

# On the potential of Raman-spectroscopy-based carbonate mass spectrometry

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The potential for using Raman spectroscopy to measure stable oxygen isotope ratios ( $^{18}\text{O}/^{16}\text{O}$ ) in carbonates is evaluated by measuring the Raman spectra and isotope ratios of a suite of 60 synthesized,  $^{18}\text{O}$ -enriched calcite crystals ranging in composition from natural abundance (0.2 mole-%  $^{18}\text{O}$ ) to 1.2 mole-%  $^{18}\text{O}$ . We determined the Raman-inferred isotopic ratios ( $R_{\text{Raman}}$ ) by fitting curves to the  $\nu_1$  symmetric stretching peak at  $1086\text{ cm}^{-1}$  and the smaller satellite peak, associated with the  $\nu_1$  stretching mode of singly substituted carbonate groups ( $\text{C}^{16}\text{O}_2^{18}\text{O}$ ) at  $1065\text{ cm}^{-1}$ . The ratio of the two peak areas shows a 1:1 correspondence with the  $^{18}\text{O}/^{16}\text{O}$  ratios derived from standard mass spectrometry methods, confirming that the relative intensities of the  $\nu_1$  symmetric stretching peaks is a direct measure of the isotopic ratio in the carbonates. The 1-sigma uncertainties of the  $R_{\text{Raman}}$  values of the individual crystals were 0.00079 (384‰ PDB) and 0.00043 (210‰ PDB) for the four-crystal sample means. This level of uncertainty is much too high to provide significant estimates of natural variability; however, there are multiple prospects for improving the accuracy and precision of the technique. Carbon isotope ratios in carbonates cannot be measured by our approach, but our results highlight the potential of Raman-based isotope ratio measurement for C and other elements in minerals and organic compounds. Copyright © 2012 John Wiley & Sons, Ltd.

**Keywords:** carbonates; isotopes; mass spectrometry; oxygen; calcite

## Introduction

Raman spectroscopy can potentially be used as a non-destructive, high-resolution isotope-ratio mass spectrometer for a variety of substances, because differences in mass within a molecule can be observed as shifts in the intensity or position of peaks in Raman spectra. Such an approach has been utilized for carbon isotopic composition in  $\text{CO}_2$  fluid inclusions in minerals,<sup>[1]</sup> and pronounced shifts in the Raman spectra of organic molecules allow  $^{13}\text{C}$  to be used as a tracer of carbon uptake in individual microbes.<sup>[2]</sup> Here, we investigate the potential of Raman spectroscopy as a method to directly measure oxygen isotope ratios in carbonates. The Raman spectra of carbonates are typically characterized by a well-defined  $\nu_1$  symmetric stretching peak near  $1100\text{ cm}^{-1}$  ( $1086\text{ cm}^{-1}$  in calcite).<sup>[3,4]</sup> A second, much smaller peak is also observed around 20 wavenumbers lower ( $1065\text{ cm}^{-1}$  in calcite). This satellite peak is the  $\nu_1$  symmetric stretching peak associated with singly substituted carbonate groups ( $\text{C}^{16}\text{O}_2^{18}\text{O}$ ).<sup>[5,6]</sup> Furthermore, the intensity of the  $\text{C}^{16}\text{O}_2^{18}\text{O}$  peak relative to the  $\text{C}^{16}\text{O}_3$  peak is higher in synthetic carbonates grown in waters enriched in  $^{18}\text{O}$ .<sup>[6]</sup> For brevity, in this paper, we will refer to the  $\nu_1$  symmetric stretching peak of the unsubstituted carbonate groups ( $\text{C}^{16}\text{O}_3$ ) as the  $\nu_1^{16}\text{O}$  peak, and the  $\nu_1$  symmetric stretching peak of the singly substituted carbonate groups ( $\text{C}^{16}\text{O}_2^{18}\text{O}$ ) as the  $\nu_1^{18}\text{O}$  peak.

The relative amount of  $^{18}\text{O}$  in carbonate is a widely used indicator of geologic and environmental variability, both in modern and past systems. The relative amount is typically expressed as the per mil deviation of the  $^{18}\text{O}/^{16}\text{O}$  ratio from a standard (typically the Pee Dee Belemnite), or  $\delta^{18}\text{O}_{\text{VPDB}}$ . The  $\delta^{18}\text{O}$  of carbonates is often used to infer past climatic and hydrologic variability from a wide variety of materials and environments, including cave deposits (speleothems), corals, carbonate shells from marine and freshwater

organisms, and inorganic and organic carbonates deposited in lake sediments and soils. A Raman-spectroscopy-based carbonate mass spectrometer could allow for rapid, non-destructive analysis at extremely high resolution (micron-scale), and this would have abundant applications in environmental and geologic research. This study takes the first step towards this goal by determining the relationship between the intensities of the two Raman peaks and calcite  $^{18}\text{O}/^{16}\text{O}$ , the precision with which this ratio can be determined from Raman spectra, and the potential application of this Raman mass spectrometer to natural systems.

