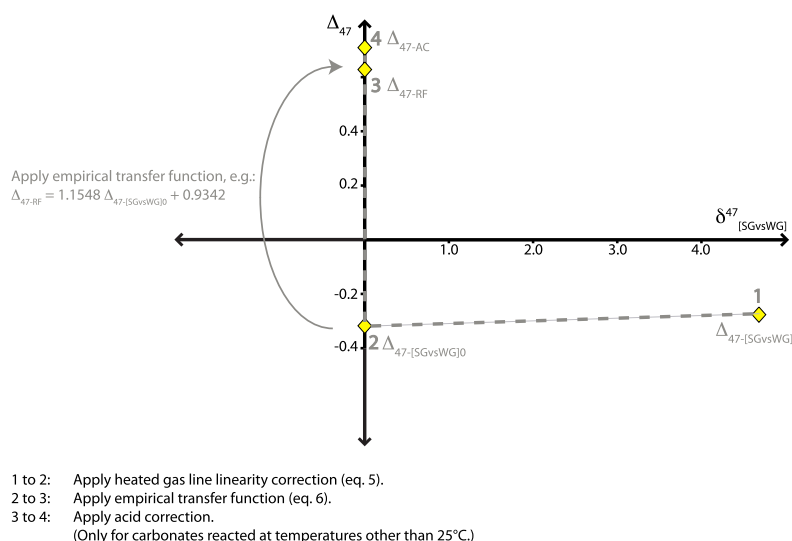


Fig. 2. Diagram to illustrate the correctional procedure for raw measurement data ($\Delta_{47\text{-[SGvsWG]}}$) using the linearity correction (1–2), empirical transfer function (2–3) and acid correction (3–4; only applied to carbonates that have been reacted at temperatures other than 25 °C; see Section 3.1 for discussion). Going forward, we suggest laboratories publishing clumped isotope data follow this correctional procedure and present all data in absolute reference frame discussed here.

high-precision H/D isotope measurements (e.g., Tobias et al., 1995; Morrison et al., 2001). To utilize this approach, one would first calculate Δ_{47} values for standards using the empirical transfer function (Eq. (6)) previously determined in one's laboratory. These values can then be plotted against the standards' accepted 'true' values. The 'true' values are those determined for the same standards, but measured during the same analytical time period as the equilibrated gas used to generate the empirical transfer function, or Δ_{47} values accepted as the 'true' value by the clumped isotope community. A linear fit through these points would then define a second transfer function that can be applied to the data, thereby enabling data to be presented in the absolute reference frame without continuously monitoring the empirical transfer function using equilibrated gases.

2.3. Tertiary reference frame

Although ideally each laboratory will now report Δ_{47} values using the absolute reference frame as defined by water-equilibrated gases, heated gases and working standards, it is challenging to re-cast previously published data into this reference frame. For data acquired prior to the development of the absolute reference frame, i.e., data that has not been corrected for scale compression due to source effects, we must attempt to convert past Δ_{47} values into the new absolute reference frame. Huntington et al. (2009) did this empirically for data produced by one mass spectrometer by accounting for variability in the heated gas line (HGL) intercept over time. Using an analogous mathematical description we develop and extend this approach to the absolute reference frame and as such enable its application to other mass spectrometers and laboratories. We refer to this as the tertiary approach, and introduce it to help laboratories project data that has already been acquired into the absolute reference frame.

First, the empirical transfer function for a given mass spectrometer and working reference gas is used to define the 'true' Δ_{47} value for that reference gas. This value is assumed to be constant (valid to first order, as long as the same reservoir of reference gas is used and no significant fractionation has occurred over time). The working reference gas's 'true' clumped isotope composition is the intercept of the empirical transfer function. This intuitively makes sense because, by definition, the working reference gas has a bulk and clumped isotopic compositions equal to zero when measured against itself ($\delta^{47} = 0$ and $\Delta_{47-[\text{WGvsWG}]} = 0$). Therefore, within the terminology of the empirical transfer function, the working reference gas has a value of $\Delta_{47-[\text{SGvsWG}]_0} = 0$ and hence is the intercept in Eq. (6) ($\Delta_{47\text{-RF}}$ plotted against $\Delta_{47-[\text{SGvsWG}]_0}$).

Next, we use CO₂ gases heated to 1000 °C to quantify variations in mass spectrometric effects. Heated gases, which have always been analyzed in laboratories making Δ_{47} measurements, are already used to account for non-linearity (Slope_{EGL} in Eq. (5)). In addition, when used together with the 'true' Δ_{47} value for the reference gas, heated gases can provide the necessary information for

quantification of ion source scrambling. If we assume the working reference gas's isotopic composition is constant with time, and therefore that the intercept of the empirical transfer function is constant, the time-dependent term in Eq. (6) is the slope of the empirical transfer function. When using heated gases ('HG'), it is possible to re-arrange Eq. (6) to calculate the slope of the transfer function by substituting $\Delta_{47\text{-RF}@1000^\circ\text{C}}$ for $\Delta_{47\text{-RF}}$, and $\Delta_{47-[\text{HGvsWG}]_0}$ (i.e., the heated gas line intercept) for $\Delta_{47-[\text{SGvsWG}]_0}$:

