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High-precision determination of ^{13}C – ^{18}O bonds in CO_2 using multicollector peak hopping

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RATIONALE: Rapid development of the CO_2 'clumped-isotope' temperature proxy (Δ_{47}) has involved both resource- and labor-intensive analytical methods. We report strategies for measuring Δ_{47} on a conventional mass spectrometer, with the usual CO_2 set of three Faraday collectors while reducing the time devoted to standardization with temperature-equilibrated CO_2 .

METHODS: We measured Δ_{47} in CO_2 on a mass spectrometer using only three Faraday cups by 'multicollector peak hopping', directing $^{46}\text{CO}_2^+$ and $^{47}\text{CO}_2^+$ ion beams into the Faraday cups normally used for m/z 44 and 45. Δ_{47} was calculated from the previously measured $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values and we then measured $^{47}\text{CO}_2^+ / ^{46}\text{CO}_2^+$. We invoke a protocol for correcting ion-beam intensities for secondary electrons to address common problems with compositional non-linearity and both long- and short-term measurement stability.

RESULTS: Our method eliminates the need for six Faraday cups for measuring Δ_{47} , a possible advantage for many laboratories. In addition, by using a correction based on removing the effects of secondary electrons, we remove the Δ_{47} dependence on $\delta^{47}\text{CO}_2$. As a result, our methods are robust against mass spectrometer operating conditions.

CONCLUSIONS: Δ_{47} can be precisely measured on mass spectrometers currently employed for measuring $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values without significant resource investment. Accounting for ion scatter during measurements of CO_2 results in improved external precision and long-term stability, while significantly increasing the machine time available for sample analysis. Copyright © 2014 John Wiley & Sons, Ltd.

'Clumped-isotope' geochemistry of carbonates, the study of multiply substituted isotopologues of CO_2 derived from acid digestion of carbonate minerals, has been applied as a geochemical tool in many investigations in geochemistry and paleoclimatology. Enthusiasm for this proxy is great, in part because the concentration of ^{13}C – ^{18}O bonds within carbonate minerals serves as an intra-mineral thermometer; one can establish carbonate growth temperatures without knowledge of the isotopic composition (i.e., $^{18}\text{O}/^{16}\text{O}$) of the water in which the carbonate formed using only the ^{13}C – ^{18}O bond ordering.^[1–14] While there has been considerable work on improving the precision and accuracy of measuring relative abundances of $^{13}\text{C}^{18}\text{O}^{16}\text{O}$ in carbonate via liberated $^{13}\text{C}^{18}\text{O}^{16}\text{O}$,^[15–20] and their isotopomers (isotopically defined symmetrical variants), these measurements remain challenging. One impediment is that modifications are required to the usual carbonate Faraday cup configuration for the multiple-collector gas-source mass spectrometer. Another is the need to correct for a compositional dependency on the measurement of the excess in $^{47}\text{CO}_2$ (i.e., all isotopomers of $^{18}\text{O}^{13}\text{C}^{16}\text{O}$) often referred to as a 'non-linearity'. These two factors pose a modest barrier for any laboratory intending to

employ the clumped isotope proxy; reconfiguring one's mass spectrometer with a new Faraday collector set is expensive and correcting for non-linearity has proven costly in time due to drifts in the dependency. Here we address both issues and demonstrate that measurement of the rare mass-47 isotopologue of CO_2 can be performed with a conventional cup configuration used for more traditional CO_2 isotope ratio measurements, and that the compositional non-linearity can be removed entirely by correcting measured rare-species ion beam intensities for off-peak backgrounds.

Conventional measurements of $^{13}\text{C}/^{12}\text{C}$ and $^{18}\text{O}/^{16}\text{O}$ in CO_2 (expressed as per mil deviations of sample, *smp*, from a standard, *std*, as $\delta^{13}\text{C}$ or $\delta^{18}\text{O} = [(R_{\text{smp}} - R_{\text{std}}) / R_{\text{std}}] \times 10^3$ where $R = ^{13}\text{C}/^{12}\text{C}$ or $^{18}\text{O}/^{16}\text{O}$), require a collection system with only three Faraday cups to measure each of three ion beams: $^{44}\text{CO}_2^+$, $^{45}\text{CO}_2^+$, $^{46}\text{CO}_2^+$. These signals are converted into ratios ^{45}R and ^{46}R , where iR is the ratio of isotopologue *i* relative to the most abundant isotopologue $^{44}\text{CO}_2$ ($^{16}\text{O}^{12}\text{C}^{16}\text{O}$), that in turn can be used to calculate $^{13}\text{C}/^{12}\text{C}$ and $^{18}\text{O}/^{16}\text{O}$ assuming a known relationship between $^{18}\text{O}/^{16}\text{O}$ and $^{17}\text{O}/^{16}\text{O}$ in the samples (e.g., Santrock *et al.*^[21]). Clumped-isotope geochemistry involves measuring the abundances of rare multiply substituted isotopologues, molecules containing more than one heavy isotope. In practice, the CO_2 isotopologue with cardinal mass 47, $^{16}\text{O}^{13}\text{C}^{18}\text{O} + ^{18}\text{O}^{13}\text{C}^{16}\text{O}$ ($>> ^{17}\text{O}^{13}\text{C}^{17}\text{O}$), is the most accessible for measurement due to its relatively high abundance among doubly substituted CO_2 species (hereafter we will use $^{13}\text{C}^{18}\text{O}^{16}\text{O}$ to represent $^{16}\text{O}^{13}\text{C}^{18}\text{O} + ^{18}\text{O}^{13}\text{C}^{16}\text{O}$ unless otherwise specified and similarly for other species).

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The convention is to report the excess of this rare isotopologue relative to the stochastic abundance prescribed by the random distribution of the ^{13}C , ^{12}C , ^{18}O , ^{17}O , and ^{16}O isotopes among the CO_2 isotopologues.^[22] The latter is the high-temperature limit for the relative abundances of the isotopic species of CO_2 . The excess in per mil is usually expressed as:

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

where ${}^i\text{R}^*$ is the stochastic isotopologue ratio.

