

# Stable Isotope Ratios Using Cavity Ring-Down Spectroscopy: Determination of $^{13}\text{C}/^{12}\text{C}$ for Carbon Dioxide in Human Breath

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**We have constructed a cavity ring-down spectrometer employing a near-IR external cavity diode laser capable of measuring  $^{13}\text{C}/^{12}\text{C}$  isotopic ratios in  $\text{CO}_2$  in human breath. The system, which has a demonstrated minimum detectable absorption loss of  $3.2 \times 10^{-11} \text{ cm}^{-1} \text{ Hz}^{-1/2}$ , determines the isotopic ratio of  $^{13}\text{C}^{16}\text{O}^{16}\text{O}/^{12}\text{C}^{16}\text{O}^{16}\text{O}$  by measuring the intensities of rotationally resolved absorption features of each species. As in isotope ratio mass spectrometry (IRMS), the isotopic ratio of a sample is compared to that of a standard  $\text{CO}_2$  sample calibrated to the Pee Dee Belemnite scale and reported as the sample's  $\delta^{13}\text{C}$  value. Measurements of eight replicate  $\text{CO}_2$  samples standardized by IRMS and consisting of 5%  $\text{CO}_2$  in  $\text{N}_2$  at atmospheric pressure demonstrated a precision of 0.22‰ for the technique.  $\delta^{13}\text{C}$  values were also obtained for breath samples from individuals testing positive and negative for the presence of *Helicobacter pylori*, the leading cause of peptic ulcers in humans. This study demonstrates the ability of the instrument to obtain  $\delta^{13}\text{C}$  values in breath samples with sufficient precision to serve as a useful medical diagnostic.**

Stable isotope ratios of the lighter elements, particularly, C, H, N, O, and S, offer an outstanding means to trace various atmospheric phenomena, geological processes, and biochemical cycles. Isotope ratio mass spectrometry (IRMS) is the means of choice for accomplishing these measurements.<sup>1</sup> We report here an alternative approach for measuring isotope ratios that is based on the ultrasensitive absorption method of cavity ring-down spectroscopy (CRDS).<sup>2</sup> The instrumentation that is required for this optical method is more compact, less expensive, and more

portable than that for IRMS. At present, the precision is ~5 times less than that of commercially available single-detector IRMS instruments at their best, but it is anticipated that with further improvements CRDS should be able to rival and in some cases surpass the ability of IRMS to measure isotope ratios present in limited samples. In IRMS, the mass of an ion is typically measured at most once, whereas in CRDS, the isotope-dependent spectra can be measured repeatedly without destruction of the sample.

Elements can exist in both stable and unstable (radioactive) forms. Most elements of biological interest (including C, H, N, O, and S) have two or more stable isotopes, with the lightest of these being present in much greater abundance. Among stable isotopes, the most useful as a biological tracer is likely the heavy isotope of carbon,  $^{13}\text{C}$ . The light isotope of carbon,  $^{12}\text{C}$ , has a natural abundance of 98.89% whereas  $^{13}\text{C}$  has a natural abundance of 1.11%. About  $1 \times 10^{-10}$ % of carbon occurs as the unstable isotope  $^{14}\text{C}$ , and its presence can be neglected in determinations of carbon isotope ratios.

Carbon isotope ratios are usually expressed in parts per thousand (or per mil, ‰) relative to a standard, using the common notation

$$\delta^{13}\text{C} = \frac{R_{\text{sample}} - R_{\text{standard}}}{R_{\text{standard}}} \times 1000 \quad (1)$$

where

$$R = ^{13}\text{C}/^{12}\text{C} \quad (2)$$

is the ratio of the heavier to the lighter stable isotope of carbon. Isotopes of the same element take part in the same chemical reactions, but because the atoms of different isotopes have different atomic weights they react at different rates, causing fractionation in the isotope abundances. Physical processes such as evaporation discriminate against heavy isotopes; enzymatic

(1) Platzner, I. T. *Modern Isotope Ratio Mass Spectrometry*; John Wiley & Sons: Chichester, U.K., 1997.

(2) Busch, K. W.; Busch, M. A. *Cavity-Ringdown Spectroscopy: An Ultratrace Absorption Measurement Technique*; ACS Symposium Series 720; American Chemical Society: Washington, DC, 1999.

