

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

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A continuous-flow cavity ring-down spectroscopy (CRDS) system integrating a chromatographic separation technique, a catalytic combustor, and an isotopic $^{13}\text{C}/^{12}\text{C}$ 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 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.

cavity ring-down spectroscopy | combustion | isotopic ratio

It is often taught in beginning chemistry classes that the atoms of an element all have the same chemistry. In one sense this statement is true in that all the atoms of a given element have the same valency, that is, make the same combinations with the atoms of other elements in forming compounds. In another sense, this statement is false in that different isotopes of an element have differing reaction rates leading to each compound possessing a different isotopic fractionation depending on its history of formation and transport. Consequently, high-precision isotope ratios have a special place in chemistry, especially in geochemistry, for determining reaction mechanisms and providing formation histories (1). Currently, the most commonly used instruments for determining high-precision isotope ratios are special-purpose isotope-ratio mass spectrometers (IRMS) (2, 3) that are fairly costly, require experts to operate and maintain, and are not portable—facts that have limited significantly the applicability of light stable isotope ratio measurements despite the technological advances achieved with online sample introduction and combustion for compound-specific isotope analysis (CSIA) (4).

Recently, optical spectroscopy-based instruments for measuring light stable isotope ratios have been developed that use the shift in the rovibrational absorption peaks that result from the different masses of the isotopomers (5, 6). The isotopes most commonly measured spectroscopically are ^{12}C and ^{13}C in carbon dioxide; however, it should be noted that the isotopes of hydrogen, nitrogen, oxygen, and sulfur have also been measured using optical spectroscopy techniques (5, 7). A salient advantage of using spectroscopy over IRMS is the ability to differentiate among isobars that are otherwise indistinguishable with mass spectrometry, caused by their identical m/z values. An example is the challenging measurement of the $^{34}\text{S}/^{32}\text{S}$ isotope ratio in SO_2 , using IRMS owing to the overlap in the masses of $^{34}\text{S}^{16}\text{O}^{16}\text{O}$ and $^{32}\text{S}^{18}\text{O}^{16}\text{O}$; however, these isotopomers can be readily distinguished using optical spectroscopy (7). Similar isobaric interferences are also encountered with doubly substituted isotopomer studies,

which proved to be very difficult to measure with IRMS (8) but can be analyzed spectroscopically.

We present a spectroscopic approach for making high-precision CSIA measurements of the $^{13}\text{C}/^{12}\text{C}$ isotope ratio of organic compounds, which is less expensive, does not require trained personnel, and is portable. The technique relies on the chromatographic separation of a mixture into individual organic compounds, the combustion of each organic compound into carbon dioxide, water, and other oxidation products, and the precise measurement of the $^{13}\text{C}/^{12}\text{C}$ isotope ratio in the carbon dioxide gas, using the absorption method of cavity ring-down spectroscopy. More specifically, each carbon atom in the compound of interest is converted to carbon dioxide, which allows the $^{13}\text{C}/^{12}\text{C}$ isotope ratio to be determined from the simple and well established infrared spectrum of carbon dioxide, using CRDS.

Short-chain hydrocarbons are used as our test compounds owing to their mud logging diagnostic significance (9) in exploratory and routine oil drilling and the suitability of our present instrumental setup for such a field measurement application. Although the $^{13}\text{C}/^{12}\text{C}$ isotope ratio could be directly measured in principle for small molecules, such as methane, this isotopic ratio becomes much more problematic to find from the parent hydrocarbon when it contains more than one type of carbon atom in the compound. This is another compelling reason why combustion followed by the spectroscopic determination of the $^{13}\text{C}/^{12}\text{C}$ isotope ratio in carbon dioxide makes this procedure so powerful and nearly universal.

Fig. 1 shows a schematic diagram of the apparatus we have used in our first studies of this new method. A gas mixture is injected into a gas chromatograph where it separates into its components based on retention time by differential interactions with the wall coating of the GC instrument. The effluent enters a catalytic combustor (C), containing 1 wire of platinum to every 3 wires of nickel of the same diameter, into which O_2 is fed by a mass flow controller (MFC). The combustor is maintained at a temperature of 1,150 °C for which complete oxidation has been 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 $^{13}\text{C}^{16}\text{O}^{16}\text{O}$ and the R (36) line of $^{12}\text{C}^{16}\text{O}^{16}\text{O}$ of the (3,0,1) – (0,0,0) combination band of carbon dioxide near 6,251 cm^{-1} at a fixed temperature and pressure from which the concentrations of $^{13}\text{C}^{16}\text{O}^{16}\text{O}$ and $^{12}\text{C}^{16}\text{O}^{16}\text{O}$ are measured and used to calculate the $^{13}\text{C}/^{12}\text{C}$ isotope ratio. We refer to this setup