Equation (1) shows that a minimum of four ion beams is required rather than the usual three used for $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ values, the additional beam being ${}^{47}\text{CO}_2^+$. In order to measure ${}^{45}\text{R}$, ${}^{46}\text{R}$ and ${}^{47}\text{R}$ (Eqn. (1)) it is current practice to utilize a minimum of four CO_2 collectors to measure simultaneously ${}^{44}\text{CO}_2^+$, ${}^{45}\text{CO}_2^+$, ${}^{46}\text{CO}_2^+$, ${}^{47}\text{CO}_2^+$ (these instruments usually have six collectors for CO_2 with the additional two cups used to measure ${}^{48}\text{CO}_2^+$ and ${}^{49}\text{CO}_2^+$ for diagnostic purposes). Most instruments are not set up for such a measurement, with only three collectors normally dedicated to CO_2 and any additional collectors positioned for other gases (e.g. N_2 , O_2 , etc.) and in most instruments the collectors are fixed, requiring the user to specify in advance the gases and ion beams to be analyzed. In addition, measurements made to date (virtually all on ThermoFinnigan MAT 253 instruments, cf. Yoshida *et al.*^[20] and Rosenheim *et al.*^[23]), exhibit spuriously high measured Δ_{47} values when $\delta^{47}\text{CO}_2$ ($\delta^{47}\text{CO}_2 \approx \delta^{13}\text{C} + 2\delta^{18}\text{O}$ for a nearly stochastic distribution of isotopes) is greater than the composition of the working standard gas, and spuriously low Δ_{47} values when sample CO_2 is isotopically lighter than the working gas.^[16,17] This compositional non-linearity varies from instrument to instrument and has been observed to change over time for the same machine on the scale of weeks to months.

Huntington *et al.*^[16] presented a method to correct for this effect by defining the non-linearity for any given mass spectrometer. This was done by comparing the reference gas with a suite of CO_2 gases of different composition (over a range of $\sim 50\%$ in $\delta^{47}\text{CO}_2$ values) that have been heated to high temperatures sufficient to impart a stochastic distribution of isotopes ($\Delta_{47} = 0\%$) (i.e. 'heated gas' correction). Building on this approach, Dennis *et al.*^[17] developed an "absolute reference frame" (more recently referred to as carbon-dioxide equilibration scale or CDES in Henkes *et al.*^[12]) to facilitate inter-laboratory comparison. The "absolute reference frame", or calibration, corrects for machine-specific shifts in scale (so-called scale compression) by comparing CO_2 samples with reference gas isotopic composition (i.e. $\delta^{47}\text{CO}_2 = 0$) re-equilibrated over a wide temperature range, ideally from ~ 0 to ~ 1000 °C. Both compositional non-linearity and scale compression have been attributed to ion fragmentation and recombination (scrambling isotopic bond ordering) in the source.^[16,17] Utilizing these two methods is labor-intensive and introduces additional assumptions and uncertainties into clumped-isotope measurements.

We find that accurate measurements of Δ_{47} can be obtained using only the three Faraday cups normally dedicated to conventional CO_2 isotope ratio measurements, with precision comparable to that of previous

studies (Δ_{47} standard error ~ 0.020 ‰). In addition, we show that the compositional non-linearity described by Huntington *et al.*^[16] is largely, if not entirely, attributable to non-zero backgrounds resulting from secondary electron scatter near the Faraday collectors, as previously shown for clumped-isotope measurements of O_2 .^[24] In this regard, our findings are similar to those of He *et al.*,^[19] Rosenheim *et al.*,^[23] and Bernasconi *et al.*,^[25] although arrived at by different sets of experiments. We apply a simple background correction that reduces Δ_{47} dependence on $\delta^{47}\text{CO}_2$ below detection, while producing a $\Delta_{47\text{measured}}/\Delta_{47\text{theoretical}}$ calibration line from temperature-equilibrated gases that is very similar to those reported in other laboratories. We note that a principal advantage of removing the compositional non-linearity is that it is possible to compare the isotopic composition of any CO_2 sample with that of a heated (approximately stochastic) aliquot of the same gas in order to determine Δ_{47} directly. This procedure circumvents the need to continually create arrays of heated gases of different compositions in order to correct for compositional dependencies. Measurements made using such a method may or may not provide greater precision than methods relying on the heated gas approach, but the possibility warrants further investigation. The methods documented here should be transferable to most mass spectrometers using conventional Faraday collector sets intended to measure only $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values in CO_2 , the only modification being replacement of resistors and capacitors on the amplifier cards.

EXPERIMENTAL

Instrumentation for Δ_{47} measurements by multicollector peak hopping (MPH)

The ThermoFinnigan MAT 253 gas-source isotope ratio mass spectrometer used in this study is fitted with a collection system consisting of nine Faraday cups (Fig. 1) to monitor several rare isotopologues of CO_2 and O_2 (m/z 32 through m/z 36). For reasons described below, the Faraday cup intended to measure m/z 47 in this configuration performed erratically, forcing us to utilize only three Faraday cups, those normally meant to monitor m/z 44, m/z 45 and m/z 46 with 3×10^8 , 1×10^{10} and 1×10^{11} Ω resistors, respectively. We used these three Faraday cups to first measure $^{13}\text{C}/^{12}\text{C}$ and $^{18}\text{O}/^{16}\text{O}$ (${}^{45}\text{CO}_2^+ / {}^{44}\text{CO}_2^+$ and ${}^{46}\text{CO}_2^+ / {}^{44}\text{CO}_2^+$, respectively), then the two higher-mass cups to measure m/z 46 and m/z 47 with 1×10^{10} Ω and 1×10^{12} Ω resistors, yielding ${}^{47}\text{CO}_2^+ / {}^{46}\text{CO}_2^+$ (the MAT 253 instrument routinely includes software switchable resistor/capacitor pairs).