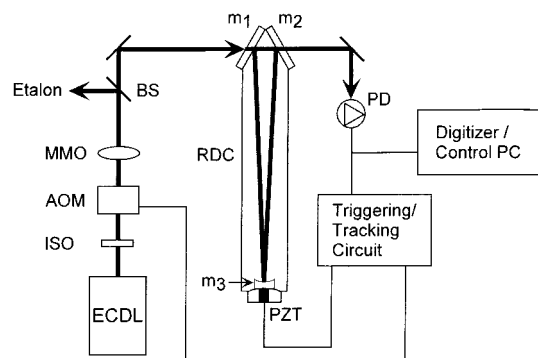


Figure 1. Schematic diagram of CRDS system. Abbreviations are as follows: ECDL, external cavity diode laser; ISO, optical isolator; AOM, acousto-optic modulator; MMO, mode-matching optics; BS, beam splitter;  $m_1$ ,  $m_2$ ,  $m_3$ , ring-down cavity mirrors; RDC, ring-down cavity; PZT, piezoelectric transducer; PD, photodiode.

discrimination and differences in kinetic characteristics and equilibria can result in reaction products that are isotopically heavier or lighter than their precursor materials. For example, the naturally occurring  $\delta^{13}\text{C}$  values for biologically interesting carbon compounds range from roughly 0‰ to  $\sim -110$ ‰ relative to the Pee Dee Belemnite (PDB) standard. The common reference for  $\delta^{13}\text{C}$  was obtained from a cretaceous marine fossil, *Belemnitella americana*, from the Pee Dee formation in South Carolina. This material has a higher  $^{13}\text{C}/^{12}\text{C}$  ratio than nearly all other natural carbon-based substances. For convenience, it is assigned a  $\delta^{13}\text{C}$  value of zero, giving almost all other naturally occurring samples negative  $\delta^{13}\text{C}$  values. All original supplies of PDB have been essentially exhausted and replaced by secondary carbonate standards calibrated against those prepared by the U.S. National Institute of Standards and Technology (NIST).<sup>3</sup>

We illustrate the power of the CRDS method for determining isotope ratios by examining  $1.0\text{ cm}^3$  of 5%  $\text{CO}_2$  in  $\text{N}_2$  in which the ratio of  $^{13}\text{CO}_2$  is compared to  $^{12}\text{CO}_2$  with a  $\delta$  value of  $-27.76\text{‰} \pm 0.22\text{‰}$ . The implications of being able to make such precise isotope ratio determinations is discussed in using this isotope ratio as a noninvasive breath test for the presence of *Helicobacter pylori*, the bacterium associated with nearly 80% of stomach ulcers.<sup>4</sup>

## EXPERIMENTAL SECTION

The design of the optical components of the ring-down spectrometer used to determine  $^{13}\text{C}/^{12}\text{C}$  isotope ratios, shown schematically in Figure 1, is similar to previous instruments.<sup>5,6</sup> Briefly, the output of a tunable external-cavity diode laser (New-Focus model 6330, 6.5 mW) passes through an isolator, preventing back reflections from reaching the diode laser. The beam is then focused into an acousto-optic modulator (AOM) capable of rapidly deflecting the beam from the optical cavity. Following the AOM, a beam splitter directs a small fraction of the laser radiation onto an etalon used to precisely measure the wavelength of the laser radiation. The remaining light passes through mode-matching

optics before coupling into a three-mirror high-finesse ring-down cavity (RDC). The RDC is a vacuum-tight monolithic design with two of the mirrors (plano/plano, 99.996% reflectivity) mounted directly onto a stainless steel support chamber. The third mirror (plano/concave 1.0-m radius of curvature,  $R = 99.996\%$ ), is mounted within the chamber on a metal diaphragm allowing the mirror's position to be adjusted with an externally mounted piezoelectric transducer (PZT). The total internal volume of the ring-down cavity is 11 mL, and the round trip path length of light within the optical cavity is 42 cm.

