Rapid and Precise Analysis of Carbon Dioxide Clumped Isotopic Composition by Tunable Infrared Laser Differential Spectroscopy

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ABSTRACT: The high precision measurement of doubly substituted (“clumped”) isotopologues in CO₂ is a topic of significant interest in isotope geochemistry. Here we describe the performance of a new isotope ratio laser spectrometer using tunable infrared laser differential absorption spectroscopy (TILDAS). The TILDAS instrument has two continuous-wave lasers to simultaneously measure the four isotopologues involved in the \( ^{12}\text{C}^{16}\text{O} + ^{13}\text{C}^{18}\text{O} \leftrightarrow ^{13}\text{C}^{16}\text{O} + ^{12}\text{C}^{18}\text{O} \) exchange reaction. CO₂ samples are trapped in a low volume (~250 mL) optical multipass cell with a path length of 36 m. Each sample is compared to a reference gas, and clumped isotope precision of 0.01‰ (SE) is achieved within 20 min for 15 µmol samples. Similar precision is also achieved for bulk isotopic composition. The degree of rare isotope clumping in excess of strictly random distribution (Δclumped(O16)) measured by this TILDAS instrument varies linearly with theoretically calculated values and shows a very weak dependence on bulk isotopic composition.

The measurement of rare isotopologues of carbon dioxide has become a topic of significant interest in multiple fields of isotope geochemistry, from geothermometry to tracing sources of CO₂ in the atmosphere. Carbon dioxide clumped isotope thermometry, focused on the formation temperatures of carbonate minerals, is the most developed application of the clumped isotope thermometry, where measurements typically require extensive sample purification, 2–4 h of machine time per sample, replication of these analyses, sample sizes of 30 µmol or greater, and analysis of many reference carbon dioxide gases and carbonate standards to achieve usefully precise data. However, a major challenge for carbonate clumped isotope thermometry is the difficulty of the measurement, due to the extremely low natural abundance and subtle variation with temperature of clumped isotopologues. Virtually all laboratories today use isotope ratio mass spectrometry (IRMS) for clumped isotope thermometry, where measurements typically require extensive sample purification, 2–4 h of machine time per sample, replication of these analyses, sample sizes of 30 µmol or greater, and analysis of many reference carbon dioxide gases and carbonate standards to achieve usefully precise data. Moreover, the clumped isotope values rely on δ18O and δ13C measurements that must be corrected for δ18O mass interference, because both the \(^{13}\text{C}^{18}\text{O}^{16}\text{O}^{16}\text{O}\) and \(^{12}\text{C}^{18}\text{O}^{17}\text{O}\) molecules have the same cardinal mass of 47 AMU. This correction is based on an assumed \(^{17}\text{O}\) abundance relationship, whose variability in nature may introduce significant errors in clumping estimates.

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requirement of 15 our average measurement time of 20 min and sample size precisions using well-established IRMS techniques. Moreover, using our new isotope ratio laser spectrometer, comparable to Laser 1 at 2250.2 cm$^{-1}$ region. The simulation was conducted at a concentration of 0.35% (balance nitrogen), a pressure of 60 Torr, and a path length of 36 m. The selection of concentration and pressure is explained in TILDAS Instrument Stability. Nitrous oxide was added to the simulation due to its potential impact on the spectral region of interest.

More efficient, direct, and broadly applicable clumped isotope measurements can potentially be achieved by laser spectroscopy, which uses an infrared laser to measure the absorption spectrum of the molecules of interest. Laser spectroscopy is now commonly used for measurement of $^{18}$O and $^6$D compositions in water and is commercially available for $^{18}$O and $^3$C analysis in carbon dioxide$^{19,20}$ Laser methods have recently been developed for high-precision $^{17}$O analysis in water$^{21,22}$ as well as for clumped isotopes of methane.$^{23,24}$ Our recent work$^{25}$ and that of Prokhorov et al.$^{26}$ have demonstrated the great promise of tunable infrared laser differential absorption spectroscopy (TILDAS) for clumped isotope measurements of carbon dioxide. In this paper, we describe the development of a new dual-laser TILDAS instrument and associated sampling system that provides high precision and rapid measurements of the clumped isotopic composition of carbon dioxide. This TILDAS instrument improves upon a prototype instrument$^{29}$ built by Aerodyne Research, Inc., allowing simultaneous and direct measurement of all four carbon dioxide isotopologues of interest. The sampling system makes use of a working reference gas to correct for instrument drift, which ensures long-term stability and allows long integration times for sample measurement. Through recent improvements in laser design and sample handling described in this paper, we can routinely achieve precisions of 0.01‰ (1 SE, standard error, $n = 4$) using our new isotope ratio laser spectrometer, comparable to precisions using well-established IRMS techniques. Moreover, our average measurement time of 20 min and sample size requirement of 15 $\mu$mol of CO$_2$ or 1.5 mg of calcite equivalent, are significantly shorter and smaller than that of well-established IRMS techniques.