Three cups are adequate for measuring the required four ion beams of CO_2 because of the relatively small differences in cup spacings. The physical distance d between any two beams at the approximate focal plane of the magnetic sector mass spectrometer can be estimated from the relationship:

$$d_{2/1} = D\Delta M / (\frac{1}{2}(m_1 + m_2)) \quad (2)$$

where D is the dispersion of the instrument, ΔM is the mass difference, and m_1 and m_2 are the masses of the ions that compose the beams. For the mass spectrometer used in this study $D = 460$ mm (~ 2 times the radius), so that

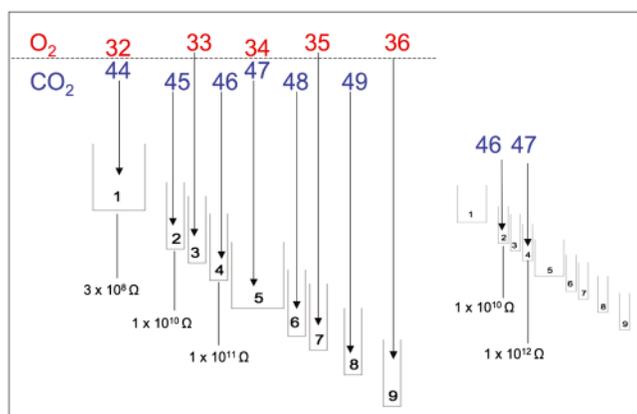


Figure 1. Schematic of O_2/CO_2 collector set used in this study showing approximate spacings and cup widths for nine collectors intended to measure ion beams for either $^{32}\text{O}_2$, $^{33}\text{O}_2$, $^{34}\text{O}_2$, $^{35}\text{O}_2$, $^{36}\text{O}_2$ or $^{44}\text{CO}_2$, $^{45}\text{CO}_2$, $^{46}\text{CO}_2$, $^{47}\text{CO}_2$, $^{48}\text{CO}_2$, $^{49}\text{CO}_2$. Inset shows cups used to measure $^{46/47}\text{CO}_2$ + using cups normally used for $^{45}\text{CO}_2$ and $^{46}\text{CO}_2$. The beams are repositioned by minor shifts in the magnetic field and accelerating voltage.

$d_{46/45} = 10.1$ mm and $d_{47/46} = 9.9$ mm. These distances are sufficiently similar (differing by ~ 0.2 mm) from the cup widths (collector slit widths are 1.5 mm) that one can use the same cups at their fixed spacing to measure $^{45/46}\text{R}$ and $^{47/46}\text{R}$ with relatively small adjustments to the accelerating voltage and magnet current. As a result, two separate measurements can be used to obtain the required ion current ratios for a complete analysis of CO_2 . Since all MAT 253 s have the same radius, and therefore the same cup spacing, the feasibility of using MPH to measure Δ_{47} is not unique to this instrument.

For convenience of presentation, our study is divided into four periods, P1 (December 2010 to May 2011), P2 (January 15 to April 10, 2012), P3 (April 13 to May 9), and P4 (May 10 to July 14). For the elapsed time between P1 and P2 the mass spectrometer was used to measure O_2 exclusively. In order to switch from O_2 to CO_2 , the mass spectrometer required no major maintenance or alterations other than changing from stainless steel capillaries used for O_2 to more highly polished nickel capillaries for CO_2 . P2, P3 and P4 were separated by major machine maintenance, necessitating breaking vacuum.

Background measurements

It has been customary in gas-source isotope ratio mass spectrometry to apply corrections for backgrounds measured with no gas entering the instrument. These 'dark' backgrounds are measured on peak. However, this method is unable to account for any change in the background signal during sample gas measurement. Work in our laboratory demonstrated that secondary electron scatter is a critical source of background in the form of negative voltages that must be accounted for when measuring rare isotopologues of O_2 .^[24] Subsequently, we found the same phenomenon when measuring rare isotopologues of CO_2 . As these secondary electrons are generated when ion beams are present, measurements of these backgrounds must be made with analyte gas flowing to the instrument. By examining off-peak positions while sample gas is present, we

monitor the negative signal produced by secondary electrons in the Faraday cups used to measure m/z 46 and m/z 47 surrounding the analyte peaks. This effect has been reported in a few other laboratories,^[19,25] and was also attributed to secondary electrons, perhaps sputtered off the metal ceiling of the flight tube. We found that background voltage is strongly affected by a hand-held magnet placed strategically near the collector array, verifying that the effect is due to electrons (much less massive than ions and therefore more easily redirected by a magnetic field). The magnitude of the negative bias is greater in more sensitive amplifiers, requiring corrections for the 1×10^{12} Ω and, in some cases, the 1×10^{10} Ω amplifiers. Correcting for secondary electron backgrounds effectively removes Δ_{47} dependence on $\delta^{47}\text{CO}_2$.

We found that three 8-s background measurements of m/z 46 and m/z 47 before and after ten 8-s cycles on peak was sufficient to assess the effect of electron scatter during an

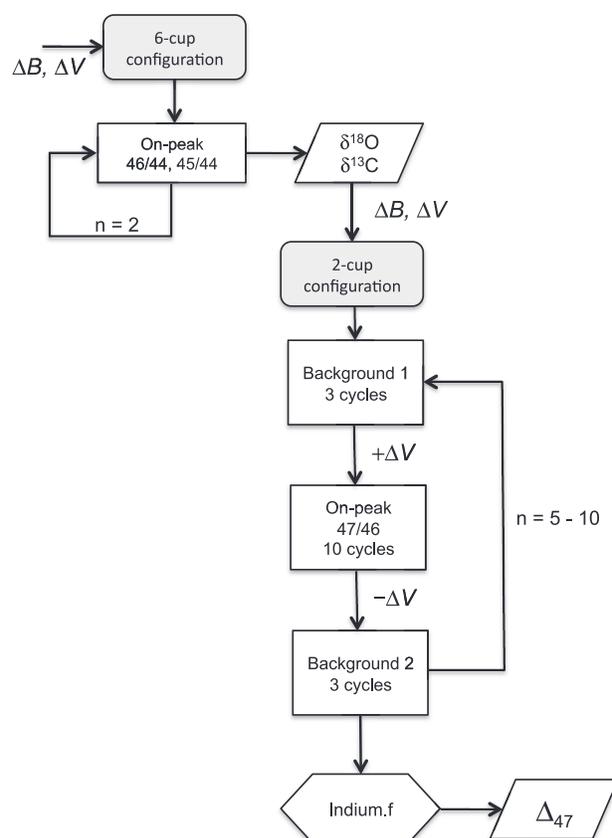


Figure 2. Schematic illustrating sample analysis procedure used in this study. After normal procedures to pressure balance, peak center, and scan 'dark' backgrounds, we measure m/z 44, 45 and 46 by running two sets of 10 cycles per gas in the six-cup configuration to determine $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values. Next we reposition the magnet (ΔB) and accelerating voltage (ΔV) to measure m/z 46 and 47 using the Faraday cups previously used for m/z 45 and 46 (two-cup configuration). Backgrounds are measured both before and after the on-peak sample analysis. Since the background voltage typically does not stabilize until after the first introduction of sample gas, we discard the first reference gas measurement in the 10-cycle analysis and in both 3-cycle sets used to determine background voltage for the correction. "Indium.f" is the Fortran code (see Supporting Information).