## Experimental procedure

### Calcite synthesis

To create a set of a calcites with a wide range of oxygen isotopic ratios, we synthesized 15 calcite samples in waters with oxygen isotope ratios ( $^{18}\text{O}/^{16}\text{O}$ ) ranging from 0.002 (natural) to 0.012. To generate the waters, we initially diluted 3 ml of 97%  $^{18}\text{O}$  water from Sigma-Aldrich with 172 ml of distilled water. After precipitation of the most-enriched calcite, the water was further diluted to produce the remaining samples.

To precipitate calcite from the enriched waters, 1–2 g of laboratory-grade calcite was dissolved in the waters while they

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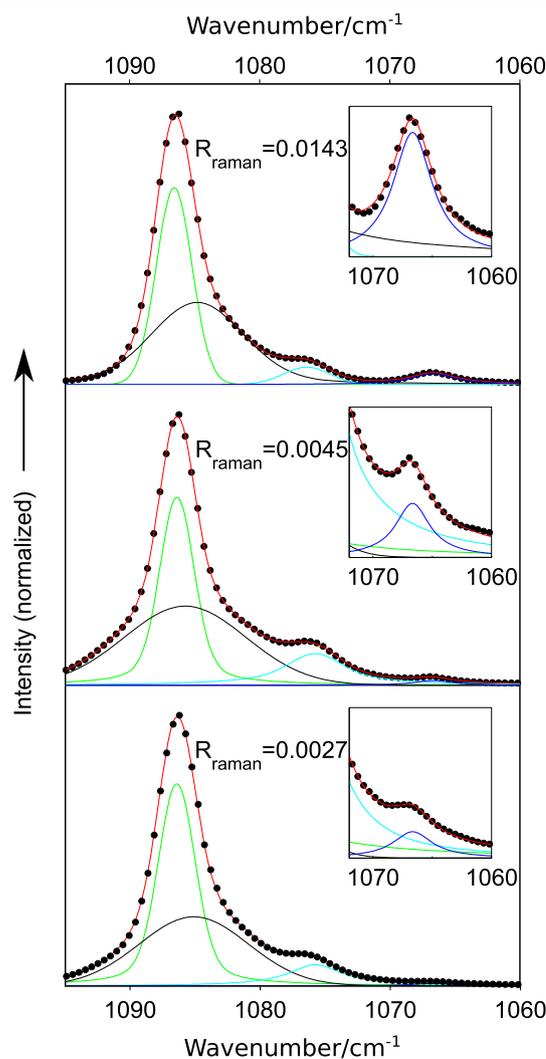
were bubbled with CO<sub>2</sub> for several hours. The waters were filtered and then capped and allowed to sit for two days to allow complete equilibration of the oxygen isotopes with HCO<sub>3</sub><sup>-</sup> in the water. Next, the water was bubbled with air for two days to drive off dissolved CO<sub>2</sub>, driving the precipitation of calcite. Finally, the waters were sonicated and filtered, and the filter was dried, while the remaining water was preserved for isotopic analysis, and further dilution and calcite synthesis. The synthesized calcites were generally euhedral crystals ranging from 25 to 50 μm in size.

### Raman spectroscopy

The Raman spectra of the synthesized calcites were collected on a Thermo Almega microRaman system, using a partially depolarized solid-state laser with an excitation wavelength of 780 nm at 100% power, and a thermoelectrically cooled CCD detector. The spectra were collected under 10× magnification, with 0.5 cm<sup>-1</sup> resolution and a 1-μm spot size, according to manufacturer specifications. For each sample, four individual calcite crystals were analyzed; Raman spectra were collected for 15 min on each crystal. To increase the signal-to-noise ratio (SNR), only crystals with one face oriented orthogonal to the laser were analyzed, beyond this constraint, crystal orientation was random. Raman peak positions and intensities were determined by fitting pseudoVoigt curves to the ν<sub>1</sub><sup>18</sup>O and ν<sub>1</sub><sup>16</sup>O peaks centered at near 1065 and 1086 cm<sup>-1</sup>, respectively, corresponding to the ν<sub>1</sub> symmetric stretching modes of the unsubstituted and singly substituted carbonate groups.<sup>[5,6]</sup> The ν<sub>1</sub><sup>16</sup>O peak, as measured by the Thermo Almega microRaman system, is asymmetric and required three pseudoVoigt curves centered on ~1086, ~1084, and ~1076 cm<sup>-1</sup>, to properly fit (Fig. 1). This asymmetry is not observed in carbonates measured on other Raman systems<sup>[5,6]</sup> and is likely due to incomplete polarization of the laser interacting with the orientation of the crystal. This, in addition to small crystal size, results in a broader peak than observed in other studies,<sup>[5,6]</sup> however, the implementation of three pseudoVoigt curves allows the peak to be fit very well (Fig. 1). The much smaller ν<sub>1</sub><sup>18</sup>O peak, centered near 1065 cm<sup>-1</sup>, can be fit with a single pseudoVoigt curve. Before fitting peaks to the spectra, the background was removed from each spectrum using the software package CrystalSleuth.<sup>[7]</sup> All four peaks in the ν<sub>1</sub> stretching region were then fit simultaneously using a weighted nonlinear least squares fitting algorithm in Matlab 7.12 (The Mathworks, Inc.; Fig. 1). The Raman-inferred <sup>18</sup>O/<sup>16</sup>O ratio (R<sup>Raman</sup>) is then calculated as

