High-precision optical measurements of $^{13}$C/$^{12}$C isotope ratios in organic compounds at natural abundance


A continuous-flow cavity ring-down spectroscopy (CRDS) system integrating a chromatographic separation technique, a catalytic combustor, and an isotopic $^{13}$C/$^{12}$C optical analyzer is described for the isotopic analysis of a mixture of organic compounds. A demonstration of its potential is made for the geochemically important class of short-chain hydrocarbons. The system proved to be linear and stable over a 3-fold injection volume dynamic range with an average precision of 0.95‰ and 0.67‰ for ethane and propane, respectively. The calibrated accuracy for methane, ethane, and propane is within 3‰ of the values determined using isotope ratio mass spectrometry (IRMS), which is the current method of choice for compound-specific isotope analysis. With anticipated improvements, the low-cost, portable, and easy-to-use CRDS-based instrumental setup is poised to evolve into a credible challenge to the high-cost and complex IRMS-based technique.

The isotopic analysis of a mixture of organic compounds was demonstrated to take place (10). The resulting combustion products are combined with dry N$_2$ before entering a cavity ring-down spectrometer. Ring-down rates are recorded for the R (12) line of $^{13}$CO$_2$ and the R (36) line of $^{12}$CO$_2$ of the (3,0,0) – (0,0,0) combination band of carbon dioxide near 6,251 cm$^{-1}$ at a fixed temperature and pressure. From the concentrations of $^{13}$CO$_2$ and $^{12}$CO$_2$ are measured and used to calculate the $^{13}$C/$^{12}$C isotope ratio. We refer to this setup

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as GC-C-CRDS. It should be appreciated that a similar approach can be followed in which the gas chromatograph is replaced by another separation technique, such as liquid chromatography with some modifications to the interface to eliminate the interfering mobile phase. If the bulk isotope ratio rather than the compound-specific isotope ratio is desired, the separation procedure can be omitted. Conversely, if the compound of interest has >1 atom of carbon occurring in unsymmetrical positions in the compound, then spectral analysis or some chemical modifications are needed before analysis to obtain the $^{13}$C/$^{12}$C site-specific isotope ratio. It is also important to realize that the same approach can be used to measure the D/H isotope ratio in water as a combustion byproduct and work is underway to measure simultaneously the D/H and the $^{13}$C/$^{12}$C isotope ratios of any compound of interest.

Cavity ring-down spectroscopy allows absorption measurements to be made on an absolute basis with a sensitivity that vastly exceeds traditional absorption measurements (11, 12). In a typical CRDS system, the optical resonator includes 2 or more mirrors in an optical cavity aligned so that incident light circulating between them. The sample of absorbing molecules is placed in the cavity for interrogation. When input light to the resonator is discontinued, the radiant energy stored in the resonator decreases over time or “rings down” in the parlance of electrical engineering. For an empty cavity, the stored energy follows an exponential decay characterized by a ring-down rate that depends on the reflectivity of the mirrors, the separation between the mirrors, and the speed of light in the resonator. If an absorbing sample is placed in the resonator, the ring-down rate decreases compared with that for the empty resonator. The corresponding absorption spectrum for the sample is obtained by plotting the reciprocal of the ring-down rate versus the wavelength of the incident light. The sensitivity gain of CRDS compared with single-pass absorption measurements comes from the much larger path length of CRDS, which is often kilometers in extent, and from the relative insensitivity of the ring-down rate to fluctuations in the intensity of the light source. Previously, carbon dioxide concentrations as low as a few parts per million have been measured (13), using CRDS and the same technique has been used to determine $^{13}$C/$^{12}$C isotope ratios (13–16), for example, as a diagnostic for ulcer-forming bacteria (*Helicobacter pylori*) by CO$_2$ breath analysis (13). CRDS has also been used successfully to analyze hydrogen and oxygen isotopes in water samples, introduced under either a liquid or vapor form, with isotopic measurements that parallel the high-precision yielded with dual-inlet IRMS systems (17, 18). Thus, CRDS is not intrinsically inferior to mass spectrometry for isotope ratio measurements, although the present study does not reach this precision.