analysis. A 20-V decrease in accelerating voltage was used to shift the measuring position off peak. This voltage was selected to position the background measurement in a flat interval adjacent to the analyte peak. The procedure that we use is represented diagrammatically in Fig. 2. We use two blocks composed of ten measurement cycles for sample and standard to determine $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values, followed by the peak hop (shift in magnet settings) to measure m/z 46, m/z 47 and adjacent backgrounds. For these measurements we use an analysis block composed of ten on-peak 8-s cycles, bracketed by three off-peak cycles to measure backgrounds both before and after the ten cycles. We repeat the analysis block five to ten times for each sample. The magnitude of the negative backgrounds changed significantly over a 55-day period, from -0.75 mV to -1.00 mV and from -24.5 mV to -29.5 mV for m/z 46 and m/z 47, respectively (Figs. 3(a) and 3(b)). On the timescale of hours the increase was effectively linear, allowing linear interpolation of backgrounds before and after on-peak analysis to obtain on-peak backgrounds.

Gas handling

Carbon dioxide gas was introduced into the mass spectrometer through a dual-inlet system. Variable-volume bellows were adjusted to provide ~ 14 V for m/z 44 (47 nanoamps on the $3 \times 10^8 \Omega$ resistor) during the first measurements, and ~ 6 V for m/z 46 (0.6 nanoamps on the $1 \times 10^{10} \Omega$ resistor) for the second set of measurements for 47/46.

We constructed a small volume (~ 200 mL) glass vacuum line backed by a turbomolecular pump for CO_2 handling and purification of all gases used in this study. Samples were passed through glass traps chilled to -78 °C by dry ice/ethanol baths to remove water, and then were entrained in helium carrier gas at a flow rate of 15 mL/min for purification through a 2-m gas chromatography (GC) column packed with Porapak Q chilled to -28 to -30 °C. The sample gas effluent from the gas chromatograph was isolated in a metal trap at liquid nitrogen temperature (approximately -196 °C). Helium carrier gas was pumped away before moving sample CO_2 into a vessel for introduction into the mass spectrometer. Multiple passes

through this system results in no changes in $\delta^{13}\text{C}$, $\delta^{18}\text{O}$ or Δ_{47} values, suggesting that contaminants are effectively removed, and that the distribution of isotopologues in low-temperature samples is not affected.

Calculating Δ_{47} from $^{47/46}\text{R}$ and $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values

We wrote a Fortran program that calculates Δ_{47} from input ion currents based on our MPH method (for code and additional details, see Supporting Information). In this scheme, measured ^{46}R and ^{45}R values from the first set of measurements yield $\delta^{18}\text{O}$ values (SMOW) and $\delta^{13}\text{C}$ values (PDB) assuming stochastic distributions of the isotopes across isotopologues (the conventional assumption with negligible loss in accuracy, e.g. Santrock *et al.*^[21]) and then the $^{47/46}\text{R}$ value measured separately is combined with the ^{46}R value of the sample to yield ^{47}R (i.e. $(^{46}\text{CO}_2/^{44}\text{CO}_2)(^{47}\text{CO}_2/^{46}\text{CO}_2) = ^{47}\text{CO}_2/^{44}\text{CO}_2$). While measured ratios from non-synchronous measurements are not comparable, we overcome this by converting all ion current ratios into absolute ratios via the usual comparison with the working reference gas. Correction for negative backgrounds produced by secondary electrons in the m/z 46 and m/z 47 cups is performed as part of the data reduction. Subtractions are made for both sample and standard gases, and are obtained from the mean of measurements taken before and after on-peak analyses of sample and standard. We found no significant difference in measured Δ_{47} values when we modeled background voltage as a non-linear time-dependent function rather than simple averages (i.e. we assume linear drifts in background in time with no obvious penalty in accuracy, similar to the " 47 interpolation method" of He *et al.*^[19]).

The code applies two versions of data reduction calculations. One is based on the slope of plots of Δ_{47} vs $\delta^{47}\text{CO}_2$ values without subtracting background voltages, and is equivalent to the "heated gas line" (hereafter HGL) or "temperature-equilibrated" correction schemes of Huntington *et al.*^[16] and Dennis *et al.*^[17] respectively. The other is based on correcting for the $\Delta_{47}/\delta^{47}\text{CO}_2$ non-linearity by subtracting the off-peak background voltages measured in Faraday cups used for m/z 47 and m/z 46. The slopes of temperature-equilibrated gas lines

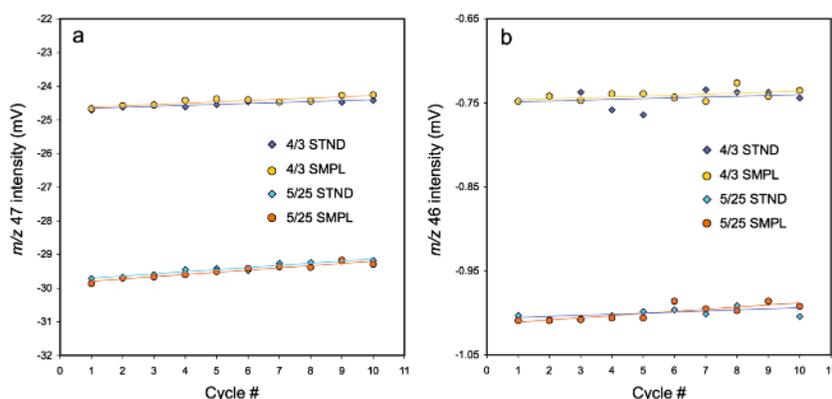


Figure 3. Off-peak voltages measured in Faraday cups used for (a) m/z of 47 and (b) m/z of 46 collected with 10 cycles of 8-s duration with CO_2 present on two different days. Background voltage is noticeably lower on May 25, but the rate of increase (~ 0.02 to 0.07 mV/cycle for m/z of 47; 0.001 to 0.003 mV/cycle for m/z of 46) is similar between sample and standard measurements and adequately described a linear function over the course of an analysis.

created after applying this background correction are in most cases indistinguishable from zero.