$$R_{Raman} = \frac{\int_{1000}^{1150} I(\nu_1^{18}O)}{\int_{1000}^{1150} \sum I(\nu_1^{16}O_{1,2,3})} * \frac{1}{3} \quad (1)$$

Where I(ν<sub>1</sub><sup>18</sup>O) are the intensities of the ν<sub>1</sub><sup>18</sup>O peak, and the intensities of the three ν<sub>1</sub><sup>16</sup>O peaks (ν<sub>1</sub><sup>16</sup>O<sub>1</sub>, ν<sub>1</sub><sup>16</sup>O<sub>2</sub>, ν<sub>1</sub><sup>16</sup>O<sub>3</sub>) are summed (I(ν<sub>1</sub><sup>16</sup>O<sub>1,2,3</sub>)) before numerically integrating using Matlab (Mathworks, Inc.). R<sub>Raman</sub> is one third of the integrated intensity ratio because only one of the three atoms in C<sup>16</sup>O<sub>2</sub><sup>18</sup>O is <sup>18</sup>O. To investigate the potential of using Raman spectra to infer changes in calcite <sup>13</sup>C/<sup>12</sup>C ratios, Raman spectra were also collected from a <sup>13</sup>C-enriched (97% <sup>13</sup>C) calcite and a natural-abundance reagent calcite (1.1% <sup>13</sup>C) calcite from J.T. Baker Chemical.



**Figure 1.** Examples of three measured Raman spectra in the ν<sub>1</sub> symmetric stretching mode region, and the associated pseudoVoigt curve fits for each (top: HC1, middle: HC4, bottom: HC11). For all panels: Raman measurements are shown as black circles, the green, black, and cyan curves show the fits to the three ν<sub>1</sub><sup>16</sup>O peaks, ν<sub>1</sub><sup>16</sup>O<sub>1</sub>, ν<sub>1</sub><sup>16</sup>O<sub>2</sub>, ν<sub>1</sub><sup>16</sup>O<sub>3</sub>, respectively; the dark blue curve shows the ν<sub>1</sub><sup>18</sup>O fit, and the red curve shows the sum of all four pseudoVoigt peaks. The Raman intensities for each sample are normalized to the maximum height of the ν<sub>1</sub><sup>16</sup>O peaks. The inset in each panel shows the ν<sub>1</sub><sup>18</sup>O region and associated curves with the Raman intensity scale amplified by a factor of 10 to better illustrate changes in ν<sub>1</sub><sup>18</sup>O peak area. The R<sub>Raman</sub> values calculated for each spectrum are shown in each panel. This figure is available in colour online at [wileyonlinelibrary.com/journal/jrs](http://wileyonlinelibrary.com/journal/jrs)

### Isotope notation

We use several notations common to isotopic studies in this paper, which we define here. R is the relative abundance of two isotopes, in this case <sup>18</sup>O/<sup>16</sup>O. Isotopic fractionation, α, is the fractional difference between the R of two substances (A and B), defined as:

$$\alpha_{A-B} = \frac{R_A}{R_B} \quad (2)$$

Finally, delta notation is used to express per mil (‰) differences from a standard, such that for oxygen:

$$\delta^{18}\text{O} = \left( \frac{R_{\text{sample}} - R_{\text{std}}}{R_{\text{std}}} \right) * 1000 \quad (3)$$

where  $R_{\text{std}}$  is the R of Vienna Standard Mean Ocean Water (VSMOW) or Vienna Standard Light Antarctic Precipitation (VSLAP) for waters and Vienna Pee Dee Belemnite (VPDB) or National Bureau of Standards (NBS) calcite standards 18 and 19 (NBS-18, NBS-19) for carbonates. Standards for calibration of both water (VSMOW, VSLAP) and calcite stable (NBS-19, NBS-18) isotope ratios were acquired from the International Atomic Energy Agency and the National Institute of Standards and Technology (USA). These standards have defined isotope ratios relative to either VSMOW for  $\delta^{18}\text{O}$  and VPDB for  $\delta^{13}\text{C}$ .

