

# On-Line Technique for Measuring Stable Oxygen and Hydrogen Isotopes from Microliter Quantities of Water

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**Detailed here is a method for extracting and analyzing oxygen and hydrogen isotopes from 10  $\mu$ L-sized water samples. Based on the traditional CO<sub>2</sub>–H<sub>2</sub>O equilibration technique, the oxygen isotope exchange reaction is done exclusively in sealed 6-mm (o.d.) Pyrex tubes at 25 °C, with full isotope exchange completed in at least 28 h. Using the same water sample employed in the <sup>18</sup>O equilibration, D/H extractions are done in separate sealed 6-mm (o.d.) Pyrex tubes by reaction with Zn at 450 °C to form H<sub>2</sub>(g). Provided that a correction factor is applied to <sup>18</sup>O analyses, accuracy and precision for both <sup>18</sup>O and D/H are comparable to standard techniques using much larger samples.**

We report here a technique for the extraction of oxygen and hydrogen isotopes from small (10- $\mu$ L) water samples. The need exists for a routine oxygen and hydrogen isotope microextraction method of water from fluid inclusions, for structural water within hydrous minerals,<sup>1,2</sup> and for use in doubly labeled water studies in the medical<sup>3</sup> and the veterinary sciences.<sup>4</sup> Our technique eliminates the need for potentially dangerous, expensive, and messy reagents (BrF<sub>3</sub><sup>5</sup> and guanidine hydrochloride<sup>6</sup>) associated with other microanalytical extraction techniques and is based on the traditional CO<sub>2</sub>–H<sub>2</sub>O equilibration technique,<sup>7</sup> later modified.<sup>8</sup> Others have attempted CO<sub>2</sub>–H<sub>2</sub>O equilibration on a microliter scale,<sup>9,10</sup> and by reduction over pure graphite.<sup>11</sup> The technique

discussed in this paper is similar to that described by Ohba.<sup>12</sup> However, unlike Ohba's technique, ours involves a reaction carried out exclusively in 6-mm Pyrex tubes which, after CO<sub>2</sub>–H<sub>2</sub>O exchange is complete, are attached directly to the inlet portion of the mass spectrometer for extraction and analysis. Thus we have eliminated the need of extracting the sample on a separate line and carrying it to the mass spectrometer for analysis. Furthermore, instead of using hot uranium, we reduce the same water samples in sealed Pyrex tubes to H<sub>2</sub>(g) by reaction with Zn turnings at 450 °C. Broken tubes and used Zn are easily discarded after analysis. Provided a correction factor is applied (scaled for degree of oxygen isotope depletion or enrichment), the accuracy and precision of this method are comparable to others using much larger samples.

## EXPERIMENTAL SECTION

A diagram of the preparation line is shown in Figure 1. A 6-mm (o.d.) tube 20 cm long, sealed at one end, which has been stored in a glassware drying oven at 80 °C, is attached below the septum port line shown in Figure 1. Whenever the breakseal port is opened and exposed to room air, the Ar purge valve is opened (valves 1 and 2 open, valves 3 and 4 closed). This will ensure the system remains dry. After the Ar purge valve is closed (valve 1), the sample tube is evacuated through a high-vacuum pump (valve 3) and flamed vigorously to remove any moisture.

The entire septum port assembly, shown in Figure 1, is attached to the vacuum line via 14/35 ground glass joints, allowing for easy removal and cleaning of the assembly when necessary. The septum is from Scott Specialty Gases (part 51-29D) and is simply pressed into a glass septum receiver. At the bottom of the septum port assembly the glass narrows to accept a 1/4-in. (6-mm) Ultra-Torr fitting (Cajon Co., Macedonia, OH, part SS-4-UT-6) where the 6-mm sample tube attaches.

Once the sample tube is sufficiently evacuated, high-vacuum valves are closed (valves 3 and 6) and CO<sub>2</sub> is expanded into the tube by slowly opening valve 5. We use inexpensive Teflon barrel valves with O-ring sealed tips that, when opened slowly, act like more expensive needling valves. Valve 2 is then closed and 10  $\mu$ L of water sample is injected through the septum. We use a Hamilton gastight syringe (Hamilton Co., Reno, NV, model 1701, 10- $\mu$ L capacity) which has been rinsed twice in the sample water.