The program corrects for potential instrument-dependent inaccuracies in absolute Δ_{47} values by applying a calibration line depicting measured Δ_{47} values at various temperatures vs theoretical Δ_{47} values for CO_2 following the recommendation of Dennis *et al.*^[17] Algorithms for stochastic CO_2 ion corrections are based on Santrock *et al.*^[21] with modifications to the input parameters (the exponent relating $^{17}\text{O}/^{16}\text{O}$ to $^{18}\text{O}/^{16}\text{O}$ is taken to be 0.526 rather than 0.516 resulting in a negligible shift in final results). Rare isotopologue ion calculations are similar to Dennis *et al.*^[17] with the exception of the background corrections.

Uncertainties in the final Δ_{47} values are calculated using a Monte Carlo approach to account for uncertainties among the blocks of unequal numbers of cycles in the acquisitions (e.g. fewer cycles are used to measure backgrounds). Sources of uncertainty considered include the inter-cycle internal precision of each measurement block, both on- and off-peak, and uncertainties in calibration slopes of temperature-equilibrated gas lines ($\Delta_{47}/\delta^{47}\text{CO}_2$ non-linearity) where such calibration lines are used in the data reduction.

Materials – standard gases

Two canisters of CO_2 reference gas were purchased from OzTech (Dallas, TX, USA) for use in this study. One canister was employed exclusively as a working gas in each calendar year. The compositions of reference gases used are: $\delta^{13}\text{C} = -47.52$ ‰ PDB, $\delta^{18}\text{O} = +2.75$ ‰ SMOW (WG1) and $\delta^{13}\text{C} = -3.58$ ‰ PDB, $\delta^{18}\text{O} = +24.96$ ‰ SMOW (WG2) used during P1, and P2 through P4, respectively. Both canisters have Δ_{47} values reflective of near room-temperature equilibration.

Standards approximating stochastic distributions of isotopes were created using methods similar to those of Huntington *et al.*^[16] Carbon dioxide gases from several different sources were mixed to produce aliquots with bulk composition ranging from approximately -60 ‰ to $+28$ ‰ in $\delta^{47}\text{CO}_2$. Aliquots were isolated in flame-sealed quartz ampules, and heated in a muffle furnace to 1000 °C for at least 3 h. We sought to minimize potential back-equilibration at lower temperature by immediately quenching ampules in cold water. Stochastic gases were passed through our purification system within 2 h of quenching with very few exceptions.

Additional standards were equilibrated at lower temperatures of 2 °C and 25 °C by allowing CO_2 to exchange oxygen isotopes with water at controlled temperature in one of several stopcock-sealed vessels. Gases equilibrated at 25 °C were held in a temperature-controlled water bath for 72 to 192 h. Gases equilibrated at 2 °C were partially submerged in a beaker of water held in a laboratory refrigerator for approximately 1 month.

RESULTS AND DISCUSSION

Initial multicollector peak hopping experiments

During P1, we found greater than expected instability (std err. frequently >0.045 ‰, occasionally >0.080 ‰) when measuring Δ_{47} using the nine Faraday cup collection system. We suspect that this is a byproduct of the Faraday cup used to measure

m/z 47. This cup is more than twice the width of the standard cups (collector slit = 3.5 mm vs 1.5 mm for other cups), and so is more vulnerable to contamination by stray non-analyte ions introduced with the CO_2 samples. We confirmed this by passing stochastic CO_2 samples contaminated with atmospheric nitrogen (N_2) through an imperfect purification system multiple times. We observed a decrease in Δ_{47} measured on the wide cup with each purification pass, and a significantly smaller such decrease using a narrow cup. Since our stochastic gases have by definition a minimum Δ_{47} , decreases in Δ_{47} with incomplete purification must be spurious due to a decrease in stray ions. In addition, the amplifier card for this cup is designed to accommodate output from two separate cups via a switch, and our investigations suggest that this switch probably adds some instability.

Comparisons between Δ_{47} values measured using the nine-Faraday cup collection system and Δ_{47} values measured with MPH for a suite of purified CO_2 samples of similar composition equilibrated at different temperatures are plotted in Fig. 4. The comparison demonstrates that measurements using the wide cup are less precise than those made with the narrower cup using MPH.

Initially, in P1, we attempted to reduce the time necessary to define the 'non-linearity' of our mass spectrometer. We hoped to do this by comparing each sample with its stochastic counterpart (we have referred to this as the 'Direct method' for determining departures from stochastic distributions of isotopes) instead of preparing and running heated gases daily as suggested by Huntington *et al.*^[16] We made stochastic counterparts by recollecting the sample gas and heating it to 1000 °C in sealed quartz ampules (as for the stochastic standards described above). The accuracy of this approach was hindered by the fact that all samples shifted in $\delta^{18}\text{O}$ value by variable amounts of between 2 and 7 ‰ during heating, presumably through exchange with the