$$\text{Slope}_{\text{ETF}} = (\Delta_{47\text{-RF}@1000^\circ\text{C}} - \text{Intercept}_{\text{ETF}}) / \Delta_{47-[\text{HGvsWG}]_0} \quad (7a)$$

where $\Delta_{47\text{-RF}@1000^\circ\text{C}} = 0.0266\text{‰}$ (see Table 1) and $\Delta_{47-[\text{HGvsWG}]_0} = \text{Intercept}_{\text{HGL}}$. It then follows that the 'true' value for a sample can be calculated as:

$$\Delta_{47\text{-RF}} = \Delta_{47-[\text{SGvsWG}]_0} \times ((\Delta_{47\text{-RF}@1000^\circ\text{C}} - \text{Intercept}_{\text{ETF}}) / \Delta_{47-[\text{HGvsWG}]_0}) + \text{Intercept}_{\text{ETF}} \quad (7b)$$

We refer to this as the tertiary approach because it makes an implicit assumption about the mechanisms of source scrambling (i.e., that fragmentation/recombination reactions move CO₂ gases towards the stochastic distribution) and relies on the stability of working reference gases. Using this approach as a day-to-day standardization method, in addition to its use for recalculating past data, thus requires regular monitoring of the stability of the working reference gas via measurements of a gas with an 'accepted' Δ_{47} value, such as a commonly measured carbonate standard or CO₂ gas equilibrated at a known temperature.

We continue to stress the importance of measuring equilibrated gases spanning a range of temperatures on a regular basis (at least every few months), as it enables each mass spectrometer's source scrambling to be rigorously monitored and minimizes the impact of other analytical errors on the empirical transfer function. However, we acknowledge the functional advantages of using a secondary reference frame. First, it enables a laboratory to indirectly measure changes in source scrambling, without having to continuously measure two or more equilibrated gases. Second, the use of a secondary reference frame allows laboratories to correct old data to the new, absolute reference frame, assuming the in-house standards used during the original measurement period can be measured today in the absolute reference frame. Once the 'true' clumped isotopic composition of the standards is known, the old data can also be re-calculated and projected into the absolute reference frame. In Section 3.2, we illustrate this procedure using data from the published synthetic calcite calibrations of the carbonate clumped isotope thermometer. Third, at a minimum, data acquired in the past can be translated to the absolute reference frame by assuming the working reference gas has not changed in clumped isotopic composition, and by using heated gas measurements to constrain changes in the slope of the empirical transfer function.

3. APPLICATION OF THE ABSOLUTE REFERENCE FRAME TO CARBONATE CLUMPED ISOTOPE THERMOMETRY

The definition of an absolute reference frame enables the comparison of clumped isotope data from different laboratories, accounting for laboratory-specific analytical or methodological artifacts. In this section, we apply the new reference frame to old measurements, and use recent data to compare measurements of inter- and intra-laboratory standards, including NBS-19 (available from NIST and IAEA) and in-house Carrara marble standards. We also re-calculate the two published synthetic calcite calibrations of the carbonate clumped isotope thermometer, thereby casting them into the new absolute reference frame, and consider reasons for discrepancies between the calibrations.

3.1. Inter-laboratory comparison of carbonates – a proof of concept

To illustrate the feasibility and utility of an absolute reference frame, we evaluate the inter-laboratory reproducibility of Δ_{47} values for carbonate standards. The Δ_{47} values have been projected into the absolute reference frame using Eqs. (5) and (6), and the empirical transfer functions defined for each laboratory (Eqs. (1)–(4)). We have also applied an empirically derived acid fractionation correction of +0.081‰ to carbonates reacted at 90 °C rather than the accepted normal calcite reaction temperature of 25 °C (this applies to data from California Institute of Technology,

Harvard University and Johns Hopkins University, whereas the Yale University laboratory uses a 25 °C reaction). This correction was determined during the development of an automated inlet system on Mass Spectrometer 2 at the California Institute of Technology (Passey et al., 2010), and is similar to a theoretically derived correction (Eq. 23 of Guo et al. (2009)) that yields an offset of +0.069‰ between 90 and 25 °C acid reactions. The empirically derived acid correction factor of +0.081‰ was calculated in the old intralaboratory reference frame, which is, on average, compressed compared to the stretched absolute reference frame. For this reason, this value is likely in error by approximately 0.01‰ but we refrain from updating it until a revised value has been determined by direct observations, calibrated in the absolute reference frame. In addition, data from Yale University have been projected into the absolute reference frame using an adjusted empirical transfer function slope calculated from monitoring changes in the heated gas line intercept over time, i.e., both recent and old data has been projected into the absolute reference frame using the tertiary approach discussed in Section 2.3. The results of the inter-laboratory comparison of carbonate standards are shown in Table 2.

Using the absolute reference frame for clumped isotope analysis, four laboratories are able to reproduce four low and high temperature standards to better than 0.017‰ (1 σ). The IAEA marble standard, NBS-19, has a clumped isotope composition of $0.392 \pm 0.017\text{‰}$. The error on this standard is driven predominantly by Harvard University's relatively low value ($\Delta_{47} = 0.373\text{‰}$ as compared to Johns

Table 2

Comparison of carbonate standards using the absolute reference frame. Temperature is calculated using Eq. (9), the synthetic calibration of Ghosh et al. (2006) projected into the absolute reference frame.