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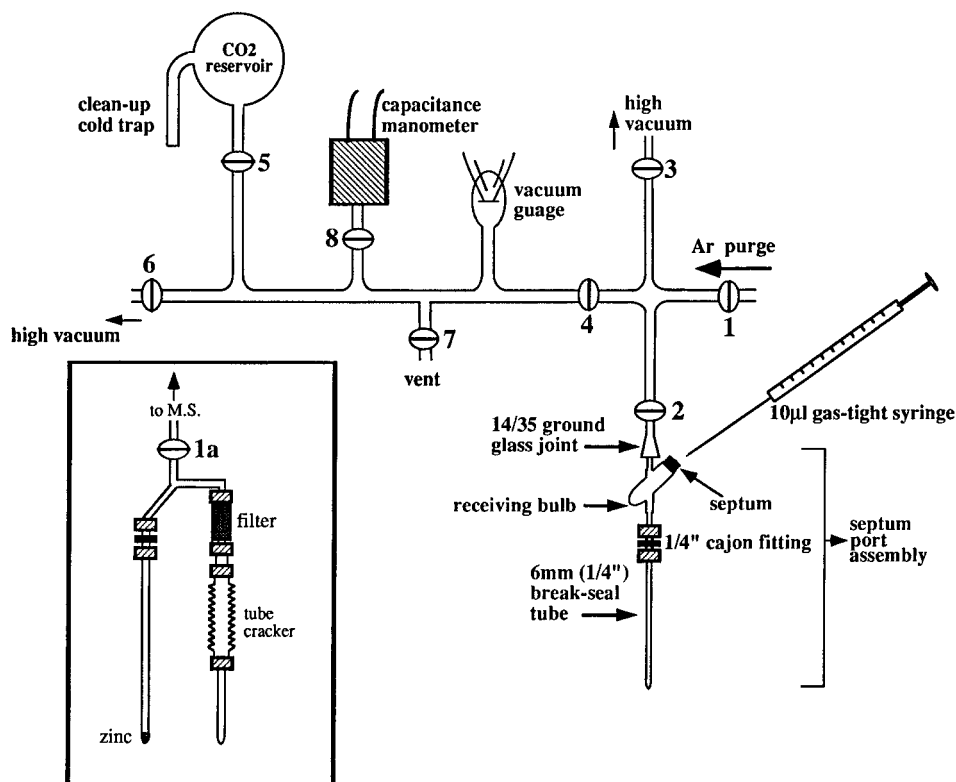


Figure 1. Microwater extraction line. A 10- $\mu$ L gastight syringe is used for injecting water samples directly.

While injecting, the water droplet is deposited on the small receiving bulb at the back of the septum port assembly. The water sample and  $\text{CO}_2$  are then frozen to the bottom of the 6-mm sample tube via liquid nitrogen by heating the receiving bulb containing the droplet with a soft flame. After 3 min the vacuum gauge is read in order to ensure complete transfer and absence of a leak (residual  $\text{CO}_2$  in the extraction line above valve 2 must first be pumped away). The tube is then sealed off with a torch and completely immersed in a 25  $^\circ\text{C}$  water bath where the  $\text{CO}_2$ - $\text{H}_2\text{O}$  isotopic exchange reaction is carried out.  $\text{CO}_2$  pressure inside the sealed tube is  $\sim 0.01$  atm.

Once equilibration is completed (see next section), the sample tube containing  $\text{CO}_2$  and  $\text{H}_2\text{O}$  is removed from the water bath and the outside is thoroughly dried. After removal from the 25  $^\circ\text{C}$  water bath, samples should not be allowed to reequilibrate at a different temperature. We place the tubes flame-sealed end down in a shallow dewar of liquid nitrogen until ready for extraction and analysis. This step ensures that the  $\text{H}_2\text{O}$  and  $\text{CO}_2$  remain frozen and thus will no longer exchange isotopically. We assume that no significant isotopic exchange will occur during the length of time needed to freeze the water sample ( $\sim 15$  s). The tube is gently scribed and inserted into the tube cracker device (Figure 1, inset) which is attached to the inlet of a Finnigan model Delta S mass spectrometer equipped with a microvolume coldfinger. The tube cracker is constructed of  $1/4$ -in. 316 stainless steel tubing and utilizes a flexible stainless steel tube (Cajon, part 321-6-X-3, with  $3/8$ -in. XBA adapters at each end) that contains a 7-mm opening stainless steel filter (Nupro Co., Wiloughby, OH, part SS-4F-7). The filter is necessary in order to prevent small glass shards from entering the inlet of the mass spectrometer and subsequently damaging internal valves. A manual valve (Whitey Co., Highland Heights, OH, part SS-43S4) separates the tube cracker device from