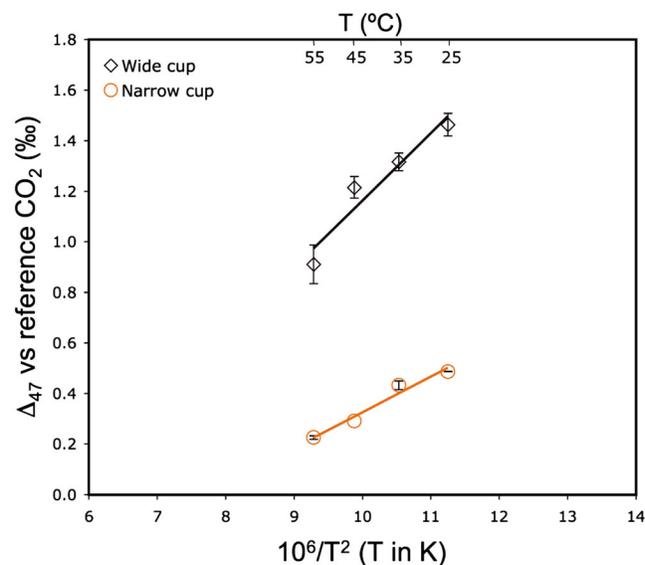


Figure 4. Comparison of six-cup configuration (black diamonds, wide cup) and two-cup configuration (MPH method, orange circles, narrow cup) Δ_{47} values of temperature-equilibrated CO_2 samples against reference CO_2 . Error bars represent one external standard error for each measurement composed of multiple blocks.

quartz ampule. This strategy is therefore limited by the ability to prevent isotopic exchange between CO_2 and SiO_2 at 1000 °C or to correct for the effect of the 2 to 7 ‰ difference in $\delta^{47}\text{CO}_2$ values on Δ_{47} values. We solved this problem by eliminating the latter, and believe that this is a sound strategy which may prove useful in circumstances where measuring the sufficient number of standards to 'define the non-linearity' and calibrate mass spectrometer 'scale compression' against theoretical values is impractical.

Exploring the Δ_{47} - $\delta^{47}\text{CO}_2$ relationship and electron scatter

The non-linearity observed when using the MPH method is similar in scale to those reported in other laboratories using six-Faraday cup collector systems, and is therefore not a product of the MPH routine. We observed $\Delta_{47}/\delta^{47}\text{CO}_2$ slopes ranging from 0.0170 ± 0.0004 (2σ) to 0.0114 ± 0.0006 (Fig. 5) compared with 0.0064 to 0.0128 at the California Institute of Technology,^[16] 0.0081 at Harvard University,^[17] 0.0059 at Johns Hopkins University,^[17] and 0.0055 at Yale University.^[17] Previously published studies report instability in the Δ_{47} - δ^{47} relationship, particularly as a consequence of the mass spectrometer being serviced. We observed one major shift in our Δ_{47} - $\delta^{47}\text{CO}_2$ relationship, which occurred at the close of time interval P2 as a result of instrument maintenance. At this time the slope of our stochastic standard line changed from 0.0170 ± 0.0004 to 0.0114 ± 0.0006 , where it remained for P3 and P4 (Fig. 5).

Before the start of P2 we employed corrections for secondary electron scatter to effectively remove Δ_{47} value dependence on $\delta^{47}\text{CO}_2$ values. Comparing measurements of temperature-equilibrated samples with (Fig. 6(b)) and without (Fig. 6(a)) electron scatter correction demonstrates that this approach is as effective for MPH as it is for machines equipped with six-Faraday cup collection systems (e.g. He *et al.*^[19]). Background corrections remained effective in

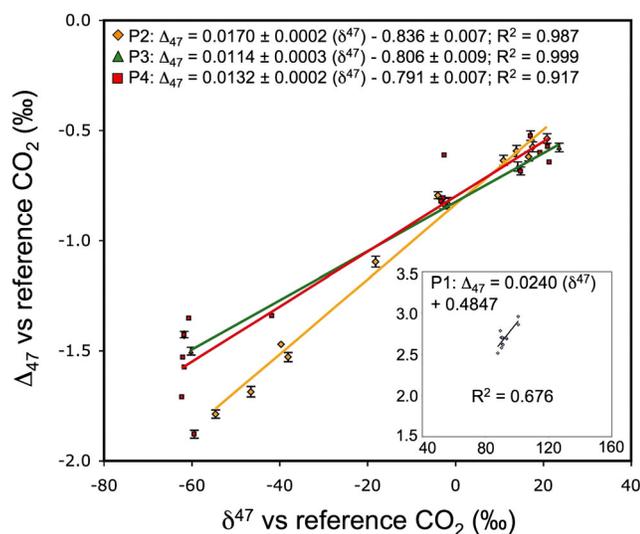


Figure 5. Variation in measured Δ_{47} with $\delta^{47}\text{CO}_2$ values relative to reference gas for CO_2 samples equilibrated at 1000 °C. Abscissa indicates difference in sample bulk composition from reference CO_2 . The slope changed significantly from P1 to P2 for reference gasses of different composition. Smaller changes occurred with the same reference CO_2 over time (represented by intervals P2–P4). Uncertainties in best fit lines are 1σ .

reducing and even eliminating compositional non-linearity despite machine maintenance activities that changed the Δ_{47} - $\delta^{47}\text{CO}_2$ relationship; during the transition from P2 to P3, the slope of the line representing stochastic gas Δ_{47} vs $\delta^{47}\text{CO}_2$ shifted as described above, while correcting these same measurements for background eliminated the slope in both periods P2 and P3. In addition, the reduced chi-squared values of the linear regressions of our temperature-equilibrated gas data improved after background correction (from 40.45 to 26.15 for 1000 °C, from 2.71 to 1.92 for 25 °C, and from 8.62 to 0.69 for 2 °C). While the Δ_{47} measurements became much more scattered during P4, the slope of the line approximating stochastic gas measurements accumulated from May 10 to July 14 remained very near zero (-0.0006 ± 0.0001) when corrected for background voltage. This supports suggestions that background contributed by secondary electron scatter is responsible for the Δ_{47} - $\delta^{47}\text{CO}_2$ non-linearity, and that changes in the amount of electron scatter measured by Faraday cups probably result in the observed changes to the slopes of temperature-equilibrated gas lines.

