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A robust and fast method of sampling and analysis of δ^{13}C of dissolved inorganic carbon in ground waters

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The stable carbon isotopic composition of dissolved inorganic carbon ($\delta^{13}C_{DIC}$) is traditionally determined using either direct precipitation or gas evolution methods in conjunction with offline gas preparation and measurement in a dual-inlet isotope ratio mass spectrometer. A gas evolution method based on continuous-flow technology is described here, which is easy to use and robust. Water samples (100–1500 μl depending on the carbonate alkalinity) are injected into He-filled autosampler vials in the field and analysed on an automated continuous-flow gas preparation system interfaced to an isotope ratio mass spectrometer. Sample analysis time including online preparation is 10 min and overall precision is 0.1 ‰. This method is thus fast and can easily be automated for handling large sample batches.

Keywords: Analysis; Carbon-13; Continuous flow; Dissolved inorganic carbon; Ground water, Hydrochemistry; Methods; Natural abundances

1. Introduction

The stable carbon isotopic composition of dissolved inorganic carbon ($\delta^{13}C_{DIC}$) is a useful parameter to constrain the terrestrial carbon sources and processes involved in the riverine [1–4] and the groundwater carbon cycle [5, 6].

Two different methods have primarily been used in the past to sample and analyse $\delta^{13}C_{DIC}$: (a) direct precipitation in the field, *e.g.* as $SrCO_3$ or $BaCO_3$, and subsequent re-release of CO_2 in the laboratory using phosphoric acid followed by offline gas separation and purification or (b) poisoning of the sample in the field (*e.g.* using $HgCl_2$) to suppress biological degradation of the sample during storage and offline gas evolution and purification in the isotope laboratory (*e.g.* [1, 7–11]. Although these classic methods produce reliable results, they have distinct disadvantages including handling a highly toxic substance ($HgCl_2$), handling chemicals in the field, filtration and being time consuming in the laboratory. In this technical note, we outline a method which combines a field-based gas evolution technique (avoiding sample poisoning) with the advantages of continuous-flow isotope ratio mass spectrometry.

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2. Description of the method

2.1 *Preparation steps*

The method employs 10 ml septum-capped Na-glass vials (Labco exetainer) that can be analysed using an autosampler. Prior to going into the field, the exetainer is preloaded with five droplets of phosphoric acid (ca. 90%), capped and the headspace is flushed with He 6.0 in the autosampler by penetrating the butyl rubber septa of the exetainers' disposable caps (Labco catalogue no. VC301) using a double-hole needle. Alternatively, the acid can also be injected at the sampling site (by penetrating the septum using a needle syringe) prior to introducing the water sample. At the sampling site, an aliquot of the water sample is injected into the exetainer using a needle syringe. Care should be taken to avoid small air bubbles that become trapped in the syringe while the sample is drawn up. The amount of sample injected depends on the carbonate alkalinity and the dynamic range of the mass spectrometer. Table 1 gives an overview of the volumes used for samples that have been analysed in our laboratory at the University of Innsbruck. The acid-water reaction begins immediately upon injection. Ideally, the exetainer should remain in upright position afterwards in order to avoid droplets of solution on the bottom of the rubber septa, which can give rise to water being entrained into the measurement system. In our experience, however, less careful handling of samples (*e.g.* shipping by courier) does not pose a problem, if the septa are carefully checked using a lamp prior to the measurement (occasional droplets can be effectively removed by gently knocking against the caps).