Standard	Laboratory	<i>n</i>	$\Delta_{47}\text{-[SGvsWG]}$	Δ_{47} (‰)	SE	Temperature (°C)
NBS-19	California Institute of Technology					
	Harvard University	7	−0.512	0.373	0.007	137.2
	Johns Hopkins University	12	−0.586	0.399	0.005	123.9
	Yale University	40	−0.407	0.404	0.006	121.1
	Mean			0.392		127.4
	1 σ			0.017		8.6
In-house Carrara Marble	California Institute of Technology	18	−0.402	0.392	0.007	127.1
	Harvard University ('CM2')	40	−0.643	0.385	0.005	130.6
	Johns Hopkins University ('UU Carrara')	8	−0.581	0.403	0.006	121.8
	Yale University ('YCM')	49	−0.445	0.400	0.004	123.3
	Mean			0.395		125.7
	1 σ			0.008		4.0
102-GC-AZ01	California Institute of Technology	9	−0.235	0.724	0.007	22.3
	Harvard University	4	−0.326	0.706	0.005	26.1
	Johns Hopkins University	17	−0.289	0.701	0.003	27.0
	Yale University	5	−0.166	0.723	0.015	22.5
	Mean			0.713		24.5
	1 σ			0.012		2.4
DSC-45923	California Institute of Technology	2	−0.098	0.797	0.009	8.4
	Harvard University	3	−0.228	0.805	0.007	7.1
	Johns Hopkins University	4	−0.074	0.775	0.016	12.4
	Yale University	4	−0.038	0.781	0.012	11.4
	Mean			0.790		9.8
	1 σ			0.014		2.5

Hopkins University and Yale University at $\Delta_{47} = 0.399\%$ and $\Delta_{47} = 0.404\%$, respectively), but there is no justification for ignoring or discounting any of these three values. In comparison, in-house Carrara marble standards yield a population mean of $\Delta_{47} = 0.395 \pm 0.008\%$. This suggests Carrara marbles typically retain consistent clumped isotope compositions, but that individual vials of NBS-19 can be heterogeneous and result in relatively poor reproducibility of clumped isotope data, even within one laboratory. The low temperature standards measured in this study are a deep-sea coral (DSC-45923) and a vein calcite (102-GC-AZ01). Both yield clumped isotope compositions reproduced between laboratories with a precision on the order of 0.010% (DSC-45923: $\Delta_{47} = 0.790 \pm 0.014\%$; 102-GC-AZ01: $\Delta_{47} = 0.713 \pm 0.012\%$). These results illustrate the benefit of using an absolute reference frame that projects each laboratory's data into a common reference frame, and demonstrates the ability to reproduce clumped isotope data *within* each laboratory on the order of $\pm 0.005\%$ (1σ SE) and *between* laboratories on the order of $\pm 0.010\%$ (1σ).

We have also conducted a simple sensitivity study for one laboratory's data to test how the selection of the linearity correction, the corresponding $\Delta_{47-[\text{EGvsWG}]_0}$ and the empirical transfer function impact the final, reported Δ_{47} value. Reported in Table 2 are the carbonate standards for Harvard University derived by applying a single slope correction to all equilibrated and heated gas data (i.e., fitting the four temperatures lines with one common slope), and then using this slope to derive values for $\Delta_{47-[\text{EGvsWG}]_0}$, construct the empirical transfer function (Eq. (2)) and correct raw carbonate data. In addition, we have derived an empirical transfer function using the best-fit slope for each one of the equilibrated and heated gas data set (i.e., four different slopes) and use these to calculate $\Delta_{47-[\text{EGvsWG}]_0}$ values to generate a second, slightly different, ETF:

$$\Delta_{47\text{-RF}} = 1.0145\Delta_{47-[\text{EGvsHG}]_0} + 0.9565 \quad \text{Harvard University ETF 2} \quad (8)$$

Table 3

Sensitivity test for Harvard University carbonate standards. Each equilibrated gas line is fit via linear least square yielding different slopes for each temperature. The corresponding $\Delta_{47-[\text{EGvsWG}]_0}$ values are then used to construct a second ETF (Harvard University ETF 2 Eq. (8)). We calculate the range in Δ_{47} values for each replicate carbonate measurement based on using the ETF for Harvard University in Table 1, ETF 2 with a single slope linearity correction, and ETF 2 with each of the four different slopes calculated for equilibrated gases. Each replicate is assigned a Δ_{47} value based on the mean of the various calculations, and an associated 1σ . The error associated with this is then propagated through to yield a weighted mean and 1σ uncertainty for each carbonate standard (columns 5 and 6) based on n replicates (column 2). This can be compared to the values reported in Table 2, and copied here in columns 3 and 4.

