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# A new online technique for the simultaneous measurement of the $\delta^{13}\text{C}$ value of dissolved inorganic carbon and the $\delta^{18}\text{O}$ value of water from a single solution sample using continuous-flow isotope ratio mass spectrometry

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**RATIONALE:** The oxygen isotope study of water reservoirs ( $\delta^{18}\text{O}_{\text{H}_2\text{O}}$  values) and the carbon isotope study of dissolved inorganic carbon ( $\delta^{13}\text{C}_{\text{DIC}}$  values) are powerful tools to decipher Earth's past and present environmental changes. This study presents a novel online analytical technique, namely the DIC evolved  $\text{CO}_2$  Gas Equilibration Method (DIC- $\text{CO}_2$ -GEM), in which the  $\delta^{18}\text{O}_{\text{H}_2\text{O}}$  and  $\delta^{13}\text{C}_{\text{DIC}}$  values can be simultaneously determined from a single solution sample.

**METHODS:** The DIC- $\text{CO}_2$ -GEM measures both  $\delta^{18}\text{O}_{\text{H}_2\text{O}}$  and  $\delta^{13}\text{C}_{\text{DIC}}$  values concurrently by combining the fundamental principles of the classic  $\text{CO}_2$ - $\text{H}_2\text{O}$  equilibration and gas evolution methods, respectively. Phosphoric acid is used to convert dissolved inorganic carbon in a solution sample of 0.2 mL into gaseous  $\text{CO}_2$ , which is then equilibrated with the solution at 25 °C. The oxygen and carbon isotope compositions are subsequently determined via continuous-flow isotope ratio mass spectrometry from the single solution sample.

**RESULTS:** Results obtained employing the DIC- $\text{CO}_2$ -GEM are in good agreement ( $<\pm 0.07\%$  for  $\delta^{18}\text{O}_{\text{H}_2\text{O}}$  values and  $<\pm 0.1\%$  for  $\delta^{13}\text{C}_{\text{DIC}}$  values) with those acquired using each of the traditional techniques. For both oxygen and carbon isotope measurements, an addition of 0.01 mL phosphoric acid yields the most consistent results between our technique and the traditional methods.

**CONCLUSIONS:** Devised to combine the traditional approaches independently assessing  $\delta^{18}\text{O}_{\text{H}_2\text{O}}$  and  $\delta^{13}\text{C}_{\text{DIC}}$  values, the DIC- $\text{CO}_2$ -GEM is less complex and highly efficient. It preserves the modern precision requirements for oxygen ( $\pm 0.09\%$ ) and carbon ( $\pm 0.18\%$ ) isotope analyses while accurately measuring both parameters simultaneously. This innovative method generates an abundance of data while minimizing resources and is suitable for a variety of practical applications. Copyright © 2014 John Wiley & Sons, Ltd.

The application of stable isotope analyses has greatly enhanced research outlining the mechanisms of Earth's processes both past and present. The oxygen isotope study of water reservoirs in the hydrosphere is important for understanding physicochemical processes in the water cycle, such as mixing, precipitation, evaporation, and condensation.<sup>[1–4]</sup> The carbon isotope study of dissolved inorganic carbon (DIC) is essential to investigate biogeochemical cycling of carbon since it has been shown to effectively trace carbon sources and sinks, such as carbon from the ocean or originated from the microbial respiration of organic matter.<sup>[5–8]</sup> As a result, significant efforts have been made by numerous scientists to improve the analytical techniques for the oxygen isotope composition of water and the carbon isotope composition of DIC by reducing sample sizes and/or increasing analytical precision (Tables 1 and 2).

For example, the oxygen isotope composition of water ( $\delta^{18}\text{O}_{\text{H}_2\text{O}}$  value) is typically measured using the  $\text{CO}_2$ - $\text{H}_2\text{O}$  equilibration technique, which was developed by Cohn and

Urey<sup>[9]</sup> in the late 1930s. This approach determines the oxygen isotope composition of  $\text{CO}_2$  via isotope ratio mass spectrometry (IRMS) after  $\text{CO}_2$  and  $\text{H}_2\text{O}$  are isotopically equilibrated at a constant temperature. Epstein and Mayeda<sup>[10]</sup> modified the Cohn and Urey's  $\text{CO}_2$ - $\text{H}_2\text{O}$  equilibration technique and successfully studied the oxygen isotope ratio of natural waters.

Several improvements have been made to this classic  $\text{CO}_2$ - $\text{H}_2\text{O}$  equilibration technique over the past 60 years, the most significant of which are (1) the use of pre-evacuated glass vials<sup>[11]</sup> that could be disposed of after use and (2) equilibration of a small quantity of  $\text{CO}_2$  with a surplus of water such that the oxygen isotope composition of  $\text{CO}_2$  is dictated by the oxygen isotope composition of the water being analyzed.<sup>(e.g. [12])</sup> Other approaches for the measurement of  $\delta^{18}\text{O}_{\text{H}_2\text{O}}$  values include (1) conversion techniques, where water is converted into  $\text{CO}_2$  by  $\text{BrF}_5$ <sup>[13]</sup> or guanidine hydrochloride<sup>[14]</sup>; (2) pyrolysis, where the oxygen in water is converted into CO at a high temperature<sup>[15]</sup>; and (3) electrolysis, where water is transformed into oxygen gas using an electrolyte (i.e.,  $\text{CuSO}_4$ )<sup>[16]</sup> (see Table 1 for details).

The carbon isotope composition of DIC ( $\delta^{13}\text{C}_{\text{DIC}}$  value) in aqueous solutions is often determined by either a direct precipitation method<sup>[17]</sup> or a gas evolution method.<sup>[18–21]</sup> The direct precipitation method involves the addition of a

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**Table 1.** Various methods of measuring  $\delta^{18}\text{O}$  value of water samples

References	Method	Principle of Method	Sample Size ( $\mu\text{L}$ )	Precision ( $\%$ , $1\sigma$ )
Epstein and Mayeda <sup>[10]</sup>	Equilibration	$\text{CO}_2\text{-H}_2\text{O}$ equilibration on custom glass extraction line	2500	0.1
O'Neil and Epstein <sup>[13]</sup>	Conversion	Using $\text{BrF}_5$ to convert $\text{H}_2\text{O}$ directly into $\text{CO}_2$	5-10	0.1-0.2
Dugan <i>et al.</i> <sup>[14]</sup>	Conversion	React $\text{H}_2\text{O}$ with guanidine hydrochloride to convert directly into $\text{CO}_2$	10	0.078
Brand <i>et al.</i> <sup>[15]</sup>	Conversion	Pyrolysis of $\text{H}_2\text{O}$ to $\text{CO}$ using CF-IRMS	2-10	0.2-0.3
Meijer and Li <sup>[16]</sup>	Electrolysis	$\text{CuSO}_4$ used as electrolyte to convert $\text{H}_2\text{O}$ into $\text{O}_2$	1000	0.1
Fessenden <i>et al.</i> <sup>[23]</sup>	Equilibration	$\text{CO}_2\text{-H}_2\text{O}$ equilibration using CF-IRMS	40-100	0.13