2.2 *Isotopic analysis*

The CO_2 in the headspace of the exetainer is analysed within a few days after injection. In the laboratory, the exetainers are loaded into the CTC Combi-Pal autosampler linked to a Gasbench II. Alternatively, exetainers can be analysed manually by putting one by one on the measurement needle of the Gasbench. Once penetrated by the double-hole needle, sample gas (He plus CO_2 and small amounts of N_2 degassed from the sample) is pushed out of the exetainer by a slow flow of He 6.0 from the Gasbench. The gas is subsequently purified (Nafion trap to remove water vapour) and separated gas chromatographically using a Poraplot Q GC column (fused silica tubing, 25 m × 0.32 mm, kept at 70 °C), followed by a second Nafion trap and then leaked into the source of the Delta$^{\text{plus}}$XL mass spectrometer. The entire procedure is fully automated and implemented into the measurement protocol under ISODAT. Each measurement commences with three injections of CO_2 reference gas, followed by nine injections of sample gas *via* an 8-port Valco valve. The software calculates the raw δ^{13}C values

Table 1. Approximate sample volumes required for DIC analysis at the University of Innsbruck Stable Isotope Laboratory, which utilizes a Delta$^{\text{plus}}$XL mass spectrometer (dynamic range 0–10 V m/z 44).

Carbonate hardness (°dH)[†]	HCO_3 (mg/l)	Sample volume (μl)
1–2	22–44	1500
2–5	44–109	700
5–8	109–174	500
8–11	174–240	300
11–15	240–327	200
15–20	327–436	100

[†] Quoted in degrees German hardness.

by taking the average of the nine consecutive sample peaks and comparing their raw ratios to those of the reference gas.

2.3 *Calibration against VPDB*

Because there are no durable DIC standards available for stable isotope analysis, we use the following procedure to calibrate the raw delta values against the VPDB: DIC samples are run intermittently with calcite samples (DIC is measured during the working day and calcite runs overnight). We use the calcite standard $\delta^{13}C$ values of the runs before and after a DIC measurement to correct and calibrate the $\delta^{13}C_{\mathrm{DIC}}$ values (that can be traced back to NBS and IAEA carbonate standards). A linearity correction is performed using the slope $\delta^{13}C$ *vs.* intensity of m/z 44 as measured during the calcite runs [12]. Alternatively, the calibration can be performed by injecting CO_2 of known isotopic composition (*e.g.* ISO TOP CO_2 of Messer) into exetainer vials and by performing a linearity correction and calibration based on these standards.

3. Results and discussion

3.1 *Precision*

Internal precision of $\delta^{13}C$ is typically between 0.05 and 0.08 ‰, depending on the CO_2 content of the sample and the linearity (m/z 44 *vs.* raw $\delta^{13}C$) of the system. External precision was measured twice within a period of 2 years by preparing nine and 20 aliquots of laboratory tap water, respectively. The standard deviation was <0.08 ‰ in both test runs (table 2). Previous tests with our instrument (using carbonate samples) have shown that precision deteriorates rapidly if the intensity of the first sample peak (m/z 44) falls below ~0.5 V. It is therefore important to adjust the amount of sample injected into the exetainer (cf. table 1).

3.2 *Accuracy*

The accuracy of our system was explored by preparing artificial DIC samples of Na_2CO_3 (Merck) whose C isotopic composition was measured using the classical reaction with dry orthophosphoric acid. Dry soda powders may experience changes in their $\delta^{13}C$ value during long storage, if they are in continuous contact with CO_2 [9, 10]. In addition, alkaline solutions absorb CO_2 giving rise to kinetic C isotope fractionation [9]. We minimized these effects by preparing Na_2CO_3 DIC solutions and Na_2CO_3 samples for the H_3PO_4 reaction rapidly from the same, homogenized aliquot of solid Na_2CO_3. Five DIC samples were prepared by dissolving ~40 mg in 100 ml of freshly produced deionized water (electric conductivity 0.08 μS/cm). Dissolution was accomplished within minutes and 1 ml each of these solutions was injected into two He-flushed exetainer each preloaded with five droplets of H_3PO_4. The entire process was completed in <1 h, and the DIC samples were analysed after 1 h. At the same time, aliquots of the same Na_2CO_3 powder were deposited in 10 ml borosilicate glass vials (between 300 and 400 μg each), capped and flushed with He within <2 h. The latter batch of samples was reacted with H_3PO_4 at 72 °C together with a set of in-house calcite standards whose isotopic composition can be traced back to NBS and IAEA standard reference materials [12]. The solid Na_2CO_3 samples yielded a mean $\delta^{13}C$ value of -4.75 ± 0.10 ‰ ($n = 12$). This value is only marginally higher than the mean $\delta^{13}C_{\mathrm{DIC}}$ value: -4.92 ± 0.08 ‰ ($n = 10$). These two data groups are indistinguishable within their 1 σ analytical error margins. The fact that the $\delta^{13}C_{\mathrm{DIC}}$ value is slightly lower than the C isotopic composition of the solid product actually

Table 2. Tests of precision using laboratory tap water.