Temperature	Slope	Intercept ($\Delta_{47-[\text{EGvsWG}]_0}$)	r^2	Harvard University empirical transfer function 2	
10	0.0074	0.0554	0.9919	$\Delta_{47\text{-RF}} = 1.0145 \Delta_{47-[\text{EGvsWG}]_0} + 0.9565$	
25	0.0066	-0.0222	0.8340		
50	0.0114	-0.1643	0.8825		
1000	0.0081	-0.9139	0.9279		
Carbonate Standard	n	Δ_{47}	SE	Weighted mean	Uncertainty in weighted mean (1σ)
NBS-19	7	0.373	0.007	0.370	0.011
CM2	40	0.385	0.005	0.382	0.007
102-GC-AZ01	4	0.706	0.005	0.705	0.003
DSC-45923	3	0.805	0.007	0.802	0.022

Upon application of this second ETF and variable linearity corrections (ranging in slope from 0.0066 to 0.0114), we calculate a new Δ_{47} value for each replicate and an associated 1σ error, which accounts for the suite of linearity corrections and use of the second ETF. We then propagate the errors of each replicate to calculate a weighted mean and 1σ uncertainty for each carbonate standard. The results are shown in Table 3. The weighted mean of the sensitivity test deviates from the original reported values by a maximum of 0.003% . The 1σ uncertainty of the mean increases for three of the four carbonate standards and the maximum error is 0.022% . This error is greatest for the carbonate with a δ^{47} value furthest from 0 (DSC-45923, $\delta^{47} = 22.8\%$), i.e., the carbonate that requires the largest linearity correction. Overall, we find the linearity correction transfers a larger error to the final Δ_{47} value than the ETF correction, especially for gases with bulk isotopic compositions far from the working reference gas ($\delta^{47} = 0\%$).

3.2. Converting carbonate Δ_{47} values to temperature

Given the advantages of an absolute reference frame, it is useful to re-calculate the published synthetic calibrations of the carbonate clumped isotope thermometer (Ghosh et al., 2006; Dennis and Schrag, 2010); i.e., so that it is straightforward to interpret carbonate Δ_{47} values reported relative to the absolute reference frame as paleotemperatures. We do so by determining a secondary transfer function for the time period during which each calibration data set was generated and projecting those data into the absolute Δ_{47} reference frame using this function. Ideally, one would do this by generating an empirical transfer function from equilibrated and heated gases from the time period of calibration using the same method as that demonstrated in Section 2.1. At the time when the two published synthetic calibration studies were conducted, fragmentation and recombination reactions were considered negligible and therefore the stochastic reference frame against which the Δ_{47} values were reported was based only

Table 4

Re-calculation of published synthetic calibration curves to convert Δ_{47} values to temperature. The re-calculations are based on constructing a secondary reference frame for each calibration time period, given observed vs. accepted values for heated gases and carbonate standards. See discussion in text.

Laboratory	Published calibration		Sample	Observed Δ_{47}	$\Delta_{47\text{-RF}}$ ('accepted')	Secondary reference frame transfer function		Re-calculation of synthetic calibration	
	Slope	Intercept				Slope	Intercept	Slope	Intercept
California Institute of Technology (Ghosh et al., 2006)	0.0592	−0.02	Equilibrated gas (1000 °C)	0.000	0.027	1.0381	0.0266	0.0636 ± 0.0049	-0.0047 ± 0.0520
			NBS-19	0.352	0.392				
Harvard University (Dennis and Schrag, 2010)	0.0337	0.2470	Equilibrated gas (1000 °C)	0.000	0.027	1.0744	0.0266	0.0362 ± 0.0018	0.2920 ± 0.0194
			CM2 (Carrara marble)	0.334	0.385				

on measurements of heated gases. This defined the trend in raw Δ_{47} vs. δ^{47} and enabled one to correct for non-linearities of a given mass spectrometer, but is insufficient to construct an empirical transfer function that accounts for 'compression' of the Δ_{47} scale due to fragmentation/recombination reactions, and which requires measurements of at least two materials having independently known Δ_{47} values. Instead, we have reconstructed the transfer functions necessary to translate these data into the absolute reference frame using carbonate standards measured at the same time as the synthetic calibration, and now having an accepted value (i.e., we derive a transfer function based on the secondary reference frame discussed in Section 2.2, and using the accepted values given in Table 2).

The old empirical transfer function for the California Institute of Technology Mass Spectrometer I (MS I) has been calculated using published data for 1000 °C heated gases and NBS-19 (Table 4). The heated gas line intercept for MS I during the Ghosh et al. (2006) study was -0.8453‰ (Huntington et al., 2009), and the published value for NBS-19 was $0.352 \pm 0.019\text{‰}$ (1 σ ; Ghosh et al., 2006). Given earlier definitions of Δ_{47} where heated gases were assumed to yield the stochastic distribution (Eiler and Schauble, 2004; Affek and Eiler, 2006), the heated gas intercept was equated to $\Delta_{47} = 0\text{‰}$, and we construct a two point secondary transfer function based on observed vs. accepted Δ_{47} values (see Table 4). This secondary transfer function is then applied to the published synthetic Δ_{47} data to yield the following re-calculation of the calibration equation (Eq. (9)). When presenting clumped isotope data in the absolute reference frame, we suggest this equation should be used to convert Δ_{47} values to temperature (in Kelvin):

$$\Delta_{47} = (0.0636 \pm 0.0049 \times 10^6)/T^2 - (0.0047 \pm 0.0520) \text{ (calibration range 1–50 °C)} \quad (9)$$

We have re-calculated the second published calibration using a similar approach. Dennis and Schrag (2010) report the heated gas intercept as -0.9244‰ during the calibration time period, and a value of $\Delta_{47} = 0.344 \pm 0.004\text{‰}$ (1 σ SE) for Harvard University's in-house Carrara marble standard ('CM2'). We plot observed vs. accepted values for heated

gas and CM2 to calculate the secondary transfer function and apply this to the published data to yield:

$$\Delta_{47} = (0.0362 \pm 0.0018 \times 10^6)/T^2 + (0.2920 \pm 0.0194) \text{ (calibration range 7.5–77 °C)} \quad (10)$$

Both re-calculations utilize a linear least square fit to the data and incorporate standard errors in replicate measurements of the synthetic carbonates. The calculation does not explicitly incorporate the error associated with using a secondary reference frame.