## Results and Discussion

Fig. 2 presents scans of the $^{12}$C$^{16}$O$^{16}$O and $^{13}$C$^{16}$O$^{16}$O line profiles in the (3,0,0)–(0,0,0) combination band used to determine the $^{13}$C/$^{12}$C isotope ratio. The cavity of the CRDS instrument is operated at 45 °C and a pressure of 140 torr (1 torr = 133.22368 pascals) consisting of the background gases helium, nitrogen, water vapor, and oxygen. The maximum amount of carbon dioxide in the cavity generated from the combustion process is typically between 0.15 and 0.70 torr. The instrument has an acquisition rate of ~330 ring-down events per second. ~30 and 130 ring-down traces are used for each determination of the $^{12}$C$^{16}$O$^{16}$O and $^{13}$C$^{16}$O$^{16}$O line shapes used to calculate the concentration, respectively, resulting in a repetition rate of 2 Hz. The start and end of the $^{12}$C$^{16}$O$^{16}$O peak are defined as the points where signal exceeded the baseline noise. The baseline noise is calculated as the average of the signal taken 2 s before the start of the chromatographic peak. The typical noise in the baseline of the $^{12}$C$^{16}$O$^{16}$O and $^{13}$C$^{16}$O$^{16}$O signals is 0.4 ppm and 0.03 ppm, respectively. The signal to noise ratio at the peak maxima generated from 5 μL of ethane, which is the minimum volume needed to produce reproducible results, is 2,600 for $^{12}$C$^{16}$O$^{16}$O and 400 for $^{13}$C$^{16}$O$^{16}$O. The signal to noise ratio for 25 μL of ethane is 14,800 for $^{12}$C$^{16}$O$^{16}$O and 1,500 for $^{13}$C$^{16}$O$^{16}$O. Volumes of >25 μL did not result in a significant improvement in the precision. Peaks are fit with an empirically transformed Gaussian function using a nonlinear least square curve fitting routine as described elsewhere (19). The repetition rate of 2 Hz limits the current setup to samples that pass through the instrument slowly relative to the few-second peak widths obtained using faster gas chromatography techniques. The current setup could be modified to operate at a faster repetition rate by using fewer ring-down events for the determination of each measurement, but this is a topic for future improvements.

The absolute concentrations of the individual carbon dioxide isotopomers are converted to isotope ratios, using the Boltzmann distribution at the known fixed temperature of the instrument. We have examined the possible temperature effect of the transit of the sample and carrier gas through the heated combustion chamber before their admission to the optical cavity. The combustion gases leave the furnace ~20 °C above room temperature. The length of the tubing between the combustor and the optical cavity is ~60 cm and we are mixing the combustion gases with dry nitrogen. We determined that there is no significant impact of the heat from the combustor on the spectroscopic analysis temperature of 45 °C, which is held at this value to a few milli-Kelvin.

Isotope ratios are conveniently quantified in parts per mil (‰) in what is called the δ notation. Specifically, δ$^{13}$C =
data for the response of $^{12}\text{C}^{16}\text{O}^{16}\text{O}$ and $^{13}\text{C}^{16}\text{O}^{16}\text{O}$ as a function of volume injected. Each data point represents the average of 5 measurements, and the error bars (1 SD) are within the size of the marker.

We also find that the instrument shows a high degree of linear response to increasing organic gas concentration. Fig. 4 present data for the response of $^{12}\text{C}^{16}\text{O}^{16}\text{O}$ and $^{13}\text{C}^{16}\text{O}^{16}\text{O}$ as a function of volume injected, where each data point represents the average of 5 measurements, and the error bars (1 SD) are within the size of the marker.