Dennis *et al.*^[17] describe a scheme to "transfer" all Δ_{47} measurements into an "absolute reference frame" by means of a calibration line between measured Δ_{47} values and theoretical Δ_{47} values (for further discussion of additional uncertainties introduced, see He *et al.*^[19]). We found that the MPH method produces calibration lines similar to those of other laboratories, and that accounting for background secondary electron scatter reduces uncertainties. Incorporating all data from temperature-equilibrated standards measured during P2, P3 and P4 produces a calibration line with background correction of $\Delta_{47}^{\text{theoretical}} = 1.119 \pm 0.016$ (2σ) $\Delta_{47}^{\text{measured}} + 0.899 \pm 0.010$ ($R^2 = 1.000$) or $\Delta_{47}^{\text{theoretical}} = 1.082 \pm 0.018$ $\Delta_{47}^{\text{measured}} + 0.921 \pm 0.008$ ($R^2 = 0.9998$) without correction. A comparison of several previously published calibrations is found in Table 1 and Fig. 7.

Electron scatter response to machine maintenance

The evolution of backgrounds with time is demonstrated by tracking the first m/z 46 and 47 background cycles taken each day over the course of 6 months (Fig. 8). In general, we found that background signals became gradually less negative during the course of the study, opposite to the observations by He *et al.*^[19]

In January 2012, the mass spectrometer was baked and sat idle under high vacuum for approximately 2 weeks. The m/z 47 backgrounds were approximately -47 mV and climbed slowly (and asymptotically) through April 10 to -23 mV. The day-to-day variability appears to correspond to the idle time, with the largest increases (less negative) in background following short idle times (<8 h) and the decreases (more negative) in background occurring after longer idle periods (12–48 h).

On April 10, the sample-side inlet valve failed and had to be rebuilt. To minimize the possibility of contamination, we vented the instrument by placing the ventilation tubes on the turbomolecular pump vent valves in Dewar flasks containing liquid nitrogen. Following the repair, we cleaned the mass spectrometer by baking for 24 h. Upon resuming operation on April 13, the background voltage had dropped approximately 10 mV to -33 mV, but resumed the P2 rate of increase within 2 days. Repairing the valve and baking the instrument resulted in a time interval, P3, during which

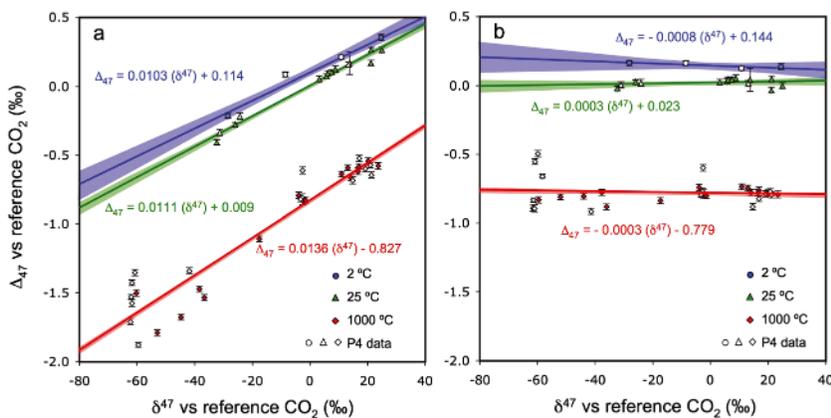


Figure 6. Plots of Δ_{47} versus $\delta^{47}\text{CO}_2$ values relative to reference gas for CO₂ samples equilibrated at different temperatures during P2, P3 and P4. 1000 °C = red diamonds, 25 °C = green triangles, 2 °C = blue circles. (A) Measurements without background correction demonstrate slopes similar to previous studies, and slightly non-parallel lines. (B) Measurements corrected for background demonstrate no discernible non-linearity and more closely approximate parallelism as expected. Shaded envelopes indicate 95% confidence interval.

Table 1. Comparison of previously published calibration lines

	Slope ^a	T (°C)	Intercept	R2	Calibration line ^b	R ²
California Institute of Technology	0.0065	8	0.0788	0.9859	$\Delta_{47t} = 1.1548\Delta_{47m} + 0.9343$	0.9986
		25	-0.0271	0.9417		
		50	-0.0957	0.9133		
Harvard University	0.0081	1000	-0.7869	0.9386	$\Delta_{47t} = 1.0105\Delta_{47m} + 0.9539$	0.9999
		10	0.0480	0.9916		
		25	-0.0216	0.9367		
Johns Hopkins University	0.0059	50	-0.1473	0.9375	$\Delta_{47t} = 1.0303\Delta_{47m} + 0.9194$	0.9997
		1000	-0.9182	0.9167		
		0	0.1412	0.8316		
Yale University	0.0055	50	-0.1002	0.9988	$\Delta_{47t} = 1.0630\Delta_{47m} + 0.9227$	0.9999
		1000	-0.8686	0.9988		
		10	0.0812	0.8086		
University of Chicago (PBL correction)	-0.0019	25	0.0013	0.8886	$\Delta_{47t} = 1.1121\Delta_{47m} + 0.9271$	1.0000
		50	-0.1094	0.4155		
		1000	-0.8431	0.9206		
(without PBL correction)	0.0144	1000	-0.8431	0.9206	$\Delta_{47t} = 1.1151\Delta_{47m} + 0.9377$	0.9992
This study [all data] (with background correction)	-0.0003	2	0.144	0.9700	$\Delta_{47t} = 1.119\Delta_{47m} + 0.899$	1.0000
		25	0.023	0.9817		
		1000	-0.779	0.9936		
(without correction)	0.0133	2	0.113	0.9700	$\Delta_{47t} = 1.082\Delta_{47m} + 0.921$	0.9998
		25	0.009	0.9817		
		1000	-0.827	0.9936		

^aSlopes of temperature-equilibrated gas lines are assumed to be parallel and are calculated by collapsing all temperature-equilibrated sample data into a single set by removing respective means to derive a common slopes.

^bCalibration line of theoretical values (Δ_{47t}) from Wang *et al.*,^[26] against measured values (Δ_{47m} ; equivalent to the “ETF” of Dennis *et al.*^[17]).