Light exiting the cavity through one of the high-reflectivity mirrors is focused onto a fast InGaAs photodetector. By scanning the length of the cavity, resonance between the laser and the RDC is periodically achieved, a condition indicated by a sudden increase in the intensity of light exiting the RDC through the cavity mirrors. This rapid increase in light intensity is used to trigger the AOM, deflecting the beam from the cavity, allowing the exponential decay of light within the RDC to be monitored. The ring-down signal generated by the photodetector is recorded using a 16-bit digitizer. This time-dependent signal intensity  $I(t)$  is fit to the exponential decay function:

$$I(t) = K + A \exp(-t/\tau) \quad (3)$$

to obtain the ring-down lifetime  $\tau$ . Losses within the cavity, including absorption losses, are inversely proportional to  $\tau$ .<sup>7</sup> By plotting  $1/\tau$  as a function of wavelength, an absorption spectrum is generated.

To increase data acquisition rates, a tracking circuit is used to control the voltage applied to the PZT. This circuit detects ring-down events and, after a short delay, reverses the motion of the PZT bringing the cavity and laser quickly back into resonance. With the tracking circuit engaged, ring-down decay waveforms can be generated at the photodetector at a rate of 1 kHz.<sup>8</sup>

The two absorption features chosen to measure the relative abundances of  $^{12}\text{C}^{16}\text{O}^{16}\text{O}$  and  $^{13}\text{C}^{16}\text{O}^{16}\text{O}$  arise from different rotational/vibrational transitions of the  $\text{CO}_2$  molecule (R(62) line of the  $^{12}\text{C}^{16}\text{O}^{16}\text{O}$   $(3,0^\circ,13) \leftarrow (0,0^\circ,1)$  band and R(30) line of the  $^{13}\text{C}^{16}\text{O}^{16}\text{O}$   $(3,0^\circ,12) \leftarrow (0,0^\circ,1)$  band). As a result, this ratio depends on temperature. To improve precision, the spectrometer is thermally regulated. The instrument itself is insulated from the room by a  $75\text{ cm} \times 60\text{ cm} \times 40\text{ cm}$  case. The temperature within the case is regulated by a pair of thermoelectric coolers controlled by a temperature regulator using feedback from a temperature sensor mounted onto the ring-down cavity. The standard deviation in the temperature of the RDC measured over a 12-day period was 0.002 K.

The ring-down cavity is evacuated using an oil-free membrane pump. The pressure within the ring-down cavity is monitored using a pressure transducer. The minimum pressure achieved in the RDC was  $\sim 1$  Torr. Absorption spectra were obtained with total cavity pressures of 5.0–70.0 Torr. Human breath samples were examined with a total RDC pressure of 70.0 Torr. Therefore, the total volume of the samples interrogated was 1.0 mL at STP.

(3) Verkouteren, R. M. *Anal. Chem.* **1999**, *71*, 4740–4746.

(4) *Helicobacter pylori: Fact Sheet for Health Care Providers*; Center for Disease Control: Atlanta, GA, 1998.

(5) Paldus, B. A.; Harb, C. C.; Spence, T. G.; Wilke, B.; Xie, J.; Harris, J. S.; Zare, R. N. *J. Appl. Phys.* **1998**, *83*, 3991–3997.

(6) Spence, T. G.; Harb, C. C.; Paldus, B. A.; Zare, R. N.; Willke, B.; Byer, R. L. *Rev. Sci. Instrum.* **2000**, *71*, 347–353.

(7) See: O'Keefe, A.; Deacon, D. A. G. *Rev. Sci. Instrum.* **1988**, *59*, 2544–2551. Zalicki, P.; Zare, R. N. *J. Chem. Phys.* **1995**, *102*, 2708–2717.

(8) Romanini, D.; Kachanov, A. A.; Stoeckel, F. *Chem. Phys. Lett.* **1997**, *270*, 538–545.

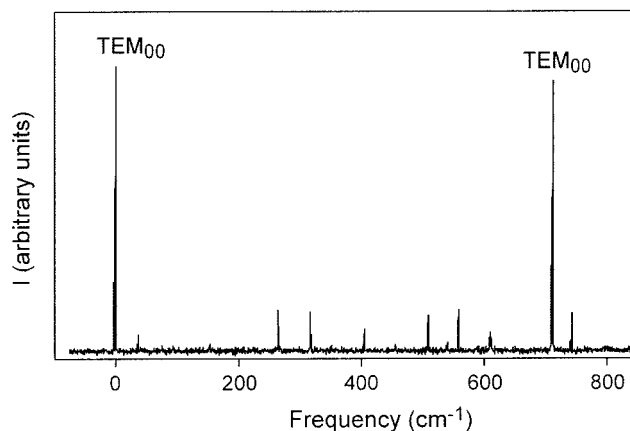


Figure 2. Intensity of light exiting the RDC as the resonant frequency of the RDC is varied by changing its length.