We present in Fig. 5 the chromatographic traces of $^{12}\text{C}^{16}\text{O}^{16}\text{O}$ (A) and $^{13}\text{C}^{16}\text{O}^{16}\text{O}$ (B) from a 25-µL mixture of methane, ethane, and propane from which the $\delta^{13}\text{C}$ value is calculated for each component (C). The large amount of noise in the baseline of C arises mainly from the noise in the measurement of $^{13}\text{C}^{16}\text{O}^{16}\text{O}$. The noise in the $^{13}\text{C}^{16}\text{O}^{16}\text{O}$ measurement is approximately independent of its concentration within the pressure regime we operate, which leads to significant fluctuations in $\delta^{13}\text{C}$ at baseline levels. As the sample passes through the instrument, the higher concentrations of CO2 allow for relatively more precise measurements that result in the $\delta^{13}\text{C}$ calculation to be made with a precision of < 1‰.

We also observe systematic variations up to 5‰ in the measured isotopic ratios between the 2 techniques that is attributed to differing levels of precision of the background gases in the CRDS cavity resulting in molecular peak broadening effects (22). The variations can be reduced in the future by modifying the peak fitting routine.

The variation from peak broadening was larger than expected. As a result, the amounts for methane, ethane, and propane in the GC-C-CRDS “From Mixture” column in Table 1 were decreased by 5.39‰ so the GC-C-CRDS and GC-C-IRMS values for propane are identical. This approach yields a reasonable estimate of the accuracy of $\delta^{13}\text{C}$ for methane and ethane. The amounts in the GC-C-CRDS “From Mixture” column in Table 1 have not been adjusted from the calculated values. In the
future, we plan to calibrate our samples to 2 known standards of carbon dioxide with a wide bracketing range of δ13C values in a background gas mixture similar to our experimental conditions. This procedure will allow for better calibration and for a scale multiplication factor to be applied for samples with different δ13C values (23). Previous analysis of carbon isotopes in carbon dioxide, using CRDS, did not show a significant variation from the standard for a wide range of δ13C values (13, 14), which leads us to believe that the scaling factor for the current work is likely below the measured error.

We conclude that GC-C-CRDS is able to make 13C/12C isotope ratio measurements of individual organic species with a calibrated accuracy of ~1–3‰, which is presently inferior to GC-C-IRMS by a factor of ~10–30, but this accuracy is sufficient in many cases of interest to make this optical technique an appealing alternative. In addition, the sample volume currently needed is ~1,000 times greater than the amounts routinely analyzed by GC-C-IRMS. However, these results represent the first efforts, and we anticipate some hardware modifications, will lead to considerable improvements in sensitivity, precision, and accuracy.

One possible modification that could lead to a reduction in the sample volume, and an improvement in the precision and accuracy, is to switch to a wavelength in the midinfrared region. The absorption of the fundamental asymmetric vibration band of carbon dioxide at 4.3 μm is ~105 times stronger than the combination band currently measured (24). Optical based techniques operating in the midinfrared region have produced impressive results for the isotopic measurement of carbon dioxide at atmospheric levels (25–27). However, note that the reduction in sample volume and improvement in precision may not scale directly with the increase in the strength of the absorbance caused by in part the quality of optical components currently available in the midinfrared region.

Another possible modification that can be implemented to our current system is to trap the peak of interest inside the optical cavity and perform repeated measurements over a longer time scale. This gain arises from the nondestructive nature of the CRDS measurement.

In conclusion, the GC-C-CRDS method presented here is a general approach for the determination of 13C/12C isotope ratios in any mixture of organic compounds that can be separated by gas chromatography. It is easy to imagine extending this approach to the isotopic ratio measurements of the combustion product of other species, such as D/H in water or 34S/32S in SO2. As long as oxygen is used for combustion, the determination of the oxygen isotopes of the compound of interest cannot be easily accomplished. Future directions also include interfacing other separation methods, such as liquid chromatography or electrophoresis, to the combustor or chemical oxidizer before optical analysis.