**Table 2.** Various methods of measuring  $\delta^{13}\text{C}$  value of dissolved inorganic carbon (DIC)

References	Method	Principle of Method	Sample Size (mL)	Precision ( $\%$ , $1\sigma$ )
Mook, <sup>[18]</sup> Kroopnick, <sup>[19]</sup> Games and Hayes <sup>[21]</sup>	Gas Evolution	Convert DIC to $\text{CO}_2$ by acidification with $\text{H}_3\text{PO}_4$ acid on vacuum line and analyze with dual inlet mass spectrometry	2-250	0.03-0.5
Gleason <i>et al.</i> <sup>[17]</sup>	Precipitation	Precipitate DIC as carbonate by adding $\text{SrCl}_2\text{-NH}_4\text{OH}$	1000	$\sim 0.1$
Torres <i>et al.</i> <sup>[20]</sup>	Gas Evolution	Convert DIC into $\text{CO}_2$ by acidification with $\text{H}_3\text{PO}_4$ acid online and analyze using CF-IRMS	0.03-0.5	0.04-0.15

$\text{SrCl}_2\text{-NH}_4\text{OH}$  or  $\text{BaCl}_2\text{-NH}_4\text{OH}$  solution directly to an aqueous sample, with subsequent instantaneous precipitation of  $\text{SrCO}_3$  or  $\text{BaCO}_3$ .<sup>(e.g.[17])</sup> This carbonate is then filtered and dried in preparation for measuring its carbon isotope composition, which reflects the  $\delta^{13}\text{C}_{\text{DIC}}$  value of the aqueous sample. The direct precipitation method requires large sample sizes, and is tedious and time consuming, involving the preparation of  $\text{SrCl}_2\text{-NH}_4\text{OH}$  or  $\text{BaCl}_2\text{-NH}_4\text{OH}$  solution, as well as filtering and drying the precipitated carbonates prior to isotopic analysis. In contrast, for the gas evolution method, the DIC in a water sample is converted into gaseous  $\text{CO}_2$  by acidification.<sup>(e.g.[22])</sup> This  $\text{CO}_2$  is then introduced into a mass spectrometer for measurement of its carbon isotope composition.

Most of the conventional offline extraction methods described above have been adapted to comply with a new generation of isotope ratio mass spectrometry (IRMS) approaches. For instance, the introduction of gas chromatography coupled with continuous-flow mass spectrometry (CF-IRMS) eliminates the cryogenic purification of  $\text{CO}_2$  from water on a glass vacuum line in the classic  $\text{CO}_2\text{-H}_2\text{O}$  equilibration technique.<sup>[23]</sup> In addition, a much smaller sample size is generally required for the measurement of  $\delta^{18}\text{O}_{\text{H}_2\text{O}}$  values, as well as  $\delta^{13}\text{C}_{\text{DIC}}$  values, in online CF-IRMS than in the conventional offline and dual-inlet IRMS methods.<sup>[20,23,24]</sup>

It should be noted that the oxygen isotope composition of water and the carbon isotope composition of DIC are measured separately in all the methods discussed above. There have also been a few attempts to develop a single method to obtain both isotopic compositions. Graber and Aharon<sup>[25]</sup> implemented an offline extraction technique that required a custom-made vacuum line and used 0.3 mL of 100% phosphoric acid to extract  $\text{CO}_2$  from a  $\sim 2$  mL DIC-containing water sample. They isolated

the liberated  $\text{CO}_2$  immediately, without equilibration with water, to determine the  $\delta^{13}\text{C}_{\text{DIC}}$  value via IRMS. In addition, they applied a correction factor of  $-1.10\%$  to the measured oxygen isotope composition of  $\text{CO}_2$  to calculate the  $\delta^{18}\text{O}_{\text{H}_2\text{O}}$  value. More recently, Yang and Jiang<sup>[26]</sup> utilized an online version of the classic  $\text{CO}_2\text{-H}_2\text{O}$  equilibration technique to determine the  $\delta^{13}\text{C}_{\text{DIC}}$  values as well as the  $\delta^{18}\text{O}_{\text{H}_2\text{O}}$  values. This was accomplished by measuring the pH, to calculate the mole fraction of carbonate ions in the solution, or  $x[\text{CO}_3^{2-}]$ , and the solution temperature, to estimate the carbon isotope fraction factor between gaseous  $\text{CO}_2$  and DIC at a given  $x[\text{CO}_3^{2-}]$ . This information was then used to calculate the  $\delta^{13}\text{C}_{\text{DIC}}$  values.

This study aims to establish a simple analytical method in which the  $\delta^{18}\text{O}_{\text{H}_2\text{O}}$  and  $\delta^{13}\text{C}_{\text{DIC}}$  values can be simultaneously determined from a single solution sample using CF-IRMS. In our DIC evolved  $\text{CO}_2$  Gas Equilibration Method, DIC- $\text{CO}_2$ -GEM, phosphoric acid ( $\text{H}_3\text{PO}_4$ ) is added to a DIC-containing aqueous sample to liberate  $\text{CO}_2$ , which then is equilibrated with the water sample itself at a constant temperature of  $25^\circ\text{C}$ . Upon completion of the isotopic equilibration between  $\text{CO}_2$  and water, the oxygen and carbon isotope compositions of the  $\text{CO}_2$  gas are measured by CF-IRMS.

## EXPERIMENTAL

### Preparation of DIC- $\text{CO}_2$ -GEM standard solutions

Standard solutions containing DIC are required to determine both the  $\delta^{18}\text{O}_{\text{H}_2\text{O}}$  and the  $\delta^{13}\text{C}_{\text{DIC}}$  values of sample solutions. Six standard solutions for the DIC- $\text{CO}_2$ -GEM were gravimetrically

prepared with ACS grade sodium bicarbonate or sodium carbonate to concentrations ranging from 5 to 10 mmolal (mmol/kg). Of the six DIC- $\text{CO}_2$ -GEM standard solutions, four were prepared with DIC-free deionized water ( $\kappa = \sim 0.6 \mu\text{S cm}^{-1}$  or  $\sim 18 \text{ M}\Omega \text{ cm}$ ), while isotopically light iceberg water from Newfoundland, Canada, was used for the other two standard solutions (Table 3). The deionized water and iceberg water were confirmed to be DIC-free, yielding no trace of  $\text{CO}_2$ , on the basis of the gas evolution method.<sup>(e.g.[20])</sup> The addition of carbonate salts at the concentrations tested in this study did not alter the oxygen isotope composition of the standard solutions. All the standard solutions were stored in sealed glass bottles.

In addition, two sets of secondary standard solutions of varying DIC concentrations (2, 5, 8, 10 and 15 mmolal) were prepared, one set with sodium bicarbonate ( $\text{NaHCO}_3$ ; pH  $\sim 8.28$ ) and the other set with sodium carbonate ( $\text{Na}_2\text{CO}_3$ ; pH  $\sim 10.87$ ). These secondary standard solutions were used not only to estimate the DIC concentration of sample solutions, but also to identify analytical limitations of the DIC- $\text{CO}_2$ -GEM, such as the accuracy of determining carbon isotope composition from various DIC concentrations and a range of pH values (Table 3).

### Stable isotope compositions of DIC- $\text{CO}_2$ -GEM standard solutions

#### Oxygen isotope composition: $\text{CO}_2$ - $\text{H}_2\text{O}$ equilibration technique

A modified version of the classic  $\text{CO}_2$ - $\text{H}_2\text{O}$  equilibration technique was employed to determine the oxygen isotope compositions of the DIC- $\text{CO}_2$ -GEM standard solutions using a Gas Bench II system (ThermoFinnigan, Bremen, Germany) with

a Thermo Finnigan Delta plus XP isotope ratio mass spectrometer at McMaster University (Hamilton, ON, Canada) (Table 3). Non-evacuated Labco RK borosilicate Exetainer<sup>®</sup> round-bottomed vials (Labco Ltd, Lampeter, UK), dimensions of 104 mm  $\times$  15.5 mm and volume of 12 mL with a screw cap and a septum, were used for  $\text{CO}_2$  equilibration with the standard solutions. The Exetainer<sup>®</sup> vials were cleaned by soaking in 5%  $\text{H}_3\text{PO}_4$  solution for 1–2 h after each use, and then rinsed three times with hot tap water, three times with reverse osmosis water, twice with deionized water, and finally dried at 70 °C overnight. The caps were cleaned using neutral pH soap, then also rinsed multiple times with deionized water, and dried at room temperature. Each screw cap was fitted with a new septum before each use.