Before entering the RDC, gas samples pass through a coaxial drying tube containing a semipermeable membrane to reduce the amount of water present in the sample. Between samples, the drying tube and RDC were evacuated to 2 Torr and the drying tube was flushed briefly with room air and reevacuated. Following drying, a fraction of the newly introduced CO<sub>2</sub> sample was used to flush the RDC twice of any residual gases from the previous sample. Purge times were adjusted until no cross contamination was observed while consecutive standard samples having  $\delta$  values of  $-25\%$  and  $-50\%$  were run.

Carbon dioxide samples with known isotopic ratios of <sup>12</sup>C<sup>16</sup>O<sup>16</sup>O and <sup>13</sup>C<sup>16</sup>O<sup>16</sup>O were obtained through the addition of excess citric acid to sodium bicarbonate standard solutions (Metabolic Solutions, Inc.). For sensitivity measurements, CO<sub>2</sub> was diluted using a computer-controlled gas mixer (Enviroincs, Inc. Series 2000). Duplicate samples of the standard carbonate samples were prepared and <sup>12</sup>C<sup>16</sup>O<sup>16</sup>O/<sup>13</sup>C<sup>16</sup>O<sup>16</sup>O ratios characterized by an independent laboratory using isotope ratio mass spectrometry with a reported precision of 0.20%.

## RESULTS

For light to couple into the RDC, the RDC and the laser must achieve resonance; i.e., the light from the laser must be resonant with one of the optical modes of the RDC.<sup>9</sup> Figure 2 is a plot of the intensity of light escaping the RDC as the resonant frequency, i.e., length, of the RDC is scanned through approximately one free spectral range. Each peak in this spectrum results when light from the laser couples into an optical mode of the RDC. The most intense features arise from light coupling into the TEM<sub>00</sub> mode of the RDC. Higher-order transverse modes are also observed but they are well separated from the TEM<sub>00</sub> and as seen in Figure 2, the TEM<sub>00</sub> peak is  $\sim 10$  times more intense than the next most intense RDC mode. With judicious selection of trigger level, the instrument measures ring-down times only for light coupling into the TEM<sub>00</sub>. Owing to variations in frequency and beam shape within the cavity, each mode has a different ring-down lifetime. Single-mode excitation of the RDC removes variations in ring-down times observed when different modes of the cavity are alternately excited and reduces mode beating at the photodetector observed when multiple modes are simultaneously excited.

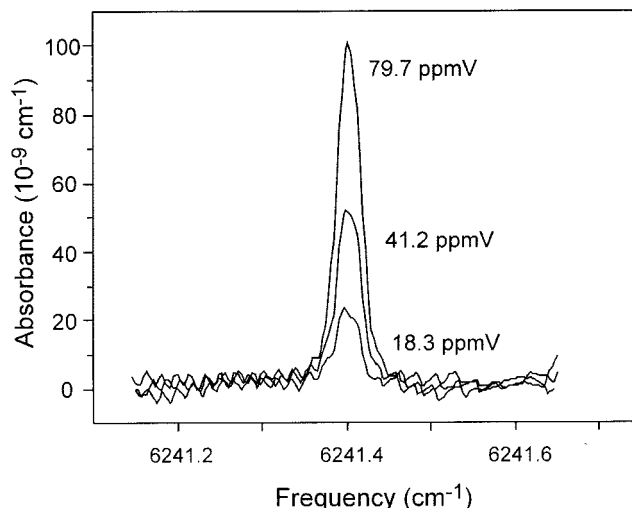


Figure 3. Absorption spectra of CO<sub>2</sub> in N<sub>2</sub>. Total pressure was maintained at 100 Torr; the CO<sub>2</sub> concentration is shown next to each absorption feature.