the inlet system (valve 1a). Also, a 6-mm Pyrex tube filled with  $\sim 300$  mg of zinc turnings is attached as a sidearm for use in collecting the water sample for later reduction to  $\text{H}_2(\text{g})$ .

After the tube cracker is pumped to a sufficient vacuum, the portion of the tube sticking out of the bottom of the cracker is placed in a small dewar containing liquid nitrogen. After 3 min, the portion of the sample tube inside the cracker, as well as the tube cracker itself, is gently heated with a heat gun. This is done to ensure that all water is driven to the bottom of the sample tube. At this point, the liquid nitrogen dewar is quickly replaced with a dewar containing a mixture of dry ice and alcohol. After 2 min, the tube is cracked and  $\text{CO}_2$  is frozen into the microinlet of the mass spectrometer for analysis. Once all the  $\text{CO}_2$  is removed from the sample tube, the manual valve is closed and the water is warmed and subsequently frozen into the sidearm tube containing the zinc which was previously evacuated. After 3 min, the zinc-containing tube is flamed sealed and removed.

Flame-sealed tubes containing both zinc and the water sample are then heated to 450  $^\circ\text{C}$  for exactly 30 min, where the water is quantitatively reduced to  $\text{H}_2(\text{g})$ . For the water reduction step, we constructed a simple furnace consisting of four rectangular aluminum blocks  $\sim 5$  in. length  $\times$  6 in. width  $\times$  2.5 in. height stacked on top of one another with six 1-in. holes bored vertically. The overall height of the furnace ( $\sim 10$  in.) allows the entire length of the sample tube to be heated at the same temperature. Tubes are inserted and removed from the block furnace using large (11 in. long) forceps. Inserted into the sides of the block furnace are nine cartridge-type heaters,  $3/8$  in. diameter, 5 in. long (Chromalox Industrial Heating Products, Pittsburgh, PA, part C-207) which are connected in parallel and plugged into a 120-V variable ac transformer. The variable ac transformer can then be adjusted to maintain the 450  $^\circ\text{C}$  temperature.

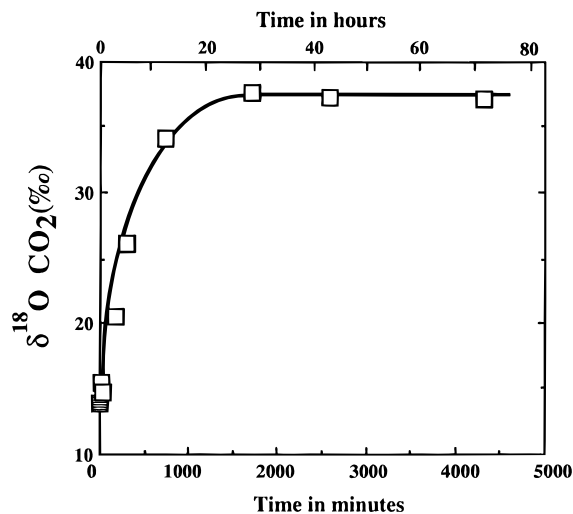


Figure 2. Variation with time of the  $\delta^{18}\text{O}$  composition of  $\text{CO}_2$  equilibrated with our laboratory working standard water. Based on these data, oxygen isotope exchange is complete in  $\sim 28$  h. The hatched symbol is the initial  $\delta^{18}\text{O}$  of the  $\text{CO}_2$ .