**EXPERIMENTAL SECTION**

**Laser-Specific Notation.** In clumped isotopes geochemistry, the variable $\Delta_i$ is used to describe the abundance of isotopologue $i$ relative to that expected if all isotopes are randomly distributed (stochastic distribution) among all isotopologues, $\Delta_i = \frac{R_i}{R_{i-r}} - 1 \times 1000$ (1)

where $R_i$ is the abundance ratio of an isotopologue of interest (i.e., $i$) relative to the isotopologue having no rare isotopes (i.e., $^{12}$C$^{16}$O$^{16}$O), and subscript $r$ in $R_{i-r}$ stands for the random distribution condition. The isotope exchange reaction involving one doubly substituted isotopologue $^{13}$C$^{18}$O$^{16}$O can be written as

$$^{12}$C$^{16}$O$^{16}$O + $^{13}$C$^{18}$O$^{16}$O $\rightleftharpoons ^{12}$C$^{18}$O$^{16}$O + $^{13}$C$^{16}$O$^{16}$O (R1)

and the temperature-dependent equilibrium constant $K$ for this reaction is expressed as

$$K = \frac{[^{12}$C$^{18}$O$^{16}$O][^{13}$C$^{16}$O$^{16}$O]}{[^{12}$C$^{16}$O$^{16}$O][^{13}$C$^{18}$O$^{15}$O]}$$ (2)

A decrease in temperature drives the reaction to the left, increasing the abundance of $^{13}$C$^{16}$O$^{16}$O. Wang et al.$^{30}$ derived the relationship between $\Delta^{^{12}$C$^{16}$O$^{16}$O}_r$ and $K_r$

$$\Delta^{^{12}$C$^{16}$O$^{16}$O}_r \approx -1000 \ln \frac{K}{K_r}$$ (3)

where $K_r$ is the equilibrium constant for the fully randomized stochastic distribution. in reaction R1, $K_r$ equals 1.

Each of the four isotopologues in eq 2 can be measured directly by the TILDAS instrument in a carefully chosen spectral region. In this study, we introduce a new notation for clumped isotopes of CO$_2$, $\Delta_{638}$, in recognition of the uniquely different way we are making the measurement by TILDAS. This laser-specific notation is based on the Air Force Geophysics Laboratory (AFGL) shorthand notation which has been used in the HITRAN database for decades. Here $^{16}$O$^{12}$C$^{16}$O, $^{16}$O$^{13}$C$^{18}$O, $^{16}$O$^{15}$C$^{17}$O, and $^{16}$O$^{16}$C$^{18}$O are referred to as CO$_2$ 626, 636, 628, and 638, respectively. Isotopologues are identified by the second digit of the atoms’ atomic mass. This $\Delta_{638}$ term is different from the $\Delta_{47}$ term used in IRMS measurement of clumped isotope because there is no isobar interference using TILDAS. $^{30}$ For this study, we will report our
results relative to a working reference gas (WR), which corrects the TILDAS instrument drift during measurement, rather than relative to $K_{\text{vs}}$ and eq 3 then becomes

$$\Delta_{638_{\text{raw}}} = -1000 \ln \frac{K}{K_{\text{WR}}} \tag{4}$$

where $\Delta_{638_{\text{raw}}}$ is defined as the $\Delta_{638}$ value of the sample gas with respect to the working reference gas.

In contrast to IRMS, each isotopologue can be measured specifically using TILDAS, we define isotopologue composition as

$$\delta_i = \left( \frac{I_{\text{sample}}}{I_{\text{standard}}} - 1 \right) \times 1000 \tag{5}$$

where $i = 636, 628, \text{and } 638$. In the more common situation in which the isotopes are not stochastically distributed among the isotopologues, $\delta_{636}$ and $\delta_{628}$ are good approximations of bulk isotopic composition, $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$, respectively, whereas $\delta_{638}$ is approximately equal to $(\delta_{636} + \delta_{628})$ and thus to $(\delta^{13}\text{C} + \delta^{18}\text{O})$.