The ring-down lifetime of the evacuated RDS is  $\sim 18.5 \mu\text{s}$ . The standard deviation in  $\tau$  for 300 measurements is typically 0.005  $\mu\text{s}$  giving a relative error ( $\sigma_\tau/\tau$ ) of  $2.7 \times 10^{-4}$ . Figure 3 shows three absorption spectra of CO<sub>2</sub> in N<sub>2</sub> obtained with CO<sub>2</sub> concentrations of 79.7, 41.2, and 18.3 ppmv with a constant total pressure of 100 Torr. The periodic noise observed in the baseline regions of this scan arises from variations in the laser path inside the RDC caused by nonlinear tracking of the piezodrive. By comparing the noise on the baseline to the integrated intensity of the observed spectral features, the minimum detectable CO<sub>2</sub> concentration was determined to be 3 ppmv. Given the data-transfer-limited acquisition rate of 300 Hz, this limit indicates a minimum detectable absorption loss of  $3.2 \times 10^{-11} \text{ cm}^{-1} \text{ Hz}^{-1/2}$ .

Two absorption features in the near-infrared were selected to measure relative abundances of <sup>12</sup>CO<sub>2</sub> and <sup>13</sup>CO<sub>2</sub>. Figure 4a shows simulated absorption spectra of <sup>12</sup>C<sup>16</sup>O<sup>16</sup>O, <sup>13</sup>C<sup>16</sup>O<sup>16</sup>O, and <sup>12</sup>C<sup>16</sup>O<sup>18</sup>O at 5.0 Torr total pressure generated using the Hitran database along with spectroscopic assignments.<sup>10</sup> Figure 4b is an absorption spectrum of CO<sub>2</sub> generated from a bicarbonate standard at a total pressure of 5 Torr over the region of interest. The spectrum in Figure 4b is the result of a single scan of 2800 points with four measurements of  $\tau$  per point and was acquired in less than 2 min. The spectra were obtained at 5 Torr to decrease pressure-broadening effects, which has the benefit of allowing the identification of possible contaminants. The peaks at 6261.83 and 6262.25 cm<sup>-1</sup> attributed to absorption by <sup>13</sup>C<sup>16</sup>O<sup>16</sup>O and <sup>12</sup>C<sup>16</sup>O<sup>16</sup>O, respectively, were chosen to measure the relative abundance of the two carbon isotopes. These peaks are close to one another in frequency and, despite the disparity in relative concentrations of each form of carbon dioxide, have about the same intensity. The <sup>12</sup>C<sup>16</sup>O<sup>16</sup>O peak at 6261.65 cm<sup>-1</sup> was not used because it overlaps an absorption feature attributed to <sup>12</sup>C<sup>16</sup>O<sup>18</sup>O as seen in the simulated spectra. However, these peaks were recorded and fit when simulated breath samples were analyzed because at a total pressure of 70 Torr (the condition under which breath samples are analyzed) a small but significant overlap occurs between these

(9) Siegman, A. E. *Lasers*; University Science Books: Mill Valley, CA, 1986.

(10) Rothman, L. S.; Hawkins, R. L.; Wattson, R. B.; Gamache, R. R. *J. Quant. Spectrosc. Radiat. Transfer* **1992**, *48*, 469–507.

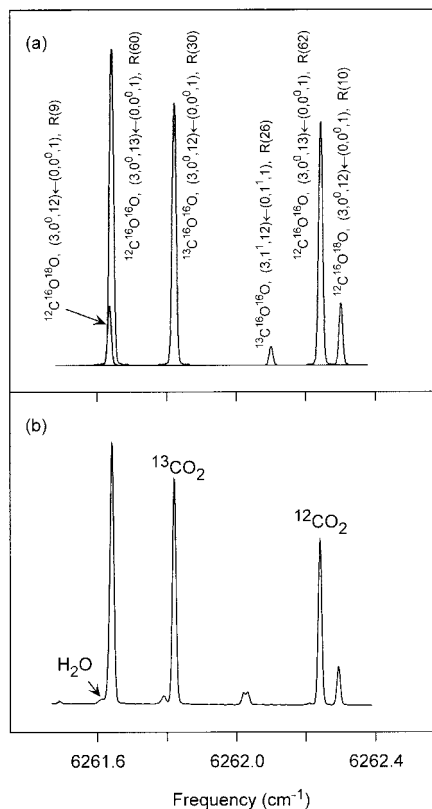


Figure 4. (a) Overlaid absorption spectra of <sup>12</sup>C<sup>16</sup>O<sup>16</sup>O, <sup>13</sup>C<sup>16</sup>O<sup>16</sup>O, and <sup>12</sup>C<sup>16</sup>O<sup>18</sup>O generated using the Hitran database with a total pressure of 5 Torr. (b) Absorption spectrum of CO<sub>2</sub> obtained via CRDS. The labeled peaks were used to determine the <sup>13</sup>C/<sup>12</sup>C isotopic ratio.

two peaks and the <sup>13</sup>C<sup>16</sup>O<sup>16</sup>O feature at 6261.83 cm<sup>-1</sup>. The small feature observed in Figure 4b at 6261.62 cm<sup>-1</sup> is attributed to absorption by H<sub>2</sub>O. This assignment is made based on the facts that the intensity of the peak does not scale with CO<sub>2</sub> concentration but increases as the amount of time the sample spends in the drying tube decreases.