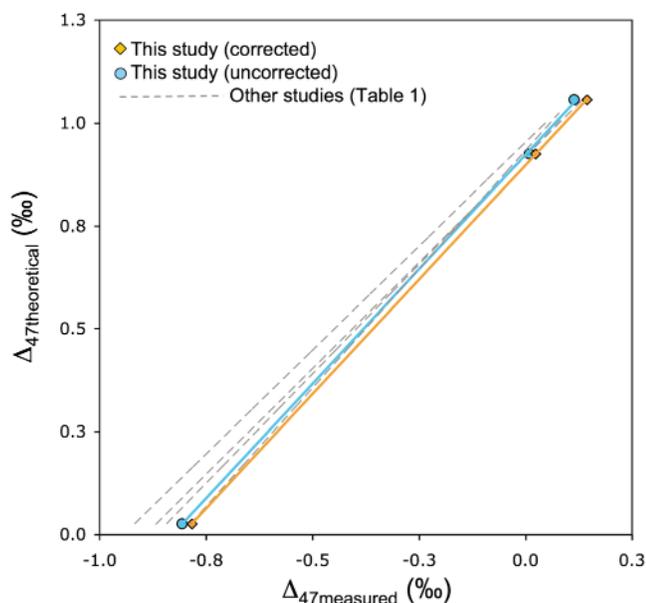


Figure 7. Comparison of $\Delta_{47}^{\text{theoretical}}/\Delta_{47}^{\text{measured}}$ calibration lines.^[17] Temperatures used in this study are 2 °C, 25 °C and 1000 °C. The MPH method results are similar to those from other laboratories. Correcting temperature-equilibrated standard gasses for measured background voltage changes the slope and y intercept of the calibration line slightly. Slopes and intercepts of these lines and those reported in other laboratories are listed in Table 1.

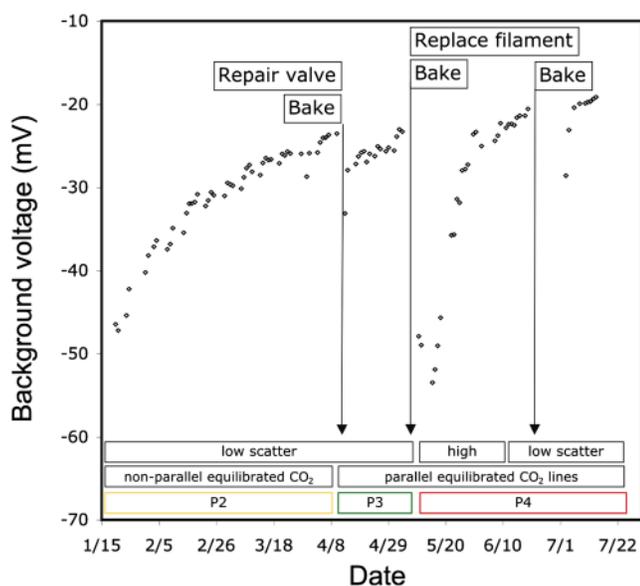


Figure 8. Evolution of background signals during the 6-month study period. Ordinate is the first m/z 47 background for reference gas for each operating day. Short idle times (<24 h) usually result in less negative backgrounds. Long idle times (>48 h) usually result in more negative backgrounds. Cleaning (by baking) results in significantly lower backgrounds. During 6 months of study, backgrounds never fully stabilized at a maximum value. Corresponding measurements for m/z 46 demonstrate almost identical patterns, although compressed to a range of ~1 mV.

we observed the lowest measurement scatter of the study. Measuring stochastic standards at four different bulk compositions (+23.6 ‰, +14.2 ‰, -1.5 ‰ and -60.3 ‰ $\delta^{47}\text{CO}_2$) produced a heated gas line of slope 0.0114 ± 0.0006 ($R^2 = 0.9999$). The change in slope brought the heated gas line closer to parallel with temperature-equilibrated lines at 25 °C and 2 °C with slopes of 0.0111 ± 0.0004 and 0.0103 ± 0.0010 , respectively, than during P2.

The transition from P3 to P4 is defined by failure of the source filament. Replacing the filament necessitates venting the machine and exposing the source to laboratory air. After replacing the source filament, we cleaned the interior by baking for 48 h. Backgrounds after this maintenance were at their lowest values of the study (-50 mV), and this change of the filament appeared to cause a severe disruption to measurement stability. Two weeks were required for the background signals to stabilize near previous levels, and it was difficult to find either a stable peak center or stable off-peak positions to measure ion beams. We later discovered that retuning partly ameliorated this effect, and recommend that extensive retuning is the best approach following a significant interruption to mass spectrometer operation.

It is clear that the magnitudes of negative background signals are sensitive to mass spectrometer baking. We suggest two possible explanations: (1) baking removes contaminants (oxides or water) from metal surfaces in the ion source that could provide a constant source of positive ions, counteracting the negative current from secondary electrons; or (2) baking removes oxides which shield metal surfaces, and cleaner metal surfaces are more liable to produce secondary electrons. The latter is less likely in that we do not normally bake the detector end of the flight tube where secondary electrons are generated. While secondary electron production is proportional to ion beam intensities, the long-term observations suggest that other internal conditions also influence electron production and scatter.

CONCLUSIONS

In this study we have demonstrated that clumped-isotope measurements of CO_2 can be made using a conventional array of three Faraday cups with a measurement strategy referred to as multicollector peak hopping (MPH). In this strategy Δ_{47} is obtained from two sets of multicollection measurements using three Faraday collectors with switchable amplifier resistors and capacitors. The first set of measurements yields $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values. The second set of measurements yields $^{47}\text{CO}_2^+ / ^{46}\text{CO}_2^+$ using two of the same Faraday collectors used in the first set of measurements but with different resistor/capacitor sets. We have shown that measurements of temperature-equilibrated standards, and calibration lines created from them, are similar to those reported from other laboratories using six-Faraday-cup collection systems. The precision of measurements made with MPH is comparable with that made with six collectors on other instruments. We also show that the $\Delta_{47}/\delta^{47}\text{CO}_2$ non-linearity reported in clumped-isotope studies employing ThermoFinnigan MAT 253 mass spectrometers is indeed predominantly caused by secondary electron scatter, and that correction for this effect eliminates the $\Delta_{47}/\delta^{47}\text{CO}_2$ non-linearity. We find that idle times and frequency of cleaning procedures both affect the background-induced $\Delta_{47}/\delta^{47}\text{CO}_2$ relationships and that correcting for background eliminates this dependency.

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