Presenting the two published synthetic carbonate calibrations in a common reference frame does not resolve the discrepancy between the two published calibrations (Fig. 3). Data from the two calibrations overlap between ca. 20 and 50 °C, but still diverge at low temperatures. Possible explanations for the divergence include, but are not limited to, differences in mineralogy, incomplete isotopic equilibration between water, dissolved inorganic carbon and carbonate, and differences in the methodology used to precipitate synthetic carbonates (Dennis and Schrag, 2010). Fig. 3 also plots the relationship between Δ_{47} and temperature based on coupling a theoretical model of the temperature dependence of ^{13}C – ^{18}O clumping in calcite (Schauble et al., 2006) to a transition state theory prediction of the kinetic isotope effects associated with phosphoric acid digestion of carbonates (Guo et al., 2009). Using the absolute reference frame we now see a larger offset between the theoretical model and the synthetic calibrations. Although the slope of the Harvard University calibration closely approaches the theoretical model, both synthetic data sets are offset to higher Δ_{47} values. One possible explanation for this is related to the fractionation associated with phosphoric acid digestion of carbonates. Using transition-state theory, Guo et al. (2009) predict an acid fractionation of 0.220‰ at 25 °C, and their published experimental results for re-crystallized carbonates support this ($\Delta_{47^*} = 0.232\text{‰}$, where Δ_{47^*} is measure of the isotopic fractionation associated with phosphoric acid digestion at 25 °C). But, if a secondary transfer function is constructed based on measurements of NBS-19 reported in Guo et al. (2009), the re-crystallization experiments yield an experimental acid

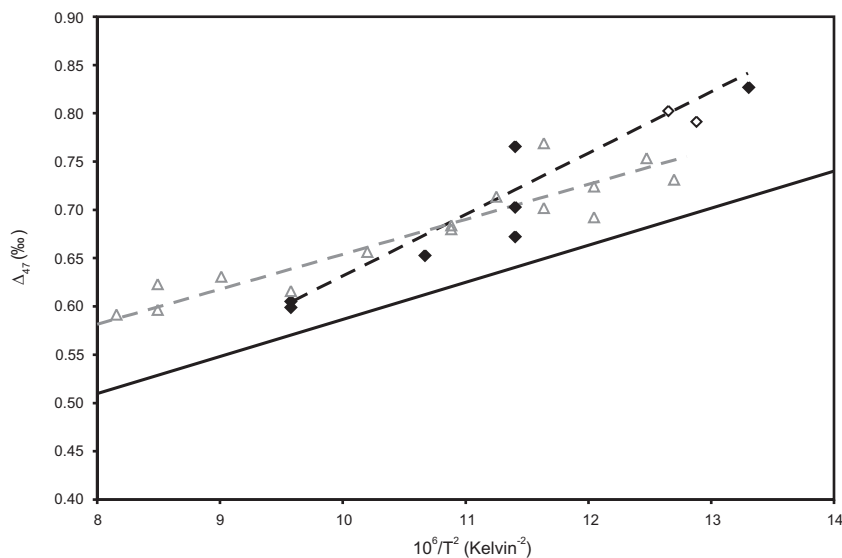


Fig. 3. Comparison of synthetic calibration curves projected into the absolute reference frame using published data and secondary transfer functions for the time period of measurement. Filled diamonds are projections of the California Institute of Technology data (Ghosh et al., 2006) into the absolute reference frame and are used to calculate Eq. (9). Open gray triangles are projections of the Harvard University data (Dennis and Schrag, 2010) into the absolute reference frame and are used to calculate Eq. (10). The black line is the relationship between Δ_{47} and temperature based on coupling a theoretical model of the temperature dependence of ^{13}C - ^{18}O clumping in calcite to a transition state theory prediction of the kinetic isotope effects associated with phosphoric acid digestion of carbonates (Schauble et al., 2006; Guo et al., 2009). The dashed black and gray lines are fits to the California Institute of Technology and Harvard University data, respectively. Coral samples with known growth temperatures and also measured by Ghosh et al. (2006) fall on to the California Institute of Technology calibration line (open diamonds).

fractionation of $\Delta_{47^*} = 0.280\text{‰}$ when projected into the absolute reference frame. As a result, the theoretical acid fractionation ($\Delta_{47^*} = 0.220\text{‰}$) diverges more significantly from their experimental results – an offset of 0.06‰ , rather than 0.01‰ . This implies some combination of the following: the re-crystallization experiments did not achieve a stochastic distribution or were subject to isotopic exchange upon cooling; or that the transition state theory does not accurately predict the fractionation associated with acid digestion of carbonates. If the acid fractionation is larger than that found by the model, consistent with larger fractionation observed by Passey et al. (2010), any prediction of equilibrium clumping in calcite, as measured by Δ_{47} , will shift to more positive values, and may help to align theory with data.