### Water mass spectrometry

To measure the oxygen isotope ratios in the waters used for calcite synthesis, we diluted the enriched waters with distilled water ( $-8.9\text{‰}$  VSMOW) in proportions resulting in a mixture with a predicted  $\delta^{18}\text{O}$  value within the calibrated range of the mass spectrometer (less than  $+50\text{‰}$ ). The diluted waters were then analyzed for  $\delta^{18}\text{O}$  using a dual inlet mass spectrometer (Delta-S, Thermo-Finnegan, Bremen, Germany) using an automated  $\text{CO}_2\text{-H}_2\text{O}$  equilibration unit. Standardization is based on internal standards referenced to VSMOW and VSLAP. Precision is better than  $\pm 0.08\text{‰}$  for  $\delta^{18}\text{O}$ . The resulting  $\delta^{18}\text{O}$  values along with the measured masses of the two waters were used to determine the oxygen isotope ratios of the waters used to synthesize calcite. Because the mass spectrometer has a linear response between the calibration points of  $-55.5\text{‰}$  (VSLAP) and  $0\text{‰}$

(VSMOW), we assume that the system maintains a linear response up to  $+50\text{‰}$  VSMOW.

### Carbonate mass spectrometry

The fractionation of oxygen isotopes during the precipitation of calcite is relatively well known ( $\alpha = 1.0288$  at  $25^\circ\text{C}$ ,<sup>[8]</sup> or  $\alpha = 1.0285$  at  $25^\circ\text{C}$ <sup>[9]</sup>), and uncertainties are small compared to the uncertainty associated with calculating the oxygen isotope ratio from Raman spectra. Given the low precision of the Raman-spectra-derived ratios, calculating the oxygen isotope composition of the precipitated calcites from the  $\delta^{18}\text{O}$  value of the waters should be sufficiently precise. To verify this assumption, we measured the  $\delta^{18}\text{O}$  of five of the synthesized carbonate samples. Samples HC14 and HC15 had oxygen isotope ratios approaching natural abundances and could be measured without dilution. The  $\delta^{18}\text{O}$  and  $\delta^{13}\text{C}$  of these carbonates were measured using an automated carbonate preparation device (KIEL-III) coupled to a gas-ratio mass spectrometer (Finnigan MAT 252). Powdered samples were reacted with dehydrated phosphoric acid under vacuum at  $70^\circ\text{C}$ . The isotope ratio measurement is calibrated based on repeated measurements of NBS-19 and NBS-18, and precision is  $\pm 0.10\text{‰}$  for  $\delta^{18}\text{O}$  and  $\pm 0.08\text{‰}$  for  $\delta^{13}\text{C}$  (1 sigma). Samples HC2, HC5, and HC8 were too enriched in  $^{18}\text{O}$  to be measured directly and were diluted with J.T. Baker calcite ( $\delta^{18}\text{O}_{\text{pdb}} = -15.65\text{‰}$ ) such that the resulting mixture would have a mean  $\delta^{18}\text{O}_{\text{pdb}}$  of  $\sim 20\text{‰}$ . To have precise dilutions, 10 mg of total calcite was reacted overnight in sealed glass tubes with dehydrated phosphoric acid at  $25^\circ\text{C}$ . The evolved  $\text{CO}_2$  gas was cleaned cryogenically and measured on a Finnigan Delta-S gas-ratio mass spectrometer. The oxygen isotope fractionation between calcite and acid-liberated  $\text{CO}_2$  was taken from Swart *et al.*<sup>[10]</sup>