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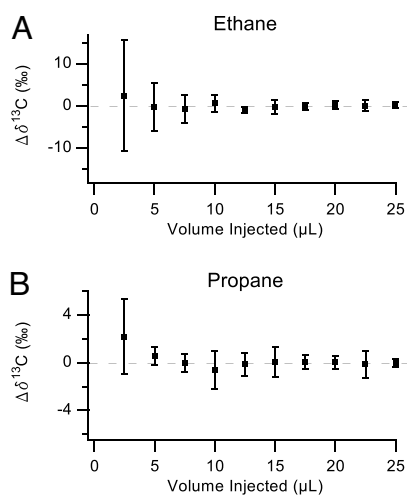


Fig. 3. Measured $\delta^{13}\text{C}$ values for ethane (A) and propane (B). The average of the measurements with volumes of 15 μL and greater is defined as zero. Error bars represent 1 SD, which have been calculated for the 5 measurements whose average is the data point shown.

$(R_{\text{sample}}/R_{\text{standard}} - 1) \times 1,000$ where R_{sample} is the $^{13}\text{C}/^{12}\text{C}$ isotope ratio of the sample and R_{standard} is 0.0112372, which is based on the standard Vienna PeeDee Belemnite value. Thus, 1 unit of $\delta^{13}\text{C}$ represents a change of ≈ 1 in the fifth decimal place of the $^{13}\text{C}/^{12}\text{C}$ isotope ratio. R_{sample} is calculated by dividing the integrated area of the $^{13}\text{C}^{16}\text{O}^{16}\text{O}$ peak by the integrated area of the $^{12}\text{C}^{16}\text{O}^{16}\text{O}$ peak from the chromatogram, in which we correct by the Boltzmann ratio of the 2 different ground-state energy levels. Fig. 3 shows the change in $\delta^{13}\text{C}$ values for different injection amounts of ethane and propane, respectively, in the GC-C-CRDS instrument. We conclude that a volume of 5 μL or larger yields highly reproducible $\delta^{13}\text{C}$ values of each compound. The precision of the 20 measurements with volumes of $>15 \mu\text{L}$ was 0.95‰ for ethane and 0.67‰ for propane.

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

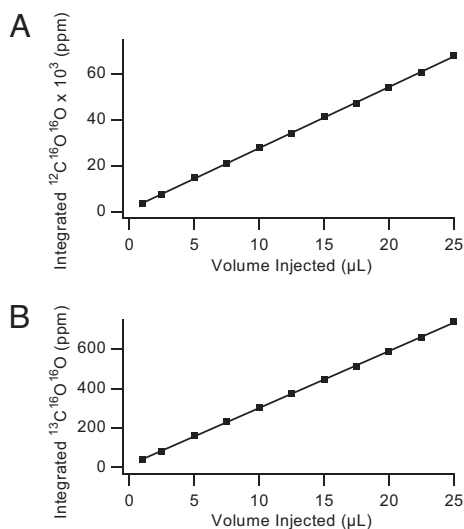


Fig. 4. Plot of the $^{12}\text{C}^{16}\text{O}^{16}\text{O}$ (A) and the $^{13}\text{C}^{16}\text{O}^{16}\text{O}$ (B) signals produced from the combustion of ethane as a function of volume injected. Each data point represents the average of 5 measurements, and the error bars (1 SD) are within the size of the marker.

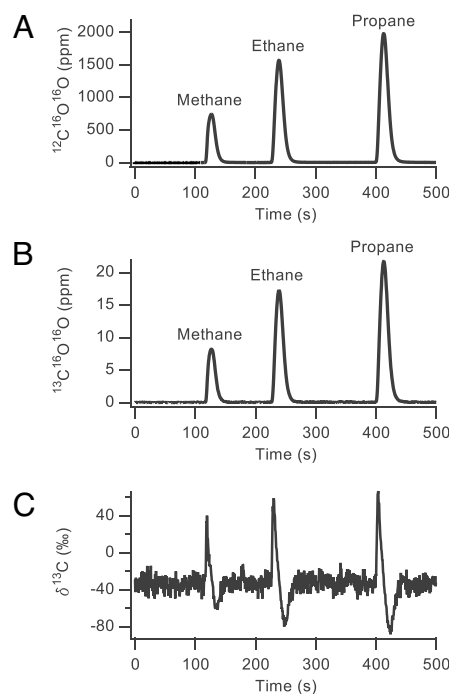


Fig. 5. Chromatographic traces of $^{12}\text{C}^{16}\text{O}^{16}\text{O}$ (A) and $^{13}\text{C}^{16}\text{O}^{16}\text{O}$ (B) from a 25- μL mixture of methane, ethane, and propane from which the $\delta^{13}\text{C}$ value is calculated for each component (C). The large amount of noise in the baseline 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 CO_2 allow for relatively more precise measurements that results in the $\delta^{13}\text{C}$ calculation to be made with a precision of $<1\%$.

of the injected amount of ethane. Please recall that 1 μL of ethane corresponds to $\approx 1 \mu\text{mol}$ of the compound.