In our modified  $\text{CO}_2$ - $\text{H}_2\text{O}$  equilibration technique at McMaster University, the Exetainer<sup>®</sup> vials were flushed and filled with a 0.2%  $\text{CO}_2$  and 99.8% He mixture. A 0.2 mL aliquot of the standard solution was subsequently injected using a 1 mL syringe, equilibrated with  $\text{CO}_2$  for a minimum of 27 h at  $25 \pm 0.1$  °C, and finally the isotopic composition of  $\text{CO}_2$  was determined by CF-IRMS. The oxygen isotope compositions of the DIC- $\text{CO}_2$ -GEM standard solutions were normalized using two laboratory water standards previously calibrated against V-SMOW and SLAP. The oxygen isotope compositions were reported on the V-SMOW scale.

#### Carbon isotope composition: Conventional McCrea and gas evolution technique

The carbon isotope compositions of DIC- $\text{CO}_2$ -GEM standard solutions were defined based upon the  $\delta^{13}\text{C}$  value of the  $\text{NaHCO}_3$  or  $\text{Na}_2\text{CO}_3$  salt used for the preparation of the

**Table 3.** Details of laboratory standards used in this study

Standard Name	Water source	$\delta^{18}\text{O}_{\text{H}_2\text{O}}$ values (‰)	1 $\sigma$	Carbonate source <sup>c</sup>	$\delta^{13}\text{C}_{\text{salt}}$ values (‰)	1 $\sigma$	DIC conc. (mmolal)
<i>Primary standards</i>							
MRSI-DIC-STD-1	Deionized water	-6.75	0.00	$\text{NaHCO}_3$ -A	-25.81	0.18 <sup>d</sup>	5
MRSI-DIC-STD-2	Deionized water	-6.74	0.01	$\text{NaHCO}_3$ -C	-2.56	0.07	5
MRSI-DIC-STD-3	Deionized water	-6.56	0.06	$\text{Na}_2\text{CO}_3$ -A	-1.99	0.13 <sup>d</sup>	10
MRSI-DIC-STD-4	Deionized water	-6.76	0.01	$\text{Na}_2\text{CO}_3$ -A	-1.99	0.13 <sup>d</sup>	6
MRSI-ICEBERG-1	Iceberg water <sup>b</sup>	-30.38	0.03	$\text{NaHCO}_3$ -A	-25.81	0.18 <sup>d</sup>	5
MRSI-ICEBERG-2	Iceberg water <sup>b</sup>	-30.38	0.03	$\text{Na}_2\text{CO}_3$ -A	-1.99	0.13 <sup>d</sup>	5
MRSI-STD-W1 <sup>a</sup>	Deep Sea water	-0.58	0.02	n/a	n/a	n/a	n/a
<i>Secondary standards</i>							
MRSI- $\text{NaHCO}_3$ -STD-2	Deionized water	-6.24	0.05	$\text{NaHCO}_3$ -A	-25.81	0.18 <sup>d</sup>	2
MRSI- $\text{NaHCO}_3$ -STD-5	Deionized water	-6.24	0.05	$\text{NaHCO}_3$ -A	-25.81	0.18 <sup>d</sup>	5
MRSI- $\text{NaHCO}_3$ -STD-8	Deionized water	-6.24	0.05	$\text{NaHCO}_3$ -A	-25.81	0.18 <sup>d</sup>	8
MRSI- $\text{NaHCO}_3$ -STD-10	Deionized water	-6.24	0.05	$\text{NaHCO}_3$ -A	-25.81	0.18 <sup>d</sup>	10
MRSI- $\text{NaHCO}_3$ -STD-15	Deionized water	-6.24	0.05	$\text{NaHCO}_3$ -A	-25.81	0.18 <sup>d</sup>	15
MRSI- $\text{Na}_2\text{CO}_3$ -STD-2	Deionized water	-6.24	0.05	$\text{Na}_2\text{CO}_3$ -A	-1.99	0.13 <sup>d</sup>	2
MRSI- $\text{Na}_2\text{CO}_3$ -STD-5	Deionized water	-6.24	0.05	$\text{Na}_2\text{CO}_3$ -A	-1.99	0.13 <sup>d</sup>	5
MRSI- $\text{Na}_2\text{CO}_3$ -STD-8	Deionized water	-6.24	0.05	$\text{Na}_2\text{CO}_3$ -A	-1.99	0.13 <sup>d</sup>	8
MRSI- $\text{Na}_2\text{CO}_3$ -STD-10	Deionized water	-6.24	0.05	$\text{Na}_2\text{CO}_3$ -A	-1.99	0.13 <sup>d</sup>	10
MRSI- $\text{Na}_2\text{CO}_3$ -STD-15	Deionized water	-6.24	0.05	$\text{Na}_2\text{CO}_3$ -A	-1.99	0.13 <sup>d</sup>	15

$\sigma$  = Standard deviation based on population (STDEVP).

conc. = Concentration.

<sup>a</sup>McMaster Research Group for Stable Isotopologues (MRSI) laboratory standard.

<sup>b</sup>Iceberg located off coast of Newfoundland, Canada.

<sup>c</sup>A and C indicate the specific salt added.

<sup>d</sup>Excludes 1 $\sigma$  outliers.

standard solutions. These carbonate salts were analyzed using a modified version of McCrea<sup>[27]</sup> at 90 °C, along with NBS 18 and NBS 19 for data normalization, on a VG-OPTIMA (Isoprime, Manchester, UK) isotope ratio mass spectrometer equipped with an automated IsoCarb system at McMaster University (Table 3). All the carbon isotope compositions were reported on the V-PDB scale. The gas evolution method was also used to obtain the carbon isotope compositions for all the DIC-CO<sub>2</sub>-GEM standard solutions, as well as sample solutions, used in this study. Exetainer<sup>®</sup> vials were preloaded with 0.2 mL 103% H<sub>3</sub>PO<sub>4</sub>, capped with a new septum and then flushed with 99.999% He. The 103% phosphoric acid was prepared from 85% phosphoric acid which was heated at 90 °C under vacuum for at least 3 days, until the density slightly exceeded 1.9 g/mL. The flushed vial was then injected with 0.2 mL of the standard solution using a 1 mL syringe. The H<sub>3</sub>PO<sub>4</sub> reacted with DIC in the standard solution to produce CO<sub>2</sub> for a minimum of 27 h at 25 ± 0.1 °C, followed by the isotopic analysis by CF-IRMS.