Figure 5 shows a plot of total cavity losses as a function of wavelength around 6262.25 cm<sup>-1</sup> with a total pressure of 70 Torr in the RDC. The CO<sub>2</sub> absorption features have been fit to Galatry and Voigt profiles with fixed temperature using a nonlinear least-squares fitting algorithm. The Voigt profile assumes that collisional broadening can be simply treated as the convolution of Gaussian and Lorentzian line shapes, whereas the Galatry profile<sup>11</sup> includes Dicke narrowing effects, which are also called motional narrowing effects.<sup>12</sup> As indicated by the residuals, the Galatry function fits the data better than the Voigt profile, leading to a 3-fold reduction in uncertainty in determining the area under the peaks. Figure 6 shows a plot of total cavity losses as a function of wavelength with a simulated breath sample in the RDC. For this spectrum, the total pressure in the RDC was 70 Torr and the CO<sub>2</sub> partial pressure was 2.1 Torr. The peaks in the spectrum have again been fit to Galatry line shapes, and the residual of the fit is shown below the spectrum. The baseline of this spectrum provides a measure of zero absorption losses within the cavity. Using molar extinction coefficients provided by Hitran, the areas under the two peaks of

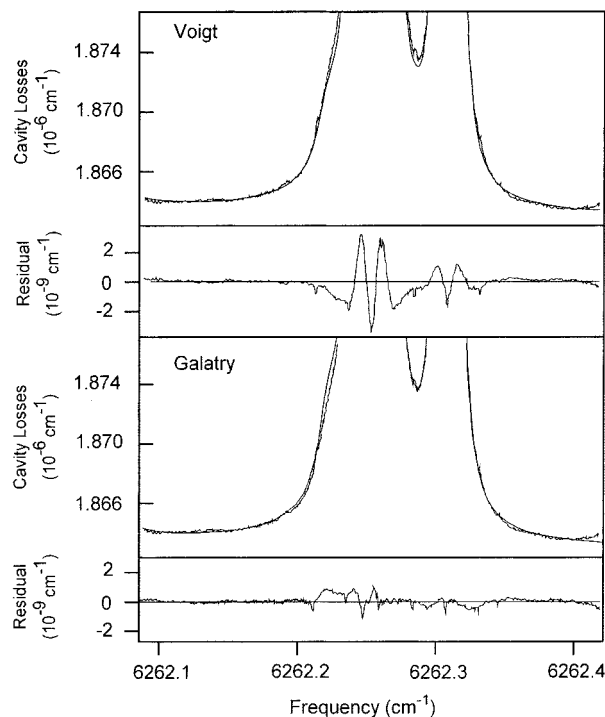


Figure 5. Absorption spectrum of 5% CO<sub>2</sub> in N<sub>2</sub> with a total pressure of 70 Torr in the RDC. Data were fit to Voigt and Galatry profiles. Residuals of each fit are also shown.

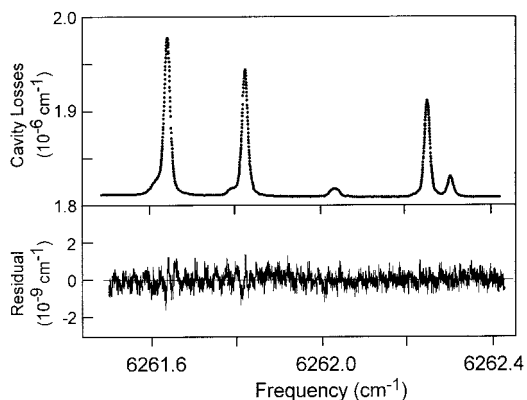


Figure 6. Absorption spectrum of CO<sub>2</sub> in N<sub>2</sub>. Total pressure in RDC is 70 Torr; CO<sub>2</sub> partial pressure is 2.1 Torr. Data points, best-fit line, and residuals are shown.