Although, theory and data appear to diverge, many modern natural carbonates (both biogenic and authigenic) have a temperature sensitivity (i.e., slope) over Earth's surface temperatures similar to the calibration of Ghosh et al. (2006), and yield Δ_{47} values within error of the original synthetic calcite calibration curve (Eiler, 2007; Came et al., 2007; Eiler et al., 2009; Passey et al., 2010; Tripathi et al., 2010). There are also examples of carbonates that do not align with this calibration, including speleothems (Affek et al., 2008; Daeron et al., 2011) and some mollusks (unpublished work). Going forward, the clumped isotope community continues to assess why the synthetic carbonate calibrations diverge, including if it is related to disequilibrium precipitation, or if there are other unrecognized artifacts related to the formation of synthetic carbonates or acid digestion reactions. In addition, future work may dem-

onstrate that some types of natural carbonates require separate Δ_{47} -temperature calibrations that account for mechanistic differences in carbonate formation. Of particular importance is the study of low temperature carbonates where the largest discrepancies between the synthetic calibrations exist. Until further inorganic carbonates can be produced and measured over the range of 0–15 °C, and the fractionation associated with acid digestion is better constrained, the reasons for the discrepancy between the synthetic calibrations will likely remain unresolved.

4. SUMMARY AND OUTLOOK

Recent work has suggested the use of high temperature carbonates, such as carbonatites and marbles, as a basis for quantifying source effects (Schmid and Bernasconi, 2010). Although using a carbonate standard directly can be advantageous because it allows a standard to be prepared and measured with identical methodology to measured carbonate samples, this approach amounts to a version of the 'secondary reference frame' discussed above. Instead, we suggest it should only be pursued in parallel with periodic characterization of the full standard reference frame using equilibrated gases. Defining the absolute reference frame based on equilibrated CO₂, rather than carbonates, is preferred for a number of reasons. First, a reference frame based on measuring equilibrated CO₂ is applicable to all laboratories measuring multiply substituted isotopologues of CO₂, regardless of the source of CO₂ (derived from carbonate minerals or occurring in the gaseous form). Second, it is not possible to theoretically predict the

clumped isotope signature of natural high temperature carbonates given uncertainties in the temperature of formation, calibration of the carbonate clumped isotope thermometer at high temperatures and solid-state isotope exchange during cooling. In addition, there are known kinetic effects that can impact the clumped isotope signature of carbonate minerals (Affek et al., 2008; Daeron et al., 2011). As such there is no reference frame in which one can compare measured values to expected values. Third, a recent study showed that there is considerable variability in the clumped isotope signatures of pristine carbonatites (i.e., carbonatites with no evidence for broad-scale fluid–rock interactions) (Dennis and Schrag, 2010). Heterogeneity was found in samples both within individual igneous suites and between suites suggesting variability in burial history, cooling rate, accessory mineralogy and water content impacts a sample's clumped isotope signature (Dennis and Schrag, 2010). Such heterogeneities make it challenging to identify carbonatites that could serve as a clumped isotope reference frame. Finally, clumped isotope signatures of carbonates have an inherent uncertainty related to the error associated with synthetic carbonate calibrations, and uncertainties related to sample preparation, including the acid digestion of carbonates.

We present an absolute reference frame based on a comparison of equilibrated CO₂ gases to quantum mechanical and statistical thermodynamic predictions of equilibrium isotopic distributions. These water-equilibrated and heated CO₂ gases span the entire range of Δ₄₇ values typically measured, and form the basis of the empirical transfer function used to project measured Δ₄₇ values into an absolute reference frame that accounts for electron ionization source effects. The absolute reference frame established here enables the direct comparison of data generated in different laboratories, with varying source conditions, and over time, i.e., it is independent of laboratory-specific analytical or methodological artifacts.

We show that when using the absolute reference frame four laboratories are able to reproduce clumped isotope data *within* each laboratory on the order of ±0.005‰ (1σ SE) and *between* laboratories on the order of ±0.010‰ (1σ). Although the initial definition of each mass spectrometer's empirical transfer function requires a number of water-equilibrated and heated gas measurements to establish the baseline 'scrambling', each individual laboratory can then define an internal secondary reference frame based on the appropriate internal standards (either carbonates or CO₂) and use this secondary reference frame for day-to-day measurements. The secondary reference frame can also be used to project previously published data into the absolute reference frame assuming a standard, which now has an accepted Δ₄₇ value, was measured during the earlier time period, or by assuming that the clumped isotopic composition of the working reference gas has not changed over time.

We have used the secondary reference frame to re-calculate two published synthetic calibration equations, and find the absolute reference frame has not improved the discrepancy in slope between the synthetic calibrations. Additionally, the discrepancy between synthetic calcite

data and theoretical predictions of clumping in carbonates has increased. In the future, the clumped isotope community must aim to better constrain the relationship between Δ₄₇ and temperature for low temperature carbonates (<15 °C). We may also benefit from improving our understanding of the fractionation associated with acid digestion of carbonates, either by conducting additional re-crystallization experiments or by re-visiting models based on transition state theory. Once established, the use of the empirical transfer function to project clumped isotope data into a common, and absolute, reference frame based on equilibrium theory will benefit the clumped isotope and larger geochemical community, and enable the comparison of data between laboratories and over time.