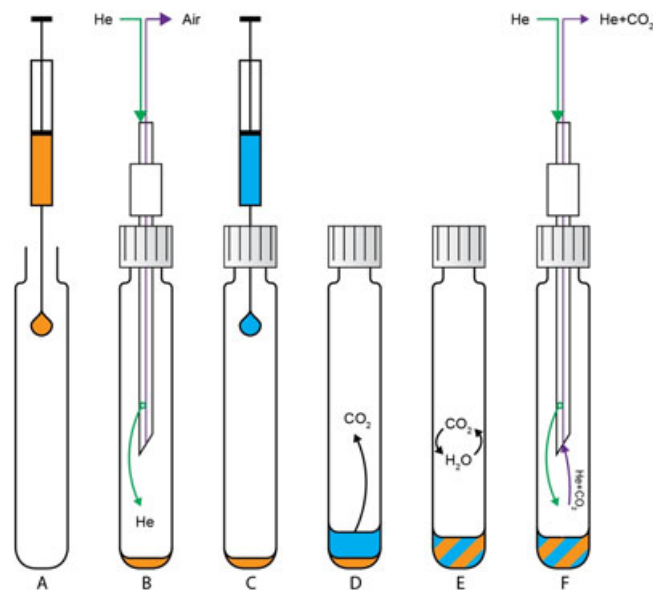
### Simultaneous measurement of $\delta^{18}\text{O}_{\text{H}_2\text{O}}$ and $\delta^{13}\text{C}_{\text{DIC}}$ values from the same aqueous solution: DIC evolved CO<sub>2</sub> gas equilibration method (DIC-CO<sub>2</sub>-GEM)

#### Overall description

This study combines the modified CO<sub>2</sub>-H<sub>2</sub>O equilibration technique and the gas evolution method to engineer the DIC evolved CO<sub>2</sub> Gas Equilibration Method (DIC-CO<sub>2</sub>-GEM), employing CF-IRMS to determine both  $\delta^{18}\text{O}_{\text{H}_2\text{O}}$  and  $\delta^{13}\text{C}_{\text{DIC}}$  values from a single solution sample. Using a 1 mL syringe, ~0.01 mL (1 drop = ~0.01 mL) of 103% H<sub>3</sub>PO<sub>4</sub> was loaded into Exetainer<sup>®</sup> vials which were then capped and flushed with 99.999% He. After being flushed with pure He gas, the Exetainer<sup>®</sup> vials were injected with a 0.2 mL DIC-containing solution sample using 1 mL syringes. The DIC is composed of four forms of inorganic carbon: aqueous carbon dioxide (CO<sub>2(aq)</sub>), carbonic acid (H<sub>2</sub>CO<sub>3</sub>), bicarbonate (HCO<sub>3</sub><sup>-</sup>), and carbonate (CO<sub>3</sub><sup>2-</sup>), with the dominant species being dictated by the pH of the solution. Upon addition of H<sub>3</sub>PO<sub>4</sub>, the DIC in the sample solution was converted into gaseous CO<sub>2</sub>. The evolved CO<sub>2</sub> gas in the headspace was subsequently equilibrated with water from the sample solution for a minimum of 27 h at 25 ± 0.1 °C prior to its isotopic analysis (Fig. 1). A constant reaction temperature (±0.1 °C) over the course of the experiment is essential for the precise determination of the  $\delta^{18}\text{O}_{\text{H}_2\text{O}}$  value.

#### Normalization of raw data: $\delta^{18}\text{O}_{\text{H}_2\text{O}}$ values

To normalize the raw oxygen isotope composition of the sample solution, a minimum of two DIC-CO<sub>2</sub>-GEM standard solutions prepared with deionized water and the two standard solutions of iceberg water origin were analyzed along with the sample solutions as outlined in the previous section. The previously determined true oxygen isotope compositions of the standard solutions, reported in Table 3, were used for normalization to calculate the oxygen isotope composition of a sample solution ( $\delta^{18}\text{O}_{\text{H}_2\text{O}}$ ) on the V-SMOW scale. It should also be noted that, at the beginning and end of each standard and sample solution set, duplicates of Exetainer<sup>®</sup> vials containing only a 0.2% CO<sub>2</sub> and 99.8% He mixture were analyzed to monitor the stability of the GasBench II CF-IRMS system. No significant shift in carbon or oxygen isotope compositions was observed.



**Figure 1.** Gas Bench II analysis using the DIC-CO<sub>2</sub>-GEM. (a) 12 mm Exetainer<sup>®</sup> vials are preloaded with 0.01 mL of H<sub>3</sub>PO<sub>4</sub>, capped and then (b) flushed and filled with helium using the double needle on the Gas Bench II. Once filled with helium, (c) a water sample is added to the vial, containing DIC with which (d) the H<sub>3</sub>PO<sub>4</sub> will react and produce CO<sub>2</sub> in the headspace. (e) A minimum of 27 h at 25 ± 0.1 °C is allotted for equilibration between CO<sub>2</sub> and H<sub>2</sub>O before (f) the headspace CO<sub>2</sub> is sampled on the Gas Bench II.

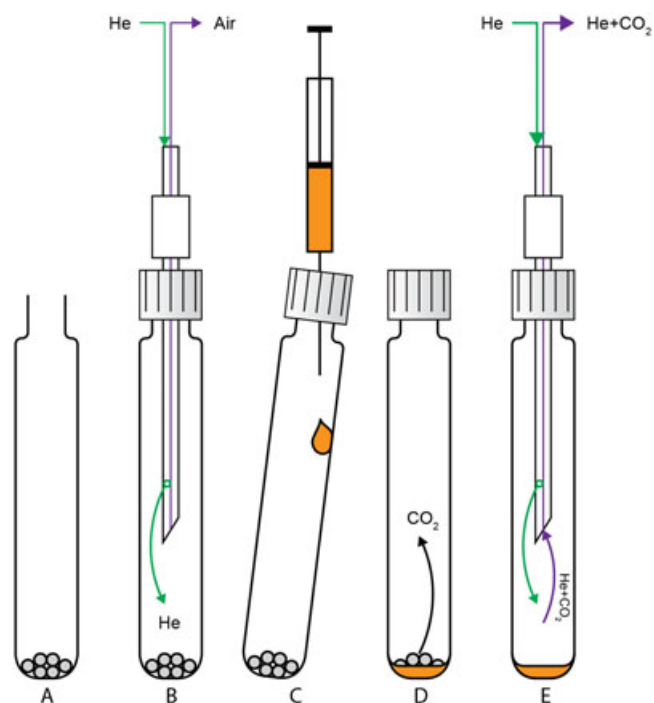
#### Normalization of raw data: $\delta^{13}\text{C}_{\text{DIC}}$ values

The raw carbon isotope compositions of the sample solutions were normalized to the defined  $\delta^{13}\text{C}$  values of the DIC-CO<sub>2</sub>-GEM standard solutions (Table 3), as well as those of two standard reference materials, NBS 18 (−5.04‰) and NBS 19 (+1.95‰), on the V-PDB scale. For each set of DIC-CO<sub>2</sub>-GEM sample sequences, along with the standard solutions, ~150 µg of NBS 18 and NBS 19 were preloaded into Exetainer<sup>®</sup> vials, capped, and flushed with 99.999% He. Upon completion of the flushing, ~0.1 mL (10 drops = ~0.1 mL) 103% H<sub>3</sub>PO<sub>4</sub> was injected into the side of the vial using a 1 mL syringe. The acid flowed down the side of the vial, reacted with the carbonate, and produced CO<sub>2</sub> for isotopic analysis (Fig. 2). The duplicate analyses of NBS 18 and NBS 19 at the beginning of each sequence were consistent with those at the end of each sequence, at which point 20–29 h had typically elapsed. The shifts observed in the oxygen isotope composition of carbonate as a function of reaction time with phosphoric acid in a previous study using a higher reaction temperature<sup>[28]</sup> were not found in this study at 25 °C.