**Selection of Absorption Lines.** Two lasers are used to measure the four isotopologues of CO$_2$ (626, 636, 628, and 638) drawn from the HITRAN database (Figure 1; Table 1). The spectral region of 2250.2 cm$^{-1}$ was chosen for $^{13}\text{C}^{18}\text{O}^{16}\text{O}$ and $^{12}\text{C}^{16}\text{O}^{16}\text{O}$ lines, which are free from spectral interference. The absorption lines for $^{13}\text{C}^{16}\text{O}^{16}\text{O}$ and $^{12}\text{C}^{18}\text{O}^{16}\text{O}$ were selected in the 2285.1 cm$^{-1}$ region because of the comparable line intensity (Table 1). Therefore, all four isotopologue lines can be simultaneously measured at the same conditions, i.e., concentration, pressure, path length, which is critical to shorten the measurement time and consequently minimize instrumental drift.

The calculation of equilibrium constant $K$ in eq 2 can be manipulated to yield an equivalent combination of isotopologue ratios that match the peak pairs in each laser:

$$K = \frac{[626][636]}{[626][638]} = \frac{[628]/[638]}{[626]/[636]} \tag{6}$$

where the isotopologue ratios $[638]/[636]$ and $[628]/[626]$ are absorption peak ratios measured in the absorption windows of the two lasers. Absorption peaks can respond strongly to changes in temperature and pressure, so each variable must be controlled and carefully monitored. However, the approach used in eq 6 uses a ratio of peak ratios, $[638]/[636]$ and $[628]/[626]$, that have similar lower-state energy differences (Table 1, $\Delta E$, 1023 vs 1193 cm$^{-1}$), which reduces the impact of temperature change in the instrument. This is because the temperature impact on absorption intensity is similar for the peaks measured in each laser, leading to a cancelation or reduction in the effects of temperature-dependent line intensities on the equilibrium constant $K$ measurement.
Therefore, lead to a measurable di
to stop isotopologue reordering. Each temperature should
were fully equilibrated, the water was removed from the sample
°
60
water or evaporatively enriched water held at 4
combustion in pyrex break-seals with South Pole ice core
that originated from corn fermentation or fossil fuel
the University of Washington, by equilibrating carbon dioxide
peak area calculation.
addition of nitrogen to the carbon dioxide broadens the

**RESULTS AND DISCUSSION**

**TILDAS Instrument Stability.** The original flow-through
optical cell of the Aerodyne CO2 isotope instrument has been
retrofitted with vacuum fittings, resulting in a leak-tight optical
cell suitable for static sample measurement at various
pressures. A 0.35% CO2 in N2 was used to test the performance of the instrument, and the pressure for optimal absorption and stability in the TILDAS instrument was found in the 50 to 60 Torr range (Figure s1(a) in Supporting Information). Larger sample sizes (i.e., higher sample pressure) did not significantly improve performance. The volume of sample needed in the TILDAS instrument for a single aliquot or replicate measurement of a sample gas is approximately 310 mL, which includes the optical sample cell, intermediate volumes, and tubing. This leads to a single replicate sample size of 3 to 4 µmol of CO2 or 0.3 to 0.4 mg of calcite equivalent. The measurement precision in terms of standard error is improved as expected by averaging multiple data points (Figure s1(b) in Supporting Information). Measuring multiple replicates and/or collecting multiple data points in each replicate increases measurement precision but also requires more sample consumption and longer analysis time. However, to achieve high precision measurements and counter the effects of instrument drift, multiple aliquots of the sample gas must be measured. Thus, there is a trade-off between measurement precision, integration time, and sample size.

To test the instrument stability, the Δ638 value of a CO2/N2 mixture gas at a concentration of 0.35% was continuously monitored for 8 h at a pressure of 60 Torr. Typical instrument drift and corresponding Allan variance plot of Δ638 are shown in Figure 3. The 1-s Allan deviation of 0.127‰ is consistent with that depicted in Figure s1(b), whereas the minimum Allan deviation of 0.013‰ is obtained with an integration time of 240 s. The instrument drift becomes significant over an integration time longer than approximately 400 s. This indicates that one measurement subcycle, which includes both a single sample replicate measurement and a single working reference measurement, should be at least 240 s to collect as many data points as possible before drift becomes significant but less than 400 s to avoid instrument drift effects. Based on extensive testing, a measurement cycle of 5 min was determined as the optimal balance between measurement precision, instrument drift, and sample size.