## RESULTS AND DISCUSSION

**Equilibration Time.** The time necessary for complete isotopic exchange between  $\text{CO}_2$  and  $\text{H}_2\text{O}$  is plotted as a function of oxygen isotope composition in Figure 2. Eight 6-mm tubes were filled with  $10\ \mu\text{L}$  of our laboratory working standards, ultramicrowater Standard (UMWS) together with  $0.57\ \mu\text{mol}$  of  $\text{CO}_2$ . All eight were completely immersed in a constant temperature ( $25\ ^\circ\text{C}$ ) water bath for up to 4300 min. The  $\delta^{18}\text{O}$  value at time 0 min is the initial oxygen isotope composition of the  $\text{CO}_2$  expanded into the tubes. With increasing time, the  $\delta^{18}\text{O}$  composition of the  $\text{CO}_2$  approaches an equilibrium value with that of the water in the vial. Our experiment indicates that oxygen isotope equilibration is completed in at least 1700 min (28 h).

**Sensitivity Analysis.** A sensitivity analysis was performed in order to determine to what extent (if any) an oxygen isotope correction needs to be applied to the microequilibrated  $\text{CO}_2$ – $\text{H}_2\text{O}$  samples. This isotope correction is based on the material balance expression from Craig<sup>13</sup> as

$$\delta = \delta' \frac{(\rho + \alpha)}{\rho} - \frac{\alpha}{\rho} (\delta^{18}\text{O}_{(\text{tank CO}_2)}) \quad (1)$$

In eq 1,  $\alpha$  is the fractionation factor between  $\text{CO}_2$  and  $\text{H}_2\text{O}$  taken to be 1.0412 at  $25\ ^\circ\text{C}$ ,<sup>14</sup>  $\delta'$  is the oxygen isotope composition of the  $\text{CO}_2$  that has been equilibrated with the water sample,  $\rho$  is the molar ratio of oxygen in the water sample to oxygen in the  $\text{CO}_2$  expanded into the tube, and  $\delta$  is the corrected value. Using eq 1, a graph (Figure 3) was constructed to show the extent of the correction for four different scenarios based on the difference between the  $\delta^{18}\text{O}$  composition of the  $\text{CO}_2$  and  $\text{H}_2\text{O}$  used during the isotopic exchange reaction. For most meteoric water samples, that is water whose  $\delta^{18}\text{O}$  composition falls between 0 and  $-55\text{‰}$ , the correction is essentially insignificant, however when working with  $^{18}\text{O}$  spiked water samples (i.e., doubly labeled water studies)

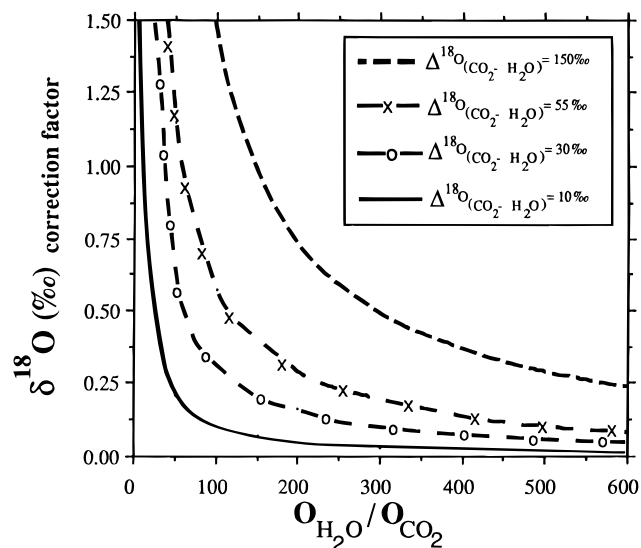


Figure 3.  $\delta^{18}\text{O}$  correction factor versus ratio of oxygen in water to oxygen in carbon dioxide. The four different scenarios show the extent of an oxygen isotope correction that must be applied based on the difference in the initial isotopic composition of the water and the carbon dioxide.