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APPENDIX A. AN EXAMPLE OF CONSTRUCTING AN EMPIRICAL TRANSFER FUNCTION

To illustrate the methodology for constructing an empirical transfer function, we show a detailed example using the heated and water-equilibrated gas data from the California Institute of Technology. First, we obtain data on heated and water-equilibrated gas measurements and construct a table of equilibration temperature, δ⁴⁷ and raw Δ₄₇ (Table A1). The raw Δ₄₇ value refers to the value calculated via:

$$\Delta_{47} = \left[\left(\frac{R^{47}}{R^{47'}} - 1 \right) - \left(\frac{R^{46}}{R^{46'}} - 1 \right) - \left(\frac{R^{45}}{R^{45'}} - 1 \right) \right] \times 1000 \quad (\text{A1})$$

where R^i represents the ratio of mass i to mass 44 measured for the equilibrated CO₂ and $R^{i'}$ represents the expected ratio given a stochastic distribution of isotopes among the isotopologues. For details on how to calculate Δ₄₇, the reader is referred to Appendix B of Affek and Eiler (2006).

Table A1

California Institute of Technology's raw water-equilibrated and heated gas data. This data has been used to calculate $\Delta_{47-[\text{EGvsWG}]0}$ values at four temperatures.

Temperature (°C)	δ^{47}	Δ_{47} (raw)
8	28.33	0.255
	3.41	0.114
	28.51	0.267
	28.63	0.261
	3.58	0.110
25	3.54	0.087
	-1.06	-0.026
	-1.58	-0.035
	22.15	0.082
	26.29	0.171
50	25.99	0.136
	-6.89	-0.160
	-4.72	-0.125
	-4.41	-0.124
	23.46	0.013
1000	22.10	0.087
	22.69	0.093
	23.66	0.036
	24.02	-0.638
	-2.25	-0.817
	24.88	-0.635
	-3.72	-0.768
	-4.79	-0.852
	24.01	-0.617
	-3.03	-0.812
25.34	-0.585	
-2.70	-0.829	

Table A2

Values used to construct the empirical transfer function for California Institute of Technology. The middle column gives the linearity-corrected Δ_{47} values relative to the working gas, while the right column gives the theoretical equilibrium values (based on interpolating a 4th order polynomial fit to equilibrium values reported in Wang et al. (2004)).

Temperature (°C)	$\Delta_{47-[\text{EGvsWG}]0}$	$\Delta_{47-\text{RF}}$
8	0.0788	1.0208
25	-0.0271	0.9252
50	-0.0957	0.8050
1000	-0.7869	0.0266

The data in Table A1 is regressed using a model that assumes different temperatures share a common slope, but have unique intercepts. This can be done using the Matlab function `mvregress`, a multivariate regression that finds the best-fit slope given data from multiple groups (here the grouping variable is temperature). We define δ^{47} as the independent variable (x -axis) and Δ_{47} as the dependent variable (y -axis). `mvregress` yields a vector, *beta*, of length five with the four intercepts ($\Delta_{47-[\text{EGvsWG}]0}$) and a

common slope: $\beta = \begin{bmatrix} 0.0788 \\ -0.0271 \\ -0.0957 \\ -0.7869 \\ 0.0065 \end{bmatrix}$ (also see Table 1). The

data and associated regressions are plotted in the left panel of Fig. 1a.

We now have values for $\Delta_{47-[\text{EGvsWG}]0}$ at each of the four temperatures, which can be plotted against the 'true' ($\Delta_{47-\text{RF}}$) value for equilibrium clumping at each temperature (Table A2, and left panel of Fig. 1b). The true values are calculated by interpolating along a 4th order polynomial fit to expected abundances of isotopologues in a thermodynamically equilibrated population of CO₂ (see Supplementary Data):

$$\begin{aligned} \Delta_{47-\text{RF}} = & 0.003 \left(\frac{1000}{T} \right)^4 - 0.0438 \left(\frac{1000}{T} \right)^3 \\ & + 0.2553 \left(\frac{1000}{T} \right)^2 - 0.2195 \left(\frac{1000}{T} \right) \\ & + 0.0616 \end{aligned} \quad (\text{A2})$$

The data was provided by Zhengrong Wang and is derived from Table 4(I) of Wang et al. (2004), which calculates equilibrium clumped isotope compositions using the zero point energies and normal mode wave numbers presented in Table 3 of Wang et al. (2004). A linear least square fit (Eq. 6.13 of Bevington and Robinson (2003)) to $\Delta_{47-[\text{EGvsWG}]0}$ vs. $\Delta_{47-\text{RF}}$, yields the empirical transfer function:

$$\Delta_{47-\text{RF}} = 1.1548 \Delta_{47-[\text{EGvsWG}]0} + 0.9343 \quad (\text{A3})$$

An empirical transfer function can be found for any set of data using the same approach.

APPENDIX B. AN EXAMPLE OF CORRECTING RAW DATA FOR A CARBONATE TO THE ABSOLUTE REFERENCE FRAME

Once an empirical transfer function has been determined for a given mass spectrometer and working reference gas, it can be used to calculate the clumped isotope composition of an unknown sample via Eqs. (5) and (6). We show a detailed calculation for a measurement of 102-GC-AZ01 at the California Institute of Technology using the equilibrated gas line slope (0.0065) and empirical transfer function (Eq. (A3)) calculated in Appendix A.