interest were used to measure directly the absolute concentrations of <sup>12</sup>C<sup>16</sup>O<sup>16</sup>O and <sup>13</sup>C<sup>16</sup>O<sup>16</sup>O. Unfortunately, the <sup>13</sup>C/<sup>12</sup>C ratio obtained from absorption spectra disagreed with the known relative concentrations of <sup>12</sup>C<sup>16</sup>O<sup>16</sup>O and <sup>13</sup>C<sup>16</sup>O<sup>16</sup>O in standard samples, indicating a relative error in the absorption line strengths of the two isotopes. However, as in IRMS, this systematic error can be eliminated by measuring the ratio of <sup>13</sup>C/<sup>12</sup>C in a sample relative to the <sup>13</sup>C/<sup>12</sup>C ratio of a standard reference material as mentioned above.

A CO<sub>2</sub> sample generated from a standard carbonate sample and characterized by IRMS was used as the reference standard. Determining δ<sup>13</sup>C for eight replicate samples of CO<sub>2</sub> with a large negative δ tested the precision of the instrument. The mean δ<sup>13</sup>C for these samples was found to be -27.76‰ with a standard deviation of 0.22‰. Table 1 shows δ<sup>13</sup>C values for nine duplicate samples having δ<sup>13</sup>C values ranging from approximately -20‰

(11) Galatry, L. *Phys. Rev.* **1961**, *122*, 1218–1223.

(12) Dicke, R. H. *Phys. Rev.* **1953**, *89*, 472–473.

Table 1.  $\delta^{13}\text{C}$  Values of Standard  $\text{CO}_2$  Samples Determined Using IRMS and CRDS

$\delta^{13}\text{C}$ (‰) by IRMS ( $\pm 0.2$ ‰)	$\delta^{13}\text{C}$ (‰) by CRDS ( $\pm 0.3$ ‰)
-20.49	-20.43
-14.91	-14.68
-9.38	-9.35
-4.19	-3.54
0.99	0.642
5.98	5.81
11.28	11.37
15.05	15.10
19.89	19.31

to +20‰ characterized by both IRMS and CRDS. As can be seen, excellent agreement exists between the two measurements. Linear regression analysis of  $\delta^{13}\text{C}$  (CRDS) versus  $\delta^{13}\text{C}$  (IRMS) data gives a slope of  $0.989 \pm 0.008$  and a  $y$ -intercept of  $7.47 \times 10^{-3}\text{‰} \pm 0.11\text{‰}$ .

It should be appreciated that a double-collector IRMS dedicated to isotopic ratio determinations of light gases has the capability of achieving an instrumental precision of 0.01‰ in the determination ( $n > 10$ ) of  $\delta^{13}\text{C}$  for carbon dioxide.<sup>13</sup> The use of such instruments, however, is not practical for routine isotope ratio measurements. Traditional single-collector IRMS instruments have a precision in the determination of  $\delta^{13}\text{C}$  of  $\sim 0.04\text{‰}$  under the most favorable conditions, with a typical precision of 0.2‰. Thus, the present CRDS instrument has a precision for the determination of  $\delta^{13}\text{C}$  that is about comparable to that of a single-collector IRMS. Further improvements in CRDS instrumentation, such as stabilization of the piezodrive, are anticipated to make CRDS determination of  $\delta^{13}\text{C}$  values comparable to or better than that of the best routine IRMS. It should be noted that the precision of the IRMS results obtained here from an independent laboratory was 0.2‰. It should also be noted that isotopic species that have nearly the same mass, such as  $^{13}\text{C}^{16}\text{O}^{17}\text{O}$  and  $^{12}\text{C}^{16}\text{O}^{18}\text{O}$ , are difficult to distinguish using IRMS techniques, whereas they are readily distinguished spectroscopically.