Table 1.  $\delta^{18}\text{O}$  and  $\delta\text{D}$  Results for Three International Water Standards and One Laboratory Working Standard

| sample name | no. of preps | std dev $1\sigma$ (‰) | $\delta^{18}\text{O}$ (SMOW)              |                         |
|-------------|--------------|-----------------------|---|-------------------------|
|             |              |                       | measured <sup>a</sup>                     | normalized <sup>b</sup> |
| V-SMOW      | 15           | $\pm 0.09$            | -0.07                                     | 0.00                    |
| GISP        | 10           | $\pm 0.10$            | -24.79                                    | -24.76                  |
| SLAP        | 8            | $\pm 0.08$            | -55.47                                    | -55.50                  |
| sample name | no. of preps | std dev $1\sigma$ (‰) | $\delta\text{D}$ (SMOW)                   |                         |
|             |              |                       | measured                                  | normalized <sup>b</sup> |
| V-SMOW      | 3            | $\pm 0.6$             | -0.5                                      | 0.0                     |
| GISP        | 5            | $\pm 0.7$             | -188.8                                    | -188.7                  |
| SLAP        | 4            | $\pm 2.5$             | -428.4                                    | -428.0                  |
| sample name | no. of preps | std dev $1\sigma$ (‰) | Laboratory Standard                       |                         |
|             |              |                       | measured $\delta^{18}\text{O}$ (SMOW) (‰) |                         |
| UMWS        | 22           | $\pm 0.04$            | -3.88                                     |                         |
| sample name | no. of preps | std dev $1\sigma$ (‰) | Laboratory Standard                       |                         |
|             |              |                       | measured $\delta\text{D}$ (SMOW) (‰)      |                         |
| UMWS        | 6            | $\pm 1.9$             | -21.6                                     |                         |

<sup>a</sup> These data were calculated using the  $\text{CO}_2$ – $\text{H}_2\text{O}$  fractionation factor of 1.0412 recommended by Friedman and O'Neil.<sup>14</sup> <sup>b</sup> These data were normalized to  $\Delta$  (V-SMOW-SLAP) =  $-55.50\text{‰}$  for  $^{18}\text{O}$  and  $-428\text{‰}$  for D.

where the difference between the oxygen isotope composition of the  $\text{CO}_2$  and  $\text{H}_2\text{O}$  could be as much as a few hundred mil, the correction does become significant. For example, a  $10\text{-}\mu\text{L}$  size water sample equilibrated with  $0.57\ \mu\text{mol}$  of  $\text{CO}_2$ , with an isotopic composition  $\sim 10\text{‰}$  greater than that of the water, will require an oxygen isotope correction of  $0.01\text{‰}$ . Alternatively,  $10\ \mu\text{L}$  of water that is  $150\text{‰}$  greater than the  $\text{CO}_2$  equilibrated under the same conditions requires an oxygen isotope correction of  $0.14\text{‰}$ .

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**Accuracy and Precision.** Data for oxygen and hydrogen isotope composition of three different international water standards and one of our laboratory working standards using the technique described in this paper are reported in Table 1. The three international standards, provided by International Atomic Energy Agency (IAEA), Vienna, include V-SMOW, GISP, and SLAP. On the basis of the number of analyses, isotope reproducibility of all three international standards and one laboratory standard using our technique is better than  $\pm 0.10\%$  for oxygen isotopes. Hydrogen isotope reproducibility ranges from  $\pm 0.6\%$  for V-SMOW to  $\pm 2.5\%$  for SLAP. Furthermore, when normalized to the V-SMOW–SLAP scale, the observed normalized values for GISP ( $\delta^{18}\text{O} = -24.76$ ,  $\delta\text{D} = -188.7$ ) are statistically indistinguishable from the mean of reported values.<sup>15</sup>

#### CONCLUSIONS

The method described in this paper satisfies the need for a technique to extract oxygen and hydrogen isotopes from small

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(10- $\mu\text{L}$ ) size water samples. Utilizing this technique not only eliminates the use of dangerous and expensive reagents commonly employed with other methods but also preserves the water sample for later use in D/H analyses. Furthermore, we have shown that good sample reproducibility over a wide range of isotopic compositions makes this method acceptable for routine oxygen and hydrogen isotope water extractions.

#### ACKNOWLEDGMENT

This study was supported by NASA's Exobiology Program. C.S.R. acknowledges support from financial assistance award DE-FC09-96-SR18546 from the U.S. Department of Energy to the University of Georgia Savannah River Ecology Laboratory.

Received for review October 19, 1998. Accepted March 16, 1999.

AC981140I