Although the overlap between 13C16O2 and the small 13C16O18O peaks in the region of 2285.1 cm⁻¹ (laser 2) could complicate our measurements, this peak is very precisely constrained based on the 13C16O2 abundance measured at 2250.267 cm⁻¹ (using laser 1). The latter peak, which is free from interference and has a stronger line intensity, is used to quantify 13C16O2 and the TDLWintel software removes a

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**Figure 2.** A schematic diagram of the sampling system installed in the TILDAS instrument. E# and P# stand for electronic and pneumatic valves, respectively, and numbers 1–8 were reserved for electronic valves. Concentric circles: critical orifice. P sensor: pressure sensor.
proportional peak area from the combined peaks at 2285.1 cm\(^{-1}\) to quantify the \(^{13}\)C\(^{18}\)O\(^{16}\)O peak for spectral fitting. This approach has proved to be robust across a range of \(\delta^{13}\)C and \(\delta^{18}\)O values in CO\(_2\) samples.

**Measurement Protocol and Long-Term Precision.** A proof-of-concept study was conducted to investigate the feasibility of a working reference gas concept in the TILDAS instrument. The CO\(_2\)/N\(_2\) sample and working reference gases were made in two identical stainless-steel canisters (Restek, USA) with the same concentration of 0.35%. A basic analytical protocol was established as follows:

1. Introduce working reference into the intermediate volume of approximately 25 mL to designated pressure;
2. Expand working reference into the optical sample cell and measure working reference for \(\sim 100\) s;
3. Pump out the intermediate volume and introduce the sample to the designated pressure while measuring the working reference;
4. Pump out the optical sample cell, flush with dry nitrogen, and pump out the nitrogen;
5. Expand the sample into the optical sample cell as in 2 above and conduct measurement of sample for \(\sim 100\) s;
6. Repeat steps 1–5 to obtain the required number of measurement subcycles.

The \(\Delta_{638raw}\) value of sample gas against working reference gas was measured for over 37 h, using a measurement subcycle of 5 min for one comparison of the reference gas and the sample gas (Figure 4). The Allan variance plot in Figure 4 illustrates that the instrument drift is successfully canceled out of the measurement through the use of a working reference gas, and the variability of Allan variance (\(\sigma^2\)) shows a declining trend obeying an ideal statistical-noise-limited behavior as the measurement proceeds. By measuring four subcycles using 20 min total time, the precision of this TILDAS instrument on CO\(_2\) clumped isotope \(\Delta_{638raw}\) measurement after the incorporation of working reference gas was improved to \(\pm 0.009\‰\) (SE, \(n = 4\)). With four measurement subcycles of the sample gas, the total sample size needed was approximately 15 \(\mu\)mol of CO\(_2\), or 1.5 mg of calcite equivalent.

**Measurement of Heated and Equilibrated CO\(_2\) Gases.** Following the analytical protocol described above, eight sample gases were analyzed with respect to a working reference gas. These are the CO\(_2\) samples equilibrated at different temperatures described above. Each sample was measured twice (Table s1 in Supporting Information, test 1 and test 2) with an interval of 8 h between measurements during which the sample remained in the canister. Four subcycles were averaged to obtain one individual meaningful measurement with a targeted precision of 0.01\‰. The results show that the two test results agree with each other, which is very encouraging in that \(\Delta_{638raw}\) values were measured with high internal precision (1 SE < 0.01\‰) across an 8-h time interval. In addition, \(\delta_{636raw}\) and \(\delta_{638raw}\) values show similar precision (0.02\‰, 1 SD), and these ratios will be used to precisely determine \(\delta^{13}\)C and \(\delta^{18}\)O values of the sample CO\(_2\) after calibration to standard materials. This minimizes the concern over clumped isotope reordering due to the usage of a dry scroll vacuum pump and canisters made of stainless steel, where the presence of adsorbed water on the container surface could be a significant concern.\(^{34}\) Note that clumping reordering was observed in only one heated CO\(_2\) sample; this is discussed in Supporting Information.