As a demonstration of the utility of the present CRDS instrumentation, we examined the determination of  $\delta^{13}\text{C}$  in human breath samples because this isotope ratio can serve as a noninvasive marker for various disease states. For example, breath tests based on  $\delta^{13}\text{C}$  were used to assess liver function and disease, gastric emptying, bacterial overgrowth, and pancreatic function. A large increase in interest for making breath tests, however, was the realization that such tests could be used to determine whether a patient was infected with the bacterium *H. pylori*,<sup>14</sup> which is the leading cause of ulcers. Most persons infected with *H. pylori* never suffer any symptoms related to the infection. It is estimated that about two-thirds of the world's population has *H. pylori*. It is also estimated that *H. pylori* accounts for more than 90% of duodenal ulcers and up to 80% of gastric ulcers.<sup>4</sup>

The presence of the bacterium *H. pylori* in humans can be detected through a well-established noninvasive breath test by having subjects ingest  $^{13}\text{C}$ -labeled urea.<sup>15</sup> The test is based on the absence of urease in the healthy human gastrointestinal tract. Urea ingested orally by a healthy human is therefore excreted unmodi-

Table 2. CRDS *Helicobacter Pylori* Breath Test Results for Known Positive (+) and Known Negative (-) Subjects

subject	$\delta^{13}\text{C}$ prior to ingestion (‰ $\pm 0.3$ ‰)	$\delta^{13}\text{C}$ following ingestion (‰ $\pm 0.3$ ‰)	DOB (‰ $\pm 0.4$ ‰)
A (-)	-21.5	-21.8	-0.3
B (-)	-23.7	-23.0	0.7
C (+)	-25.8	-10.4	15.4
D (+)	-23.5	29.6	53.1

fied. *H. pylori*, however, has a high urease activity, and converts urea ( $\text{CO}(\text{NH}_2)_2$ ) into  $\text{HCO}_3^-$  and  $\text{NH}_4^+$ . The  $\text{HCO}_3^-$  finds its way through the blood stream to the lungs and is exhaled as  $\text{CO}_2$ . Hence, individuals harboring *H. pylori* will exhibit an increase in the concentration of  $^{13}\text{C}$  in their breath following ingestion of the  $^{13}\text{C}$ -labeled urea. Results are reported as the difference over baseline (DOB) value, that is, the difference in the  $\delta^{13}\text{C}$  value over that of the baseline  $\delta^{13}\text{C}$  value, which is recorded before the consumption of the  $^{13}\text{C}$ -labeled urea. A DOB value less than 2–3 generally indicates the absence of *H. pylori* whereas a DOB value greater than 2–3 is strongly associated with the presence of *H. pylori*. Thus, the demand on the  $^{13}\text{C}/^{12}\text{C}$  ratio measurement is that the instrument has a precision in its  $\delta^{13}\text{C}$  determination of better than 1‰. This requirement is well met by the present CRDS instrumentation.

Four members of the research team, two of whom were known to test positive for the bacterium and two of whom tested negative, were assayed for the presence of *H. pylori* by measuring  $\delta^{13}\text{C}$  in samples of their breath using CRDS before and 30 min after consuming  $^{13}\text{C}$ -enriched urea. Table 2 lists the results of this study. The larger error in the breath tests (0.30‰) is caused by the slightly lower concentration of  $\text{CO}_2$  in the real breath samples than in simulated samples (2%–3% vs 5%, respectively). The precision of the CRDS instrument is clearly sufficient to identify the increase in  $\delta^{13}\text{C}$  observed for subjects known to carry *H. pylori*.

## CONCLUSION

We have demonstrated the use of an instrument employing the CRDS technique capable of measuring  $\delta^{13}\text{C}$  values for human breath samples to high precision. The instrument employs semiconductor laser technology and is in principle smaller and less expensive than its IRMS counterparts. The current instrument, which has a precision of 0.22‰ for a minimum of eight replicate samples, is sufficient to identify subjects harboring *H. pylori*. CRDS appears to be an appealing alternative to routine isotope ratio mass spectrometry for determining the ratio of stable isotopes present in limited quantities of permanent gases.

## ACKNOWLEDGMENT

Blueleaf Networks, T.G.S., and R.N.Z. gratefully acknowledge support from the Advanced Technology Program sponsored by National Institute of Standards and Technology. Blueleaf Networks also thanks DARPA and the National Institutes of Health for support.

Received for review January 8, 2002. Accepted February 18, 2002.

AC025511D

(13) Craig, H. *Geochim. Cosmochim. Acta* **1957**, *12*, 133–139.

(14) Marshall, B. J.; Warren, J. R. *Lancet* **1984**, 1311–1315.

(15) Klein, P. D.; Graham, D. Y. *Am. J. Gastroenterol.* **1993**, *88*, 1865–1869.