## RESULTS AND DISCUSSION

### Influence of acid volume on oxygen isotope exchange kinetics

The oxygen isotope compositions,  $\delta^{18}\text{O}$  values, of MRSI-DIC-STD-1, MRSI-DIC-STD-2, and MRSI-DIC-STD-3 are −6.75‰, −6.74‰, and −6.56‰, respectively, using the conventional method (Table 4). However, they were determined to be −6.05‰, −5.98‰, and −8.23‰ when 0.2 mL of phosphoric



**Figure 2.** Running NBS-18 and NBS-19 standards on the Gas Bench II for the normalization of carbon isotope compositions of DIC. (a) 12 mm Exetainer<sup>®</sup> vials are preloaded with ~150 µg of carbonate then (b) flushed and filled with helium. (c) Using a 1 mL syringe, 20 drops of 100% H<sub>3</sub>PO<sub>4</sub> are added to the vial, tilting it slightly to ensure the acid flows down the glass side, thus extending the travel time and ensuring thermal equilibration of the phosphoric acid. (d) The flowing acid contacts and reacts with the carbonate, yielding CO<sub>2</sub> gas, which is then (e) analyzed following the Gas Bench II sampling procedure.

acid was used in an early stage of the development of the DIC-CO<sub>2</sub>-GEM. This unexpected deviation from the true oxygen isotope composition was therefore investigated by testing the effect of the amount of phosphoric acid on oxygen isotope exchange between CO<sub>2</sub> and water. For a simple interpretation of experimental data, two types of DIC-free water, deionized water from the McMaster Research Group for Stable Isotopologues (MRSI) and iceberg water from Newfoundland, Canada, were used in the test. The DIC-CO<sub>2</sub>-GEM was employed as described before except that the Exetainer<sup>®</sup> vials were flushed with a CO<sub>2</sub>-He gas mixture, instead of ultrapure He gas, because DIC-free water does not yield CO<sub>2</sub> in the reaction upon addition of phosphoric acid.

Figure 3 and Table 5 show that, as the volume of phosphoric acid decreases, the oxygen isotope composition of the deionized water-equilibrated CO<sub>2</sub> ( $\delta^{18}\text{O}_{\text{CO}_2\text{-H}_2\text{O}}$  value) diverges further from the initial oxygen isotope composition (−4.70‰) of the CO<sub>2</sub>-He gas mixture, illustrating faster oxygen isotope exchange kinetics between CO<sub>2</sub> and water. This trend also exists for the iceberg water, but is much less obvious because the equilibrium oxygen isotope composition (−3.43‰) of the iceberg-water-equilibrated CO<sub>2</sub> happened to be very close to the initial oxygen isotope composition of the CO<sub>2</sub>-He gas mixture. The potential effect of the isotopic signature of phosphoric acid on the  $\delta^{18}\text{O}_{\text{CO}_2\text{-H}_2\text{O}}$  value was

investigated by equilibrating only CO<sub>2</sub> with H<sub>3</sub>PO<sub>4</sub> and it was found to be negligible, confirming that the observed trend was not a result of the different volume of phosphoric acid used.

The oxygen isotope exchange reaction between gaseous and aqueous CO<sub>2</sub> is the rate-determining step in the hydration of CO<sub>2</sub>, with the rate being influenced by a variety of factors, such as the chemical composition and pH of the sample solution. Mills and Urey<sup>[29]</sup> alluded to the relationship between pH and oxygen isotope exchange in the CO<sub>2</sub>-H<sub>2</sub>O system. However, to the best of our knowledge, no prior study has acknowledged the implications of oxygen isotope exchange kinetics when the solution pH is significantly low. Our experimental data clearly demonstrates that lowering the pH of a sample solution by adding phosphoric acid into the CO<sub>2</sub>-H<sub>2</sub>O system slows down the oxygen isotope exchange kinetics between CO<sub>2</sub> and water. This is thought to be due to the conversion of the majority of DIC into gaseous CO<sub>2</sub>, leaving virtually no aqueous CO<sub>2</sub> to mediate oxygen isotope exchange between CO<sub>2</sub> and water in the solution. Therefore, our experimental finding suggests that CO<sub>2</sub>-H<sub>2</sub>O equilibration time can be reduced significantly when the acid volume used for the DIC-CO<sub>2</sub>-GEM is optimized.

#### Acid volume optimization for analytical reliability

##### Oxygen isotopes

In the DIC-CO<sub>2</sub>-GEM, the volume of phosphoric acid that was used to convert DIC into gaseous CO<sub>2</sub> proved to be an important parameter for the accuracy (deviation from the true value) and precision (reproducibility) of the  $\delta^{18}\text{O}_{\text{H}_2\text{O}}$  measurement. Table 4 shows that a decrease in the volume of phosphoric acid displays a higher analytical accuracy or a better agreement between the results obtained by the DIC-CO<sub>2</sub>-GEM and the modified CO<sub>2</sub>-H<sub>2</sub>O equilibration technique. For example, in the test using 0.2 mL of phosphoric acid for MRSI-DIC-STD-3, the difference in the  $\delta^{18}\text{O}_{\text{H}_2\text{O}}$  value between the two methods was 1.67‰. However, this difference was significantly reduced to 0.28‰ and 0.08‰ as the amount of phosphoric acid decreased to 0.05 and 0.01 mL, respectively. In addition, based upon repeated analyses of six DIC-CO<sub>2</sub>-GEM standard solutions, the overall precision (1 $\sigma$ ) of  $\delta^{18}\text{O}_{\text{H}_2\text{O}}$  measurement was enhanced from 0.29 to 0.09‰ as the volume of phosphoric acid was reduced from 0.2 to 0.01 mL. A similar improvement in analytical precision was observed from three sample solutions. As a result, 0.01 mL was chosen as an optimal volume of phosphoric acid for the measurement of  $\delta^{18}\text{O}_{\text{H}_2\text{O}}$  values in the DIC-CO<sub>2</sub>-GEM.

##### Carbon isotopes

The effect of phosphoric acid volume on the accuracy and precision of  $\delta^{13}\text{C}_{\text{DIC}}$  measurement was first investigated by analyzing six standard solutions with 0.2, 0.05, and 0.01 mL of phosphoric acid. In order to quantify the overall analytical accuracy, the  $\delta^{13}\text{C}_{\text{DIC}}$  values determined by the DIC-CO<sub>2</sub>-GEM using different volumes of phosphoric acid were compared with the previously determined true carbon isotope compositions of the corresponding standard solutions (Table 3). Unlike oxygen isotopes, no systematic relationship between analytical accuracy and the volume of phosphoric