We present in Fig. 5 A and B the ^{12}C and ^{13}C chromatographic traces from a mixture of methane, ethane, and propane in approximately equal proportions that was injected into the GC-C-CRDS instrument. In Fig. 5C, we show the experimentally derived $\delta^{13}\text{C}$ values. Please note the characteristic S-shape caused by what is known as the inverse isotope effect (20, 21) in which the ^{13}C isotopomer is not as retained as the ^{12}C isotopomer in the GC separation. This separation time shift is ~ 100 ms.

Table 1 shows the $\delta^{13}\text{C}$ measured from a separation of a 75- μL mixture consisting of approximately equal amounts of methane, ethane, and propane. Table 1 also compares the $\delta^{13}\text{C}$ values of individually analyzed gases obtained from our GC-C-CRDS instrument to those from a GC-C-IRMS instrument (University of California, Davis, Stable Isotope Facility, Davis, CA) referenced to the same Vienna PeeDee Belemnite standard. We observed systematic variations up to 5‰ in the measured isotopic ratios between the 2 techniques that is attributed to differing levels 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 “Individual Components” 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

Table 1. Comparison of $\delta^{13}\text{C}$ 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)

Compound	GC-C-IRMS $\delta^{13}\text{C}$, ‰, individual components	GC-C-CRDS $\delta^{13}\text{C}$, ‰, individual components	GC-C-CRDS $\delta^{13}\text{C}$, ‰, from mixture
Methane	-44.07 ± 0.46	-47.16 ± 0.68	-45.76 ± 0.61
Ethane	-37.68 ± 0.41	-39.63 ± 0.89	-36.15 ± 0.86
Propane	-39.57 ± 0.20	-39.57 ± 0.82	-38.75 ± 1.04

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-CRDS. 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.

future, we plan to calibrate our samples to 2 known standards of carbon dioxide with a wide bracketing range of $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ 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 $^{13}\text{C}/^{12}\text{C}$ isotope ratio measurements of individual organic species with a calibrated accuracy of $\approx 1\text{--}3\%$, which is presently inferior to GC-C-IRMS by a factor of $\approx 10\text{--}30$, 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 $\approx 1,000$ 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 $\approx 10^5$ 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 $^{13}\text{C}/^{12}\text{C}$ 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 isotope ratio measurements of the combustion product of

other species, such as D/H in water or $^{34}\text{S}/^{32}\text{S}$ in SO_2 . 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 \times 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 $^\circ\text{C}$ for 3 min, then increased at a rate of 80 $^\circ\text{C}/\text{min}$ to 80 $^\circ\text{C}$, and held there for 4 min. For individual samples of methane, ethane, and propane the temperature was held constant at 30 $^\circ\text{C}$, 40 $^\circ\text{C}$, and 80 $^\circ\text{C}$, respectively. The injector temperature was set to 80 $^\circ\text{C}$ 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. \times 0.040-inch i.d. dual bore, 20-inch length) held in a resistively heated furnace with a feedback temperature control (Extex Instruments) operating between 1,000 $^\circ\text{C}$ and 1,150 $^\circ\text{C}$. 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, GVFB-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 $\text{cm}^3/\text{minute}$ 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 cm^3/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 CO_2 standard (Oztech Trading Corporation; $\delta^{13}\text{C} = -40.86\%$).

1. Biegeleisen J (1965) Chemistry of Isotopes: Isotope chemistry has opened new areas of chemical physics, geochemistry, and molecular biology. *Science* 147:463–471.
2. Becker JS (2005) Recent developments in isotope analysis by advanced mass spectrometric techniques. *J Anal At Spectrom* 20:1173–1184.
3. Sessions AL (2006) Isotope-ratio detection for gas chromatography. *J Sep Sci* 29:1946–1961.
4. Matthews DE, Hayes JM (1978) Isotope-ratio-monitoring gas chromatography-mass spectrometry. *Anal Chem* 50:1465–1473.

5. Kerstel E (2004) in *Handbook of Stable Isotope Analytical Techniques*, ed de Groot PA (Elsevier, Amsterdam), Vol 1, pp 759–787.
6. Kerstel E, Gianfrani L (2008) Advances in laser-based isotope ratio measurements: Selected applications. *Appl Phys B* 92:439–449.
7. Christensen LE, et al. (2007) Measurement of sulfur isotope compositions by tunable laser spectroscopy of SO_2 . *Anal Chem* 79:9261–9268.
8. Eiler JM (2007) “Clumped-isotope” geochemistry—The study of naturally-occurring, multiply-substituted isotopologues. *Earth Planet Sci Lett* 262:309–327.