Test 1 Exetainer no.	m/z 44	δ^{13}C (‰)	Test 2 Exetainer no.	m/z 44	δ^{13}C (‰)
1	4.03	−3.90	1	5.77	−4.06
2	3.97	−3.89	2	5.87	−4.07
3	4.15	−3.79	3	5.59	−4.08
4	4.07	−3.78	4	5.82	−4.07
5	4.04	−3.77	5	5.45	−4.15
6	4.10	−3.69	6	5.96	−3.98
7	4.10	−3.74	7	5.78	−4.02
8	4.01	−3.85	8	6.49	−3.93
9	4.14	−3.79	9	4.08	−3.99
10	4.03	−3.78			
11	4.05	−3.84			
12	4.09	−3.79			
13	4.13	−3.84			
14	4.08	−3.86			
15	4.00	−3.86			
16	3.96	−3.87			
17	4.01	−3.86			
18	4.05	−3.85			
19	4.08	−3.82			
20	4.02	−3.82			
Mean		−3.82	Mean		−4.04
SD		0.05	SD		0.07

Test 2 was performed ~2 years after test 1. The intensity of mass 44 of the first sample peak is also given.

demonstrates that the DIC solutions did not experience significant exchange with the laboratory air during solution preparation; otherwise its δ^{13}C value would have increased (the δ^{13}C value of the laboratory air value is ca. −10 ‰ and would give rise to a $\delta^{13}C_{DIC}$ value of ~9 ‰ higher than that in case of complete exchange).

3.3 *Storage effects*

Previous studies using glass autosampler vials with butyl rubber caps reported evidence of changes in the δ^{18}O and – to a lesser extent also – in the δ^{13}C value during storage, depending on the CO_2 content and its isotopic composition [13–16]. We have systematically studied storage effects of Labco autosampler vials and found only insignificant (*i.e.* <0.1 ‰) C isotope shifts within a period of up to 2 weeks of storage at room temperature [17]. We routinely ship He-flushed exetainer to foreign field locations by courier, where phosphoric acid is being injected followed by injection of an aliquot of the water sample. The exetainer is shipped back to the laboratory (again by courier) and analysed within 2 days. Blank exetainer vials (with and without acid) are routinely included in these sample batches, but no evidence of significant leakage was found. We therefore conclude that the butyl rubber septa are gastight, provided that the caps are properly tightened. In addition, the relative insensitivity of the C isotopic composition of DIC samples in exetainer to minor gas exchange over periods of up to 2 weeks reflects the much higher pCO_2 in the exetainer when compared with the outside air pCO_2. Nevertheless, we recommend to keep the time interval between flushing the samples, injection the water and analysing them as short as possible (*i.e.* within 1 week) and to include blank exetainer within each sample batch as monitors of possible gas leakage. The use of Kel-F discs in addition to butyl rubber septa [cf. ref. 16] is not applicable for the technique presented in this paper, because Kel-F discs loose their sealing function once they are penetrated by a needle.

4. Summary

Given that the total analysis time is 10 min per sample, the method described earlier is well suited for large sample batches run by an autosampler. Its advantages include minimal sample handling in the field, avoidance of toxic substances, small sample volumes, automated continuous-flow online sample preparation and analysis using an autosampler. The method has so far been used primarily to analyse water samples from karst aquifers. Its application to other water types (*e.g.* very low-DIC or high-DOC surface water samples) appears feasible, but should be thoroughly tested first.

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