**Table 4.** Testing various amounts of acid on the potential effects of measured oxygen and carbon isotope compositions

Name	DIC-CO <sub>2</sub> -GEM												Conventional Method							
	δ <sup>18</sup> O <sub>H<sub>2</sub>O</sub> values (‰)						δ <sup>13</sup> C <sub>DIC</sub> values (‰)						δ <sup>18</sup> O <sub>H<sub>2</sub>O</sub> values (‰) <sup>b</sup>	δ <sup>13</sup> C values (‰) <sup>c</sup>						
	Amount of H <sub>3</sub> PO <sub>4</sub> added (mL)						Amount of H <sub>3</sub> PO <sub>4</sub> added (mL)													
	0.2	1σ	n	0.05	1σ	n	0.01	1σ	n	0.2	1σ	n	0.05	1σ	n	0.01	1σ	n		
<i>Standards</i>																				
MRSI-DIC-STD-1	-6.05	0.38	7	-6.59	0.17	8	-6.74	0.12	17	-25.91	0.07	7	-26.14	0.07	8	-26.33	0.08	17	-6.75	-25.81
MRSI-DIC-STD-2	-5.98	0.30	7	-6.81	0.08	2	-6.78	0.18	14	-2.64	0.08	7	-2.57	0.06	2	-2.43	0.06	14	-6.74	-2.56
MRSI-DIC-STD-3	-8.23	0.09	7	-6.84	0.35	4	-6.64	0.05	10	-2.05	0.06	7	-2.04	0.05	4	-1.98	0.13	10	-6.56	-1.99
MRSI-DIC-STD-4	-	-	-	-6.75	0.05	4	-6.76	0.07	17	-	-	-	-2.46	0.03	4	-2.48	0.19	17	-6.76	-1.99
MRSI-ICEBERG-1	-29.57	0.06	4	-30.26	0.22	8	-30.37	0.06	17	-25.78	0.02	4	-25.34	0.12	8	-25.11	0.28	17	-30.38	-25.81
MRSI-ICEBERG-2	-31.24	0.04	2	-30.53	0.26	6	-30.39	0.09	17	-2.23	0.06	2	-2.37	0.13	6	-2.55	0.31	17	-30.38	-1.99
MRSI-STD-W1 <sup>a</sup>	-0.59	0.87	7	-0.60	0.07	6	-0.58	0.09	9	n/a	-	-	n/a	-	-	n/a	-	-	-0.58	n/a
Average		0.29			0.17			0.09			0.06			0.08			0.18			
<i>Samples</i>																				
#CKG-Sept2512-D2-S	-	-	-	-6.53	0.26	8	-6.51	0.04	8	-	-	-	-2.43	0.07	8	-2.41	0.17	8	-6.59	-2.37
#CKG-Oct2912-E-E	-	-	-	-6.44	0.22	8	-6.52	0.04	8	-	-	-	-18.23	0.06	8	-17.80	0.09	8	-6.49	-17.80
#CKG-Apr313-D-E	-	-	-	-6.53	0.18	8	-6.71	0.05	8	-	-	-	-20.48	0.06	8	-20.13	0.09	8	-6.73	-19.95
Average					0.22			0.04						0.06			0.12			

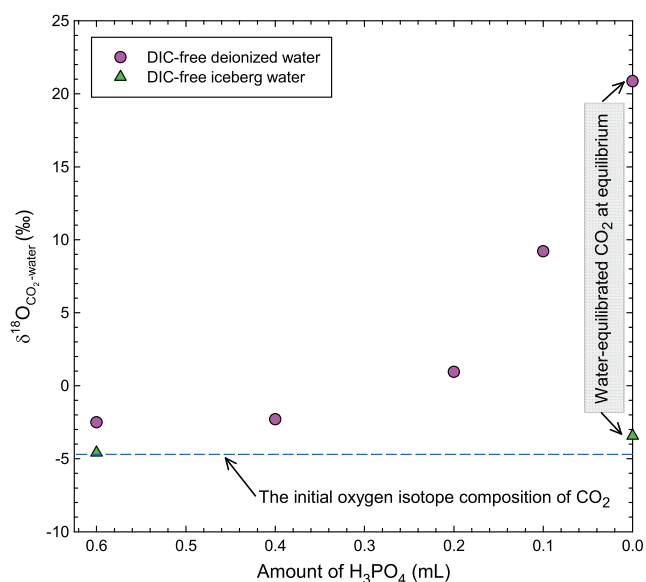
σ = Standard deviation based on population (STDEV).

n = Number of samples.

<sup>a</sup>McMaster Research Group for Stable Isotopologues (MRSI) laboratory standard.

<sup>b</sup>Determined using the modified CO<sub>2</sub>-H<sub>2</sub>O equilibration technique (see the text).

<sup>c</sup>For standard solutions determined via isotopic analysis of carbonate salt used, for sample solutions determined via the gas evolution method (see the text).



**Figure 3.** Effect of varying amounts of 103%  $\text{H}_3\text{PO}_4$  acid on the oxygen isotope composition of water-equilibrated headspace  $\text{CO}_2$  ( $\delta^{18}\text{O}_{\text{CO}_2\text{-H}_2\text{O}}$  values) for two types of DIC-free water. With decreasing acid amount, the oxygen isotope composition of water-equilibrated  $\text{CO}_2$  approaches its equilibrium  $\delta^{18}\text{O}_{\text{CO}_2\text{-H}_2\text{O}}$  value, showing an increase in the rate of oxygen isotope exchange between  $\text{CO}_2$  and  $\text{H}_2\text{O}$ . Note that  $\delta^{18}\text{O}_{\text{CO}_2\text{-H}_2\text{O}}$  values are reported relative to the oxygen isotope composition of a laboratory  $\text{CO}_2$  standard at McMaster University.

acid was detected. Nonetheless, a slightly larger discrepancy (i.e.,  $>0.2\text{‰}$ ), indicating lower analytical accuracy, was found from some standard solutions irrespective of the volume of phosphoric acid used. Given that analytical accuracy in  $\delta^{13}\text{C}_{\text{DIC}}$  measurement can also be influenced by the degree of  $\text{CO}_2$  degassing, which is often inevitable with prolonged sample storage, the observed low analytical accuracy might be attributed to potential  $\text{CO}_2$  degassing of some standard solutions. For this reason, additional tests were carried out with three solution samples using 0.05 and 0.01 mL of phosphoric acid (Table 4). In this test, the  $\delta^{13}\text{C}_{\text{DIC}}$  values of the samples were measured by the DIC- $\text{CO}_2$ -GEM within a maximum of 2 weeks after their  $\delta^{13}\text{C}_{\text{DIC}}$  values were defined on the basis of the gas evolution method. This practice was to minimize  $\text{CO}_2$  degassing of the sample solutions so that the

pure effect of phosphoric acid volume on  $\delta^{13}\text{C}_{\text{DIC}}$  values could be quantified. The best overall analytical accuracy was achieved when 0.01 mL of phosphoric acid was used. In the meantime, analytical precisions ( $1\sigma$ ) of 0.06 and 0.12‰ were estimated based upon  $\delta^{13}\text{C}_{\text{DIC}}$  measurements of the three solution samples when 0.05 and 0.01 mL of phosphoric acid were used, respectively (Table 4). These values are better than the 0.08 and 0.18‰ which were determined using the standard solutions for these same volumes. Higher analytical precisions from the analyses with the larger volume of phosphoric acid suggest less variability in DIC into gaseous  $\text{CO}_2$  conversion. After considering the experimental results for both  $\delta^{18}\text{O}_{\text{H}_2\text{O}}$  and  $\delta^{13}\text{C}_{\text{DIC}}$  values, the use of 0.01 mL phosphoric acid was adopted for the DIC- $\text{CO}_2$ -GEM.

### Comparison with conventional methods

#### *Oxygen isotopes: DIC- $\text{CO}_2$ -GEM vs the modified $\text{CO}_2$ - $\text{H}_2\text{O}$ equilibration technique*

Twelve solution samples of seawater-like ionic strength and various pH values were analyzed by the DIC- $\text{CO}_2$ -GEM, as well as by the modified  $\text{CO}_2$ - $\text{H}_2\text{O}$  equilibration technique, for the comparison of their respective oxygen isotope compositions ( $\delta^{18}\text{O}_{\text{H}_2\text{O}}$ ). Table 6 shows that the analytical precision of DIC- $\text{CO}_2$ -GEM estimated from this test was  $\pm 0.05\text{‰}$ . This short-term analytical precision was better than the overall DIC- $\text{CO}_2$ -GEM precision of  $\pm 0.09\text{‰}$ , which was determined by long-term analyses of DIC- $\text{CO}_2$ -GEM standard solutions (Table 4). More importantly, the oxygen isotope compositions obtained by the DIC- $\text{CO}_2$ -GEM were in good agreement with those determined by the modified  $\text{CO}_2$ - $\text{H}_2\text{O}$  equilibration technique, the most popular method for the determination of  $\delta^{18}\text{O}_{\text{H}_2\text{O}}$  values in stable isotope geochemistry (Table 6). The average absolute difference in  $\delta^{18}\text{O}_{\text{H}_2\text{O}}$  values between the two techniques turned out to be 0.07‰, with a maximum difference of 0.15‰. Although the reason for the large discrepancy for several samples is unclear, it does not appear to be related to the pH or DIC concentration of the aqueous solution tested. Considering its short-term analytical precision ( $\pm 0.05\text{‰}$ ) and the overall difference ( $\pm 0.07\text{‰}$ ) between the two techniques estimated by using six sample solutions (Table 6), the DIC- $\text{CO}_2$ -GEM is believed to be a viable alternative to the classical  $\text{CO}_2$ - $\text{H}_2\text{O}$  equilibration technique for most geochemical applications.