**Table 1.** Water and calcite oxygen isotope data, measured by traditional mass spectrometry and Raman spectroscopy

Sample	Water and calcite mass spectrometry					Raman-based mass spectrometry				
	$R \times 10^3$		$\delta^{18}\text{O}_{\text{PDB}}$	$R \times 10^3$		$R_{\text{Raman}} \times 10^3$				Mean
$R_{\text{waters}}$	$R_{\text{calc-inf}}$	$R_{\text{calc-meas}}^*$		$\delta^{18}\text{O}_{\text{PDB}}$	$X_1$	$X_2$	$X_3$	$X_4$		
HC01	12.1	12.5	5042.4			14.3	12.8	13.5	12.7	13.3
HC02	8.56	8.81	3258.2	8.30	<i>3074.9</i>	8.19	9.16	9.33	9.26	8.99
HC03	5.37	5.53	1672.8			5.48	5.31	5.98	5.57	5.58
HC04	4.83	4.97	1406.1			4.51	5.26	5.23	4.95	4.99
HC05	3.75	3.86	868.2	3.56	<i>721.8</i>	3.60	2.22	4.40	3.99	3.55
HC06	3.26	3.36	623.7			5.41	6.49	3.55	2.88	4.58
HC07	2.79	2.87	386.9			2.87	1.23	2.82	2.93	2.46
HC08	2.67	2.75	329.2	2.71	<i>310.5</i>	3.06	1.48	3.61	3.64	2.95
HC09	2.42	2.49	206.3			3.05	2.11	2.94	2.78	2.72
HC10	2.35	2.42	169.6			3.76	2.34	3.32	3.50	3.23
HC11	2.23	2.29	110.2			2.69	2.12	2.08	2.67	2.39
HC12	2.14	2.20	66.4			2.34	2.43	2.41	2.17	2.34
HC13	2.12	2.18	53.2			3.31	2.65	1.14	2.99	2.52
HC14	2.07	2.13	29.6	2.13	32.0	1.47	2.94	1.80	1.63	1.96
HC15	2.02	2.08	4.7	2.08	6.6	2.07	2.61	2.96	2.98	2.66

note: All R values are shown as  $R \times 10^3$

note:  $R_{\text{calc-inf}}$  is the  $^{18}\text{O}/^{16}\text{O}$  ratio of the calcite inferred from water composition<sup>[9]</sup>

$R_{\text{calc-meas}}$  is the  $^{18}\text{O}/^{16}\text{O}$  ratio of the calcite measured by mass spectrometry.  $R_{\text{Raman}}$  values are shown for each of the four individual crystals measured, and their mean ratio.

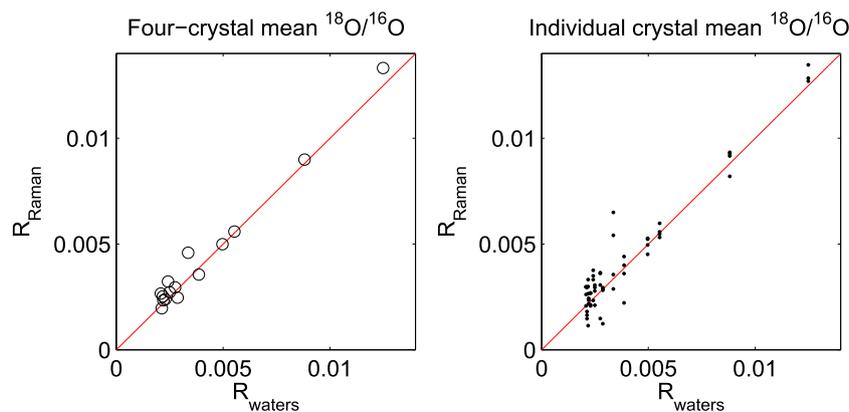
\* *Italicized values were measured offline using mass dilution, and are less certain*

## Results and discussion

The Raman-inferred, and mass-spectrometer-measured water and calcite  $^{18}\text{O}/^{16}\text{O}$  ratios and  $\delta^{18}\text{O}$  values for the 15 samples are presented Table 1. The five calcite samples measured on the mass spectrometer generally agree with the values predicted from the waters, within the uncertainty associated with the mass dilution analysis procedure. In samples HC14 and HC15, the  $\delta^{18}\text{O}$  values predicted from the waters underestimate those directly measured from the calcites by 2.4 and 1.9‰, respectively. These offsets may be associated with the extrapolation of the water and carbonate calibrations towards more enriched values, but may also be due to non-equilibrium  $^{18}\text{O}$  enrichment in the calcites associated with high dissolved  $[\text{Ca}^{2+}]$  during calcite synthesis.<sup>[9]</sup> Overall, the  $\delta^{18}\text{O}$  and  $R$  values inferred from the water and carbonate mass spectrometry appear to be sufficiently consistent for comparison with the less-certain Raman-inferred values. For consistency, in the rest of this paper, we compare the Raman-inferred isotope ratios ( $R_{\text{Raman}}$ ) with the calcite isotope ratios inferred from the water measurements ( $R_{\text{calc-inf}}$ ).