The reported TILDAS results are proportional to the theoretical equilibrium \(\Delta_{638}\) values from Petersen et al.\(^{35}\) (Figure 5). We used an approach analogous to the absolute reference frame of Dennis et al.,\(^{14}\) or carbon dioxide equilibrium scale (CDES), for normalizing IRMS clumped CO\(_2\) measurements to theoretical equilibrium \(\Delta_{47}\) values.\(^{30}\) The theoretical difference in clumping between 4 °C and 1000 °C samples is expected to be 1.048\‰, whereas that reported by TILDAS in fermented corn and fossil fuel CO\(_2\) samples is 1.073\‰ and 1.144\‰, respectively. Interestingly, this shows that the TILDAS instrument exhibits a slightly expanded scale, such that the TILDAS measurement precision of <0.01\‰ will
be further improved when the measured $\Delta_{638}$ values are converted to the CDES.$^{14}$

Equilibration of CO$_2$ with water resets the clumping in the CO$_2$ to a value that is controlled by temperature, and each of the gases equilibrated at the same temperature therefore should have identical $\Delta_{638}$ values (Figure 5). Although the values produced in this test are not identical for each temperature, they are very similar (Figure 6). This implies that there is minimal to insignificant dependence on the bulk isotopic composition in the measurement of $\Delta_{638raw}$ values. If a single average slope is calculated based on the 4 °C, 60 °C, and 1000 °C lines, we obtain $-6.23 \times 10^{-5}$, with intercepts of 0.22447, -0.07291, and -0.88429, respectively. Using this slope, the temperature of sample equilibrated at 22 °C can be calculated as a quality control assessment,$^{14}$ which results in 25 ± 4 °C (1 SD). Additional testing is needed to determine if the variance in observed slopes in Figure 6 is due to noise in our first set of measurements or whether this is systematic. The relatively weak correlation between $\Delta_{638raw}$ and $\delta_{638raw}$ demonstrated in this preliminary study is very encouraging, in that it implies that working reference gases (which almost always show clumping associated with room temperature equilibration with water vapor) can have bulk isotopic compositions that are significantly different from the sample compositions without strongly affecting the $\Delta_{638raw}$ measurement.

**Testing Isotopic Fractionation during Sample Handling.** Compared to IRMS, the carbon dioxide sample handling and analysis technique are very different in the TILDAS approach. The TILDAS instrument and sample inlet system were operated across a range of conditions to investigate potential fractionation risk, especially due to the usage of critical orifices, regulators, large-diameter tubing (1/4–1/2”), and storage canisters. Three working references with the same concentration of 0.35% CO$_2$ in N$_2$, i.e., WR1, WR2, and WR3, were made and stored in three canisters. There were small differences in the final $\Delta_{638}$ values of these three gases resulting from the preparation process and perhaps from slight differences in the final concentration of CO$_2$ (e.g., WR1 0.346% and WR2 0.354%). We varied our sample handling procedures systematically in an attempt to isolate potential artifacts. The experimental parameters and results are summarized in Table 2.

Experiments 1 and 2 (expt 1 and expt 2) compared differences in the size of the critical orifices used to restrict sample flow. Both were 100 μm in expt 1, and one was increased to a diameter of 125 μm in expt 2. The TILDAS-reported $\Delta_{638raw}$ values suggest that the usage of different sizes of critical orifices did not cause an observable fractionation because (1) the result is comfortably within the uncertainty range of zero enrichment in both cases, and (2) expt 1 result agrees with expt 2. Analogously, the results of expt 3 and expt 4 indicate that no observable fractionation was caused by a significant increase in regulator output pressure (5 vs 15 psig).

Experiment 5 was conducted to investigate the possible occurrence of fractionation in the gas stored in a canister and throughout a prolonged (11 h) measurement of $\Delta_{638}$. Before WR3 was introduced into the TILDAS instrument, some of it was pumped away for 30 s (analogous to the gas consumption of three subcycles) through the sample critical orifice, valve E1, P12, and P14 (Figure 2). This reduced the amount of total gas in WR3 relative to WR1, leading to a large difference in the proportional rate of consumption of the two gases with the WR3 gas being depleted much more rapidly than WR1. Figure 7(a) shows the continuous variation of $\Delta_{638raw}$ (WR3 vs WR1) values over 11 h until WR3 ran out, during which the isotopic composition did not change as WR3 was consumed. In summary, the above experiments demonstrate that no observable fractionation occurs within the sampling system and TILDAS instrument.