**Table 5.** Effect of phosphoric acid volume on oxygen isotope compositions

Sample Name	$\delta^{18}\text{O}_{\text{CO}_2}$ values (‰)														
	Amount of $\text{H}_3\text{PO}_4$ added (mL)														
	0.0	1 $\sigma$	<i>n</i>	0.1	1 $\sigma$	<i>n</i>	0.2	1 $\sigma$	<i>n</i>	0.4	1 $\sigma$	<i>n</i>	0.6	1 $\sigma$	<i>n</i>
0.2% $\text{CO}_2$ + 99.8% He	-4.70	0.13	2	—	—	—	-4.70	0.04	2	—	—	—	—	—	—
Deionized water	20.86	0.00	2	9.20	0.31	2	0.95	0.33	2	-2.31	0.02	2	-2.51	0.10	2
Iceberg water	-3.43	0.03	2	—	—	—	—	—	—	—	—	—	-4.59	0.04	2

$\sigma$  = Standard deviation based on population (STDEVP).  
*n* = Number of samples.

**Table 6.** Comparing the measured oxygen isotope compositions between the two different methods

Sample Name	DIC-CO <sub>2</sub> -GEM			CO <sub>2</sub> -H <sub>2</sub> O Equilibration			Difference <sup>a</sup> (‰)
	$\delta^{18}\text{O}_{\text{H}_2\text{O}}$ values (‰)	1 $\sigma$	<i>n</i>	$\delta^{18}\text{O}_{\text{H}_2\text{O}}$ values (‰)	1 $\sigma$	<i>n</i>	
#CKG-Aug312-D2-S	-6.75	0.03	3	-6.70	0.01	2	0.05
#CKG-Aug312-D2-E	-6.64	0.04	3	-6.79	0.05	2	0.15
#CKG-Aug3012-E-S	-6.68	0.03	2	-6.68	0.02	2	0.00
#CKG-Aug3012-E-E	-6.56	0.03	3	-6.59	0.02	2	0.03
#CKG-Sept2512-D2-S	-6.51	0.04	8	-6.59	0.03	2	0.08
#CKG-Sept2512-D2-E	-6.51	0.05	3	-6.48	0.01	2	0.03
#CKG-Oct2912-E-S	-6.67	0.03	3	-6.56	0.01	2	0.11
#CKG-Oct2912-E-E	-6.52	0.04	8	-6.49	0.00	2	0.03
#CKG-Apr313-D-S	-6.81	0.04	3	-6.71	0.00	2	0.10
#CKG-Apr313-D-E	-6.71	0.05	8	-6.73	0.02	2	0.02
#CKG-Apr313-E-S	-6.91	0.15	3	-6.76	0.00	2	0.15
#CKG-Apr313-E-E	-6.80	0.05	3	-6.70	0.07	2	0.10
Average		0.05			0.02		0.07

$\sigma$  = Standard deviation based on population (STDEVP).  
*n* = Number of samples.  
<sup>a</sup> Absolute difference between DIC-CO<sub>2</sub>-GEM and the modified CO<sub>2</sub>-H<sub>2</sub>O equilibration technique.

*Carbon isotopes: DIC-CO<sub>2</sub>-GEM vs the gas evolution method*

The sample set used for oxygen isotope analyses in the previous section was examined for their carbon isotope composition of DIC ( $\delta^{13}\text{C}_{\text{DIC}}$  values) to evaluate the analytical compatibility of the DIC-CO<sub>2</sub>-GEM with the conventional gas evolution method. In this evaluation, all the carbon isotope analyses completed using the two methods were carried out within 7 days in order to minimize any potential CO<sub>2</sub> degassing of the sample solutions. Subsequently, the  $\delta^{13}\text{C}_{\text{DIC}}$  values determined by the DIC-CO<sub>2</sub>-GEM were compared with those obtained from the gas evolution method. The estimated analytical precision based on the isotopic analyses of six solution samples in this evaluation was  $\pm 0.1\%$  which, as with the oxygen isotopes, was better than the long-term

analytical precision of  $\pm 0.18\%$  obtained from the DIC-CO<sub>2</sub>-GEM standard solutions (Tables 4 and 7). However, it should be noted that the difference between the two methods ranged from 0.0 to 0.22‰ with an average of 0.1‰. This varying discrepancy between the two methods is believed to have originated from incomplete (<100%) conversion of DIC in the solution into CO<sub>2</sub> gas, which is key, but an inevitable part of experimental design for the DIC-CO<sub>2</sub>-GEM. Although the gas evolution method still remains the method of choice for the highest precision and accuracy for  $\delta^{13}\text{C}_{\text{DIC}}$  measurement, given the reasonable analytical accuracy ( $\pm 0.1\%$ ) and precision ( $\pm 0.1\%$ ) estimated from this short-term evaluation, there is no doubt that the DIC-CO<sub>2</sub>-GEM can be useful for many applications in various DIC studies of carbon isotopes.

**Table 7.** Comparing the measured carbon isotope compositions between the two different methods

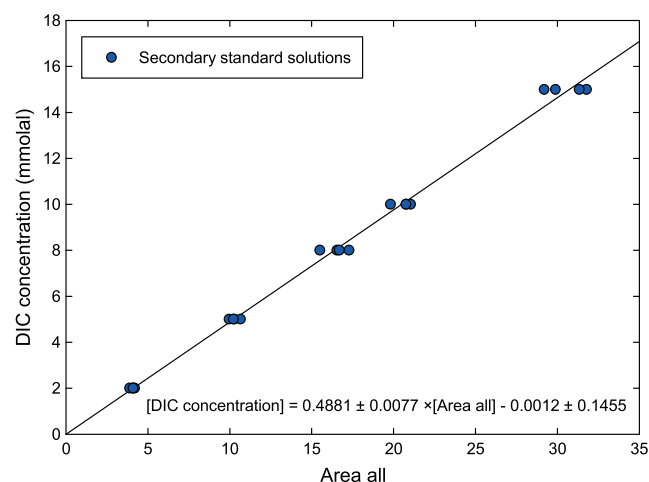
Sample Name	DIC-CO <sub>2</sub> -GEM			Gas Evolution			Difference <sup>a</sup> (‰)
	$\delta^{13}\text{C}_{\text{DIC}}$ values (‰)	1 $\sigma$	<i>n</i>	$\delta^{13}\text{C}_{\text{DIC}}$ values (‰)	1 $\sigma$	<i>n</i>	
#CKG-Aug312-D2-S	-3.61	0.03	3	-3.56	0.02	2	0.05
#CKG-Aug312-D2-E	-2.78	0.17	3	-2.62	0.00	2	0.16
#CKG-Aug3012-E-S	-13.87	0.04	2	-13.87	0.01	2	0.00
#CKG-Aug3012-E-E	-17.98	0.06	3	-17.97	0.00	2	0.01
#CKG-Sept2512-D2-S	-2.41	0.17	8	-2.37	0.01	2	0.04
#CKG-Sept2512-D2-E	-2.39	0.16	3	-2.27	0.01	2	0.12
#CKG-Oct2912-E-S	-14.04	0.13	3	-13.96	0.03	2	0.08
#CKG-Oct2912-E-E	-17.80	0.09	8	-17.80	0.00	2	0.00
#CKG-Apr313-D-S	-24.65	0.05	3	-24.48	0.02	2	0.17
#CKG-Apr313-D-E	-20.13	0.09	8	-19.95	0.00	2	0.18
#CKG-Apr313-E-S	-24.80	0.15	3	-24.58	0.02	2	0.22
#CKG-Apr313-E-E	-22.79	0.04	3	-22.62	0.00	2	0.17
Average		0.10			0.01		0.10

$\sigma$  = Standard deviation based on population (STDEVP).  
*n* = Number of samples.  
<sup>a</sup> Absolute difference between DIC-CO<sub>2</sub>-GEM and the gas evolution method.