### Raman-inferred isotope ratios ( $R_{\text{Raman}}$ )

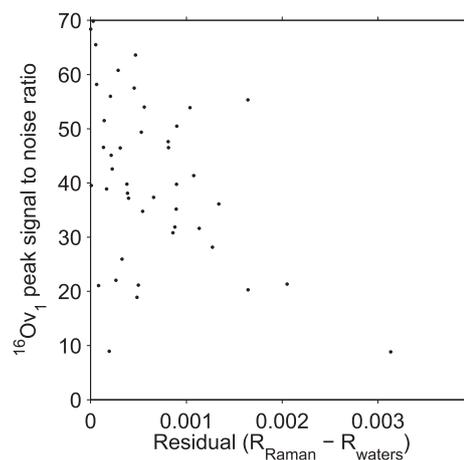
The  $R_{\text{Raman}}$  values as calculated by Eqn (1) are reasonable for both the individual crystals and sample means (Table 1). The variability  $R_{\text{Raman}}$  between crystals of the same batch displays a range in standard deviations from 0.00012 to 0.00166. The  $R_{\text{Raman}}$  values demonstrate a clear 1:1 relationship with the  $R_{\text{calc-inf}}$  values, especially when comparing the four-crystal mean  $R_{\text{Raman}}$  values (Fig. 2). The residuals between the  $R_{\text{Raman}}$  and  $R_{\text{calc-inf}}$  values are normally distributed, with a standard deviation of 0.00079 (corresponding to 384‰ PDB) for the individual crystals, and 0.00043 (210‰ PDB) for the four-crystal means values. This range of uncertainty is about an order of magnitude outside the range of natural variability, and therefore this approach is not yet sufficiently resolved for most geologic and environmental applications. However, it should be noted that this is an exploratory study, and a number of existing-technology refinements could be applied that would significantly improve the accuracy and precision of our approach. These will be discussed below.



**Figure 2.** Relation between Raman-inferred  $^{18}\text{O}/^{16}\text{O}$  ratios ( $R_{\text{Raman}}$ ) and the  $R$  values predicted from the  $^{18}\text{O}/^{16}\text{O}$  measured in the waters ( $R_{\text{calc-inf}}$ ), for both the four-crystal mean of each sample (left) and measurements of the individual crystals (right). For each plot, a 1:1 relationship is also shown.

### Potential sources of error and bias

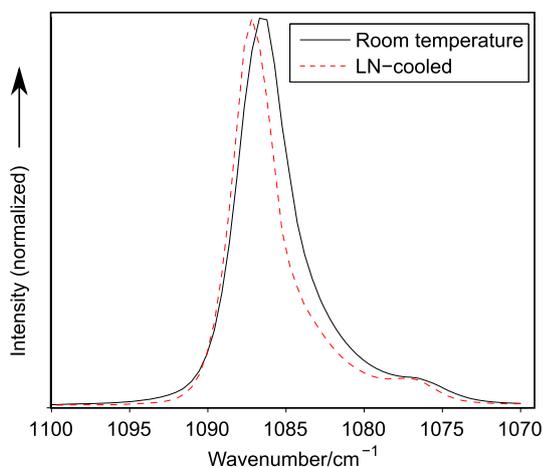
Because the  $\nu_1^{18}\text{O}$  peak is small, particularly at lower, closer-to-natural  $R$  values, it would be expected that the SNR in the  $\nu_1^{18}\text{O}$  peak would be a primary factor affecting the accuracy and precision of the  $R_{\text{Raman}}$  values. However, this does not appear to be the case in these samples. There are no significant relationships between the residuals and the  $\nu_1^{18}\text{O}$  peak to background ratio, the  $\nu_1^{18}\text{O}:\nu_1^{16}\text{O}$  ratios, or even the root-mean squared error of the fit. Additionally, the size and breadth of the third  $\nu_1^{16}\text{O}$  peak centered at  $1076\text{ cm}^{-1}$  ( $\nu_1^{16}\text{O}_3$ ) controls the separation between the  $\nu_1^{16}\text{O}$  and  $\nu_1^{18}\text{O}$  peaks, and in some cases, such as for spectra from HC6, which have the highest residuals of any samples, clearly affects the quality of the  $\nu_1^{18}\text{O}$  peak fit. Despite this, various metrics of the relative size and influence of the  $\nu_1^{16}\text{O}_3$  peak show no consistent relationship with the accuracy of  $R_{\text{Raman}}$ , either for sample means or individual crystals. The only metric of SNR that shows a clear relationship with the residuals, for the samples with small relative  $\nu_1^{18}\text{O}$  peaks ( $R < 0.005$ ), is the ratio between  $\nu_1^{16}\text{O}$  height and background (the height of the nearby baseline, between  $1020$  and  $1040\text{ cm}^{-1}$ , before adjustment by CrystalSleuth<sup>[7]</sup> (Fig. 3). This result suggests that the overall signal strength of the  $\nu_1$  symmetric stretching mode is a primary control on the precision and accuracy of the  $R_{\text{Raman}}$  estimates.