**Cancelation of Diffusion Effects on $\Delta_{638}$ Ratio.** Using Chapman–Enskog theory, the diffusion coefficient of gas a through gas b can be expressed as

Figure 5. Reported TILDAS $\Delta_{638raw}$ values versus theoretical equilibrium CO$_2$ $\Delta_{638}$ values. Numbers and symbols in blue: fermented corn CO$_2$; in red: fossil fuel CO$_2$. Residuals for either gas, below, do not show a trend.

Figure 6. Observed relationship between $\Delta_{638raw}$ and $\delta_{638raw}$ in the heated and equilibrated gases using TILDAS instrument.
Table 2. Summary of Experimental Conditions and Results Regarding Potential Isotopic Fractionation Investigation*

<table>
<thead>
<tr>
<th>experiment no.</th>
<th>gas no.</th>
<th>regulator output pressure (psig)</th>
<th>critical orifice diameter (μm)</th>
<th>Δδ638raw (%ε) mean ± 1 SE</th>
<th>Δδ636raw (%ε) mean ± 1 SE</th>
<th>Δδ628raw (%ε) mean ± 1 SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>expt 1</td>
<td>WR1</td>
<td>5</td>
<td>100</td>
<td>-0.004 ± 0.005</td>
<td>0.005 ± 0.003</td>
<td>-0.002 ± 0.002</td>
</tr>
<tr>
<td></td>
<td>WR1</td>
<td>5</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>expt 2</td>
<td>WR1</td>
<td>5</td>
<td>100</td>
<td>0.003 ± 0.006</td>
<td>0.021 ± 0.003</td>
<td>0.014 ± 0.002</td>
</tr>
<tr>
<td></td>
<td>WR1</td>
<td>5</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>expt 3</td>
<td>WR1</td>
<td>5</td>
<td>100</td>
<td>-0.035 ± 0.003</td>
<td>0.047 ± 0.003</td>
<td>0.071 ± 0.002</td>
</tr>
<tr>
<td></td>
<td>WR1</td>
<td>5</td>
<td>125</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>expt 4</td>
<td>WR1</td>
<td>5</td>
<td>100</td>
<td>-0.037 ± 0.004</td>
<td>0.050 ± 0.003</td>
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<tr>
<td></td>
<td>WR2</td>
<td>5</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>expt 5</td>
<td>WR1</td>
<td>5</td>
<td>100</td>
<td>-0.021 ± 0.003</td>
<td>0.043 ± 0.001</td>
<td>0.017 ± 0.001</td>
</tr>
<tr>
<td></td>
<td>WR3</td>
<td>5</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*In experiment 2 (expt 2), the regulator output pressure of WR1 was set to 5 psig, and it was connected to both the sample inlet (critical orifice diameter of 100 μm) and the working ref inlet (critical orifice diameter of 125 μm). In expt 4, WR1 was connected to the sample inlet and its regulator output pressure was set to 5 psig. WR2 was connected to the working ref inlet, and its regulator output was 15 psig. Both critical orifices had a diameter of 100 μm.

\[ D \propto \sqrt{1/\mu} \]  

where \( \mu \) is the reduced mass given by \( \mu = m_1 m_2 / (m_1 + m_2) \). In this study, carbon dioxide is mixed with nitrogen for clumped isotope measurement, and two isotopologue ratios, \([638]/[636]\) and \([628]/[626]\), are independently measured by two lasers, such that the diffusion coefficient ratios can be calculated as

\[
\frac{D_{638}}{D_{636}} = \frac{\sqrt{\frac{45 \times 28}{45 + 28}}}{\sqrt{\frac{47 \times 28}{47 + 28}}} = 0.991806
\]

\[
\frac{D_{628}}{D_{626}} = \frac{\sqrt{\frac{44 \times 28}{44 + 28}}}{\sqrt{\frac{46 \times 28}{46 + 28}}} = 0.991510
\]

while the diffusion coefficient ratio of four isotopologues used to calculate the equilibrium constant \( K \) is

\[
\frac{D_{628}D_{636}}{D_{632}D_{638}} = \frac{\sqrt{\frac{44 \times 28}{45 + 28}}}{\sqrt{\frac{47 \times 28}{46 + 28}}} = 0.999702
\]