### DIC concentrations in samples

The DIC concentration of a solution sample can be estimated by analyzing a series of secondary standard solutions of known DIC concentrations along with the sample. In this study, two sets of secondary standard solutions were used to establish an equation that relates the amount of  $\text{CO}_2$  liberated by  $\text{H}_3\text{PO}_4$  from a solution sample to its DIC



**Figure 4.** Calibration between the known DIC concentrations (mmolal) of secondary standard solutions and the 'Area all' of eleven  $\text{CO}_2$  peaks evolved from the standards.

**Table 8.** Experimental details of DIC concentration calculation

Standard Name	DIC conc. (mmolal)	Area all <sup>a</sup>
<i>Set #1: NaHCO<sub>3</sub></i>		
MRSI-NaHCO <sub>3</sub> -STD-2-r1	2	4.07
MRSI-NaHCO <sub>3</sub> -STD-2-r2	2	4.18
MRSI-NaHCO <sub>3</sub> -STD-5-r1	5	10.26
MRSI-NaHCO <sub>3</sub> -STD-5-r2	5	10.65
MRSI-NaHCO <sub>3</sub> -STD-8-r1	8	16.54
MRSI-NaHCO <sub>3</sub> -STD-8-r2	8	17.27
MRSI-NaHCO <sub>3</sub> -STD-10-r1	10	20.77
MRSI-NaHCO <sub>3</sub> -STD-10-r2	10	21.04
MRSI-NaHCO <sub>3</sub> -STD-15-r1	15	31.78
MRSI-NaHCO <sub>3</sub> -STD-15-r2	15	31.34
<i>Set #2: Na<sub>2</sub>CO<sub>3</sub></i>		
MRSI-Na <sub>2</sub> CO <sub>3</sub> -STD-2-r1	2	3.88
MRSI-Na <sub>2</sub> CO <sub>3</sub> -STD-2-r2	2	4.09
MRSI-Na <sub>2</sub> CO <sub>3</sub> -STD-5-r1	5	9.96
MRSI-Na <sub>2</sub> CO <sub>3</sub> -STD-5-r2	5	10.22
MRSI-Na <sub>2</sub> CO <sub>3</sub> -STD-8-r1	8	15.49
MRSI-Na <sub>2</sub> CO <sub>3</sub> -STD-8-r2	8	16.69
MRSI-Na <sub>2</sub> CO <sub>3</sub> -STD-10-r1	10	19.81
MRSI-Na <sub>2</sub> CO <sub>3</sub> -STD-10-r2	10	20.76
MRSI-Na <sub>2</sub> CO <sub>3</sub> -STD-15-r1	15	29.19
MRSI-Na <sub>2</sub> CO <sub>3</sub> -STD-15-r2	15	29.88

conc. = Concentration.  
<sup>a</sup>Average of eleven  $\text{CO}_2$  peaks evolved from secondary standards.

concentration in mmolal (Fig. 4). A sample measurement sequence consisted of four  $\text{CO}_2$  reference gas injections with the third injection designated as the reference, followed by eleven sample  $\text{CO}_2$  injections. The average of the "Area all" values (or the sum of the peak areas for mass 44, 45, and 46 of  $\text{CO}_2$ ) for eleven individual  $\text{CO}_2$  peaks evolved from each standard solution was calculated and these average values were then calibrated against the respective DIC concentrations. For precision comparison purposes, the "Area all" values for an individual  $\text{CO}_2$  peak (#3 and #8) among the eleven peaks were also examined through independent and cumulative application of the two sets of secondary standard solutions. The most accurate estimation of DIC concentration was achieved by combining all eleven  $\text{CO}_2$  peaks using  $\text{NaHCO}_3$  solutions as the standard, although using two sets of standard solutions, one set with  $\text{NaHCO}_3$  and the other set with  $\text{Na}_2\text{CO}_3$ , did not sacrifice the analytical accuracy significantly (Table 8). Therefore, using the two sets of standard solutions is recommended when natural samples of unknown pH values are analyzed for their DIC concentration. While this approach<sup>(e.g.[30])</sup> may not be as accurate as other established methods (e.g., coulometry<sup>[31])</sup> because quantitative recovery of  $\text{CO}_2$  from DIC in the solution is not expected in the DIC- $\text{CO}_2$ -GEM, the use of proposed secondary standards allows for a broad range of samples to be tested with confidence and enables us to determine DIC concentrations within an error of  $\pm 0.32$  mmolal.

### CONCLUSIONS

Traditionally, the  $\text{CO}_2$ - $\text{H}_2\text{O}$  equilibration and gas evolution methods are independently employed to determine the oxygen isotope composition of water ( $\delta^{18}\text{O}_{\text{H}_2\text{O}}$  value) and the carbon isotope composition of dissolved inorganic carbon ( $\delta^{13}\text{C}_{\text{DIC}}$  value), respectively. The innovative DIC- $\text{CO}_2$ -GEM developed in our study combines fundamental principles associated with these classical techniques, affording fewer resources and maximizing efficiency. Our DIC- $\text{CO}_2$ -GEM provides a simple way to obtain both oxygen and carbon isotope compositions in a single analysis and thus allows quick identification of samples requiring further in-depth study when screening for isotopic changes in a large suite of water samples.

The DIC- $\text{CO}_2$ -GEM data indicate that oxygen isotope exchange kinetics between  $\text{CO}_2$  and water are pH-dependent; therefore, the  $\text{CO}_2$ - $\text{H}_2\text{O}$  equilibration time can be minimized through addition of an optimal phosphoric acid volume to the  $\text{CO}_2$ - $\text{H}_2\text{O}$  system. Under the experimental conditions tested in this study, the addition of 0.01 mL phosphoric acid yielded the best overall analytical accuracy and precision for both  $\delta^{18}\text{O}_{\text{H}_2\text{O}}$  and  $\delta^{13}\text{C}_{\text{DIC}}$  measurements. For different experimental conditions, the optimal volume of phosphoric acid can easily be quantified as described in this study. While unable to claim the highest analytical precision achieved by the gas evolution method, the DIC- $\text{CO}_2$ -GEM is a practical substitution for many geochemical applications requiring a slightly lower accuracy in  $\delta^{13}\text{C}$  measurements. Particularly for those studies in which both carbon and oxygen isotope analyses are desired, the novel DIC- $\text{CO}_2$ -GEM offers an efficient alternative for researchers by measuring both isotopic compositions in a single solution sample. Furthermore, our

tests employing two sets of standard solutions of known DIC concentrations demonstrate an excellent capacity for determining DIC concentrations of aqueous samples with an error of  $\pm 0.32$  mmolal.

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