First, we determine the bulk (δ^{47}) and raw clumped (Δ_{47}) isotopic composition of an aliquot of 102-GC-AZ01 sample gas relative to the working gas: $\delta_{[\text{SGvsWG}]}^{47} = 2.924\text{‰}$ and raw $\Delta_{47-[\text{SGvsWG}]} = -0.239\text{‰}$. We then apply Eq. (5) to correct for non-linearity:

$$\begin{aligned} \Delta_{47-[\text{SGvsWG}]0} &= -0.239 - (2.924 \times 0.0065) \\ &= -0.258\text{‰} \end{aligned} \quad (\text{B1})$$

This is followed by application of the empirical transfer function:

$$\Delta_{47-\text{RF}} = (-0.258 \times 1.1548) + 0.9343 = 0.636\text{‰} \quad (\text{B2})$$

Finally, carbonates reacted at 90 °C must be normalized to the 25 °C acid digestion scale:

$$\Delta_{47-\text{RF-AC}} = 0.636 + 0.081 = 0.717\text{‰} \quad (\text{B3})$$

where the value 0.081 is the apparent difference in Δ_{47} between CO₂ gas evolved from 90 °C reactions to 25 °C

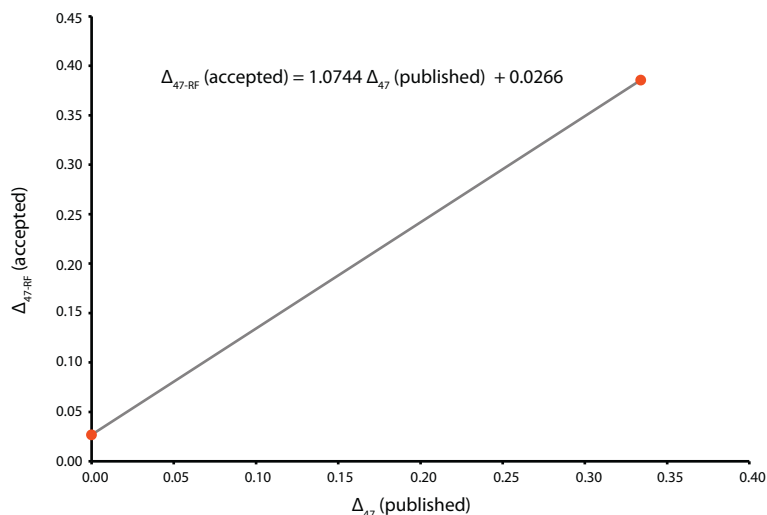


Fig. C1. Secondary transfer function used for projecting Harvard University's synthetic calcite calibration (Dennis and Schrag, 2010) into the absolute reference frame.

reactions (Passey et al., 2010). If one wishes to convert the clumped isotopic composition of a carbonate to temperature using one of the two synthetic calcite calibrations, rearrangement of Eqs. (9) and (10), respectively, yield:

$$T(^{\circ}\text{C}) = \left(\frac{0.0636 \times 10^6}{0.717 + 0.0047} \right)^{0.5} - 273.15 = 23.7 \quad (\text{B4})$$

$$T(^{\circ}\text{C}) = \left(\frac{0.0362 \times 10^6}{0.717 - 0.2920} \right)^{0.5} - 273.15 = 18.7 \quad (\text{B5})$$

APPENDIX C. AN EXAMPLE OF CONSTRUCTING A SECONDARY TRANSFER FUNCTION FOR PROJECTING DATA INTO THE ABSOLUTE REFERENCE FRAME

Finally, we demonstrate how data can be projected into the absolute reference frame using a secondary transfer function (Section 2.2). This was the approach taken for re-calculating the two previously published synthetic calcite calibrations of the clumped isotope thermometer. We demonstrate this for a synthetic calcite precipitated at Harvard University (Dennis and Schrag, 2010). First, we construct a secondary transfer function using the published value of Harvard University's in-house Carrara marble standard ('CM2'; $\Delta_{47} = 0.334\text{‰}$) and the past assumption regarding the clumped isotopic composition of heated gases ($\Delta_{47} = 0\text{‰}$). In the new reference frame, these materials now have accepted values. For CM2, the accepted $\Delta_{47\text{-RF}}$ value is 0.385‰ (Table 2), and for 1000°C heated gases the accepted $\Delta_{47\text{-RF}}$ value is 0.0266‰ (Table 1). We plot published vs. accepted values, and determine the slope and intercept of a line connecting the two points (Fig. C1). The slope is 1.0744 and the intercept is 0.0266. We now apply the secondary transfer function to the published synthetic calcite data. Dennis and Schrag (2010) report a Δ_{47} value of 0.6398‰ for calcite precipitated at 25°C . We now project this into the absolute reference frame via:

$$\Delta_{47\text{-RF}} = (0.6398 \times 1.0744) + 0.0266 = 0.7140\text{‰} \quad (\text{C1})$$

This approach can be taken for all published data where there are also two, or preferably more, standards available from the same analytical period.

APPENDIX D. SUPPLEMENTARY DATA

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.gca.2011.09.025](https://doi.org/10.1016/j.gca.2011.09.025).

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