**Figure 3.** Relation between  $^{18}\text{O}/^{16}\text{O}$  residuals ( $R_{\text{Raman}} - R_{\text{calc-inf}}$ ) and the  $\nu_1^{16}\text{O}$  peak height to background ratio in the  $R_{\text{Raman}}$  spectra, for samples with  $R$  values  $< 0.005$ .

### Potential for improving the accuracy and precision of $R_{\text{Raman}}$ estimates

There is considerable potential for reducing the uncertainty of the  $R_{\text{Raman}}$  estimates and increasing the utility of Raman-spectroscopic carbonate mass spectrometry, even with existing techniques and technology. First, it should be emphasized that the Raman spectra collected here were preliminary and that little effort was made to screen crystals based on orientation or signal strength. Still, the fact that the Raman intensity ratios show a 1:1 relationship with the values acquired through standard mass spectrometric methods, with no apparent biases, is extremely encouraging for future applications of the technique. There are several simple ways to potentially reduce uncertainty in the estimates. The incomplete polarization of the microAlmega Raman system causes the  $\nu_1^{16}\text{O}$  peak to be split into three smaller peaks, including a small peak near  $1076\text{ cm}^{-1}$  that can influence the  $\nu_1^{18}\text{O}$  peak. Using a fully polarized laser that works in one transversal mode (TEM00) and only one longitudinal mode would suppress the depolarized scattered light and lower the background. This would likely reduce the overlap between the peaks resulting in a better fit. Second, the result that the  $\nu_1^{16}\text{O}$  peak height-to-background ratio affects the accuracy indicates that efforts to increase the overall SNR of the Raman spectra, even screening crystals by signal strength, would likely reduce uncertainty in  $R_{\text{Raman}}$ . Increasing the spectral resolution by focusing the CCD on a smaller area of the spectrum should increase the quality of the peak fit, and eliminate aliasing driven by the precise position of the peak.<sup>[1]</sup> Another approach could be resonance Raman (RR) scattering, which occurs when the wavelength of the exciting laser is chosen such that its energy corresponds to the electronic transition of specific atomic bonds within a molecule.<sup>[11]</sup> RR spectroscopy can greatly increase (by a factor of  $10^3$  to  $10^5$ ) the signal strength of a Raman spectrum,<sup>[11]</sup> and could potentially allow for much more precise determination of area of the  $\nu_1^{18}\text{O}$  peak. A simpler approach is immersing the sample in liquid nitrogen, which increases the separation of nearby peaks and increases the sharpness of the peaks (Fig. 4). The accuracy of  $R_{\text{Raman}}$  would likely improve with frequent comparison of Raman-inferred values to an internal standard during sample collection. The use of relative measurements in isotope ratio mass spectrometry

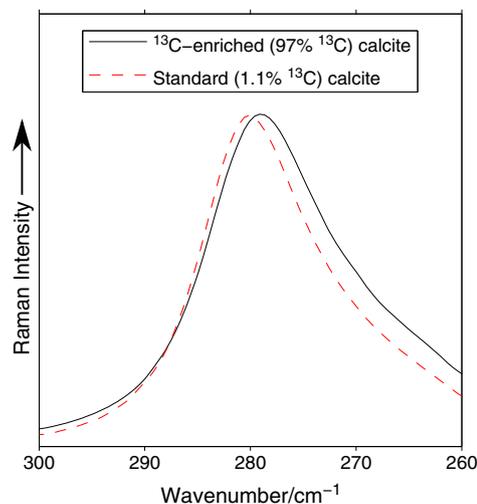


**Figure 4.** The  $\nu_1$  symmetric stretching mode region of Raman spectra of optical calcite before and after immersion in liquid nitrogen.

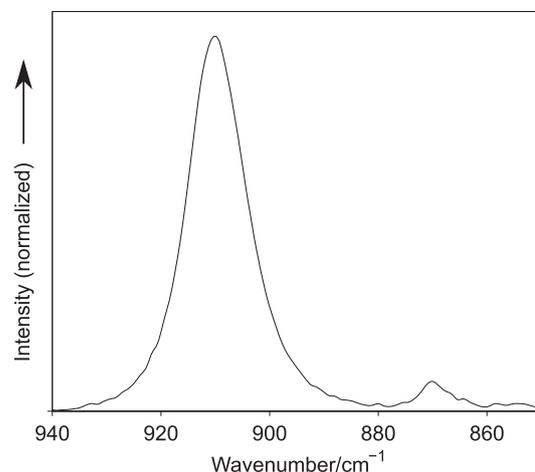
has allowed greatly improved precisions to be attained.<sup>[12]</sup> Relative deviations from a standard can be measured far more accurately than the determination of the absolute  $^{18}\text{O}/^{16}\text{O}$  ratio, as we did here.