**Materials and Methods**

A Hewlett Packard 5890 Series II gas chromatograph (Agilent) was connected to a prototype CRDS instrument through a homebuilt combustion chamber for this study. Injections of a mixture of methane, ethane, and propane (all 99.5% or higher grade purity) were separated on a GC capillary column (Agilent J&W, HP-PLOT Q, 30 m × 0.53-mm i.d., 40-μm film thickness). The helium carrier gas linear velocity (ultra-high purity 99.999%) through the column was 30 cm/s. For mixtures, the GC oven temperature program was as follows: 40 °C for 3 min, then increased at a rate of 80 °C/min to 80 °C, and held there for 4 min. For individual samples of methane, ethane, and propane the temperature was held constant at 30 °C, 40 °C, and 80 °C, respectively. The injector temperature was set to 80 °C for all of the analyses.

The output of the column was connected with a stainless steel Tee-connector (Swagelok, 1/8 inch) to a nonporous alumina tube (McMaster-Carr, 0.125-inch o.d. × 0.040-inch i.d. dual bore, 20-inch length) held in a resistively heated furnace with a feedback temperature control (Extech Instruments) operating between 1,000 °C and 1,150 °C. One platinum and 3 nickel wires (Elemental Microanalysis, 0.125 mm) were inserted into each bore of the combustion tube, and the nickel wires were initially oxidized at an elevated temperature with oxygen passing over the wires (10). Vespel ferrules (SGE, GVF8–8) were used to seal the connections between the combustion tube and the connectors. The combustion tube was positioned so the ends of the tube were at least 5 cm from the furnace to reduce heating of the fittings on the end of the tube. A flow of 1 cm3/minute of oxygen (99.6% purity) was passed through the combustion tube, via the orthogonal port of the Tee-connector, during analysis runs to ensure the availability of excess oxygen supply for the complete oxidation of the sample and to regenerate the catalyst. All gases used were supplied by Praxair.

The combustion products from the oxidation reactor were fed directly into a prototype CRDS fast analyzer (Picarro), using a stainless steel Tee-connector (Swagelok, 1/4 inch). A flow of 10–20 cm3/min of either nitrogen (99.95% purity) or ~500 ppm carbon dioxide in nitrogen was concurrently fed into the instrument to help control the flow rate of the sample passing through the CRDS cavity.

The chromatograms from the instrument were analyzed using third party software (WaveMetrics Igor or Microsoft Excel). Details of the peak integration algorithm are discussed in Results and Discussion.

The values obtained through IRMS were referenced to a calibrated CO2 standard (Oztech Trading Corporation; δ13C ~ −40.86‰). The GC-C-CRDS individual component amounts are calibrated to have the same value for propane as for GC-C-IRMS. Also shown are the results from running a 75-μL mixture of approximately equal amounts of methane, ethane, and propane on the GC-C-IRMS. These results have not been calibrated with the measurements obtained using GC-C-IRMS. Owing to variations in the sample preparation process, differences in isotopic composition between samples run individually and as a mixture are expected. Errors represent 1 standard deviation based on 5 measurements in each case.

**Table 1. Comparison of δ13C values for methane, ethane, and propane run individually on the GC-C-IRMS (2.5-μL injection; split ratio 1:30) and the GC-C-CRDS (25 μL, splitless)**

<table>
<thead>
<tr>
<th>Compound</th>
<th>GC-C-IRMS δ13C, %e</th>
<th>GC-C-CRDS δ13C, %e from mixture</th>
<th>GC-C-CRDS δ13C, %e, individual components</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methane</td>
<td>−44.07 ± 0.46</td>
<td>−47.16 ± 0.68</td>
<td>−45.76 ± 0.61</td>
</tr>
<tr>
<td>Ethane</td>
<td>−37.68 ± 0.41</td>
<td>−39.63 ± 0.89</td>
<td>−36.15 ± 0.86</td>
</tr>
<tr>
<td>Propane</td>
<td>−39.57 ± 0.20</td>
<td>−39.57 ± 0.82</td>
<td>−38.75 ± 1.04</td>
</tr>
</tbody>
</table>