Equation 9 shows that the fractionation due to the different diffusion coefficients among the CO₂ isotopologues would result in a deviation of 0.3‰ in \( \Delta_{638}^{\text{ppm}} \), which is 28 times less than that in the \([638]/[636]\) and \([628]/[626]\) ratios (eq 8). This implies that diffusional fractionation in CO₂ would lead to large changes in bulk \( \delta^{13}C, \delta^{18}O \) but only small changes in \( \Delta_{638} \) because of diffusion cancelation. In this study, no observable fractionation in the \([638]/[636]\) and \([628]/[626]\) ratios was found. For example, Figure 7(b) demonstrates that the \([628]/[626]\) ratio remained constant in terms of \( \delta_{628}\text{raw} \) (WR3 vs WR1) values while the WR3 gas was depleted at a significantly faster rate than WR1. In practice, two features help to reduce the isotope fractionation caused by diffusion: (1) the carbon dioxide and nitrogen were well-mixed before being introduced into the sampling system, and (2) turbulent mixing during the gas expansion assists in homogenizing the mixture.

**CONCLUSION AND FUTURE WORK**

We have developed an isotope ratio laser spectrometry method to precisely determine the carbon dioxide clumped isotopic composition, \( \Delta_{638}^{\text{ppm}} \), using a TILDAS instrument. In addition, this system can rapidly measure the stable oxygen isotopic composition, \( \delta_{628} \), and stable carbon isotopic composition, \( \delta_{636} \) (both terms are relatable to conventional \( \delta^{18}O \) and \( \delta^{13}C \) after calibration), at high precision. The TILDAS instrument directly and simultaneously measures four isotopologues, \( ^{16}O^{12}C^{16}O, ^{16}O^{13}C^{16}O, ^{16}O^{12}C^{18}O, \) and \( ^{16}O^{13}C^{18}O \), in the mid-infrared spectral regions of 2250.2 and 2285.1 cm\(^{-1}\). One aliquot measurement yielded a precision of 0.02‰ (1 SE, 5 min) in \( \Delta_{638}\text{raw} \) values (with respect to an uncharacterized working reference gas). With a specially designed sampling system, the concept of which is adapted from dual-inlet IRMS, long-term instrument drift was corrected by using a working reference gas. A precision of 0.01‰ was achieved with four repeated measurements within 20 min, and the total sample size needed was approximately 15 μmol of CO₂ or 1.5 mg of calcite equivalent. With enough sample, additional comparisons will improve the precision of the measurement and ±0.002‰ (1 SE) can be achieved in 10 h (Figure 4). Heated CO₂ samples and samples equilibrated with water at different temperatures demonstrate that the TILDAS \( \Delta_{638}\text{raw} \) values match theoretical values in a linear relationship, at a slightly expanded scale. A weak dependence of \( \Delta_{638}\text{raw} \) on \( \delta_{638}\text{raw} \) was observed with a slope of -0.0003 to 0.0002 over a range of 100‰ in \( \delta_{638}\text{raw} \) and heated CO₂ samples had a slope of +0.0009 over a 45‰ range of \( \delta_{638}\text{raw} \). This suggests that accurate measurements of samples across a broad range in bulk isotopic composition should be feasible with only a few working reference gases spanning that range.

Work with this TILDAS instrument is ongoing, including additional calibration efforts and improvements to the TILDAS instrument. Calibration work includes (A) comparison to the international carbonate reference materials NBS-19, NBS-18, and IAEA-603, as well as (B) the clumped isotope interlaboratory comparison carbonates, ETH-1, ETH-2, ETH-3, and ETH-4, and (C) measurement of more carbon dioxide equilibrium scale gases. In addition we are (D) developing
TILDAS instrument thermometry relationships in carbonates using newly created synthetic calcites as well as those already included in publications. These four exercises are the four pillars of IRMS clumped isotope measurement and will be necessary to cross calibrate TILDAS-based Δ638 and IRMS-based Δ47 measurements. The performance of the TILDAS instrument demonstrated in this study is competitive with the best IRMS systems and surpasses typical IRMS measurements in several key respects, especially in measurement duration, which is typically 20 min or less.

**ASSOCIATED CONTENT**

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.analchem.9b04466.

Figure s1. Determine optimal conditions in terms of TILDAS instrument stability. Figure s2. One clumped isotope reordering case. Table s1. Δ638raw, Δ636raw, and Δ28raw measurement results using a TILDAS instrument.

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**Notes**

The authors declare no competing financial interest.

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