### Carbon isotopes

The displacement of the carbon atom in the  $\nu_1$  symmetric stretching mode is not very large, so there is no significant shift of frequency in the  $1050\text{--}1100\text{ cm}^{-1}$  region between the normal and  $^{13}\text{C}$ -enriched calcites. However, the substituted C should be apparent in stretching modes where the C atom does undergo large motions, such as the  $\nu_{13}$  active translational mode near  $281\text{ cm}^{-1}$ . Here, the  $^{13}\text{C}$ -enriched calcite appears shifted to lower wavenumbers (centered near  $279\text{ cm}^{-1}$ ) (Fig. 5). This shift is consistent with theoretical expectation that the fractional change in position is a function



**Figure 5.** Raman spectra of  $^{13}\text{C}$ -enriched and standard calcite in the  $\nu_{13}$  active translational mode region.



**Figure 6.** Raman spectrum of the C–C stretching mode region in the calcium oxalate weddellite ( $\text{CaC}_2\text{O}_4 \cdot 2\text{H}_2\text{O}$ ). Based on its position (following Eqn (4)) and relative intensity, the satellite peak at  $\sim 870\text{ cm}^{-1}$  likely corresponds to the  $^{13}\text{C}\text{--}^{12}\text{C}$  bond.

of the square root of the changes in mass.<sup>[6]</sup> In this case for the  $\nu_{13}$  mode of  $\text{Ca}^{13}\text{CO}_3$ :

$$\nu_{13}/\nu_{13}^* = \sqrt{(m_{13c16o}/m_{12c16o})} = 0.9918 \quad (4)$$

corresponding to a theoretical  $\nu_{13}$  peak at  $279\text{ cm}^{-1}$ . Because the change in mass is small, and the peak is at low wavenumbers, small changes in the size of the  $\text{Ca}^{13}\text{CO}_3$   $\nu_{13}$  peak would be completely obscured by the  $\text{Ca}^{12}\text{CO}_3$  peak. This does not mean that the  $^{13}\text{C}/^{12}\text{C}$  ratios could not be measured in other minerals or molecules. For example, the C–C stretching mode in the calcium oxalate weddellite ( $\text{CaC}_2\text{O}_4 \cdot 2\text{H}_2\text{O}$ ) has a satellite peak at  $\sim 870\text{ cm}^{-1}$  (Fig. 6). The position (following Eqn (2)) and intensity of the peak suggest that the satellite peak is associated with a  $^{13}\text{C}$ – $^{12}\text{C}$  bond.

### Potential for quantifying isotope ratios in other molecules

Given that we are able to use Raman spectra to quantify differences in  $^{18}\text{O}/^{16}\text{O}$  ratios in calcite, but are not able to distinguish large changes in  $^{13}\text{C}/^{12}\text{C}$  ratios, it makes sense to generalize the conditions necessary to directly quantify isotope ratios from Raman spectra. There are three primary criteria necessary to effectively fit and quantify peaks in Raman spectra, and to relate those peaks to isotopic ratios in the compound of interest. First, to avoid the complication of the effect of sample orientation on relative intensities, the two peaks must be associated with the same vibrational mode. This constraint drives the second criterion: the shift in the wavenumber associated with the change in mass must be large enough so that the secondary peak is distinct and identifiable from the primary peak. Following Eqn (4), this is a function of both the relative change in mass in the molecule, and the position of the stretching peak, where larger changes in mass and vibrational modes at higher wavenumbers result in larger shifts. Finally, the rare isotope of interest must be sufficiently abundant that the associated peak is above the background intensity. Despite these constraints, it is likely that isotopic ratios in a wide variety of organic and inorganic compounds could be quantified from Raman spectra, with variable precision.

### Conclusions

We have synthesized a suite of  $^{18}\text{O}$ -enriched calcites and measured their  $^{18}\text{O}/^{16}\text{O}$  ratios with both traditional mass

spectrometry and through their Raman spectra. The Raman-inferred ratios show a clear 1:1 relationship with the ratio calculated from traditional mass spectrometry measurements, suggesting that oxygen isotope ratios can be measured directly in Raman spectra. The measurements of individual crystals were precise to within a ratio of 0.00079 (384‰) and the four-crystal means were precise to within 0.00043 (210‰). These uncertainties are too large to be useful in natural systems; however, this is a preliminary study, and the accuracy and precision could be greatly improved by increasing the SNR of the Raman spectra, using a spectrometer that maintains the polarization of the laser, and refining our curve-fitting techniques. Carbon isotope ratios in carbonates cannot be measured by our approach; however, our results do highlight the potential of Raman-based mass spectrometry for C and other elements in minerals and organic compounds.

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