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Gas IRMS

Carbon isotope analyses on dissolved inorganic carbon of seawater samples:

Sample preparation and analysis using the GasBench Plus System

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Introduction

The $\delta^{13}C$ isotope composition of the dissolved inorganic carbon (DIC) content of natural water samples helps to better understand carbon cycling and gas exchange in various hydrogeological systems (e.g., oceans, lakes, sedimentary porewaters). Hydrogeological carbon reservoirs are nowadays influenced by the rising anthropogenic CO_2 content of the atmosphere, and this can be studied by analyzing e.g., DIC water samples. The methodical development for this application was guided by Torres et al., 2005 towards the use of Thermo Scientific™ GasBench II IRMS System in continuous flow mode.

The biggest challenges of the $\delta^{13}C$ analysis of DIC samples are often connected to sample storage and sample preparation before the analysis with the IRMS and this is based on the fragility of the carbonate balance in water samples. The contact between atmospheric CO_2 and the DIC needs to be prevented efficiently, directly after sampling to avoid contamination and the disruption of the intrinsic carbonate balance of the sample. This can be achieved by using large sample volumes, sample containers filled-up to the top, robust and air-tight sealing (e.g., wax) and cooling of the samples (~8 °C). However, also organisms within natural water samples can affect the carbonate balance by their metabolic processes (e.g., respiration), hence the samples are often sterilized using toxic substances (e.g., mercury chloride ($HgCl_2$)) if the samples cannot be analyzed directly after sample taking. This makes the handling of DIC water samples demanding.

This technical note focuses on the sample preparation and analysis of DIC seawater samples describing the established workflow and highlighting possible pitfalls during sample preparation.

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Equipment

Extraction and analysis of $\delta^{13}C$ of DIC water samples was done using the GasBench Plus universal on-line gas preparation and introduction system connected via a Thermo Scientific™ConFlo IV Universal Interface to a Thermo Scientific™ DELTA™ Q IRMS. The instrument is operated using Thermo Scientific™ Qtegra™ Intelligent Scientific Data Solutions (ISDS) Software, with all isotope measurements carried out in continuous flow mode. The GasBench Plus System is equipped with a Thermo Scientific™ TriPlus™ RSH SMART Autosampler featuring the improved needle guide design and automatic tool changing option which enabled automatic Helium flushing and sampling. The temperature during the gas extraction is stabilized using a Thermo Scientific™ Thermostatted sample tray for GasBench Plus System.

Analytical setup

The fundamental principle of the sample preparation for DIC analyses is that the carbonate balance in the aqueous DIC water sample is shifted to the gaseous phase (CO_2), by acid addition, and the released CO_2 accumulates in the headspace of the vials (which was flushed with Helium (He)) and equilibrates before the CO_2+He mixture is sampled and processed using the GasBench Plus System.

Two sample preparation procedures were tested on DIC water samples to investigate their effect on the $\delta^{13}C$ results (Figure 1). The selected four DIC water samples comprised authentic seawater (spiked with $HgCl_2$).

A) 5 drops of 98% H_3PO_4 were manually added with a syringe into open borosilicate sample bottles of 12 mL capacity and the vials were closed with screwcaps fitted with fresh septa. The closed vials were placed in a temperature regulated autosampler tray that was maintained at 26 °C. An autosampler-assisted flushing procedure was used to remove air from the vials using a He stream. Flushing was carried out at a flow of 120 mL/min for 6 minutes. 1000 µL of aqueous DIC sample was added with a syringe and the samples were allowed to equilibrate for 24h at an equilibration temperature of 26 °C. This is the typical procedure for DIC water samples (Figure 1A).

B) Aqueous DIC samples of 1000 µL aliquots were added into the open borosilicate sample bottles of 12 mL capacity using a syringe and the vials were closed with screwcaps fitted with fresh septa. The closed vials were placed in a temperature regulated autosampler tray that was maintained at 26 °C. An autosampler-assisted flushing procedure was used to remove air from the vials using a He stream. Flushing was carried out at a flow of 120 mL/min for 6 minutes. 5 drops of 98% H_3PO_4 were manually added with a syringe and the samples were allowed to equilibrate for 24h at a temperature of 26 °C. This is the typical procedure for solid carbonate samples (Figure 1B).
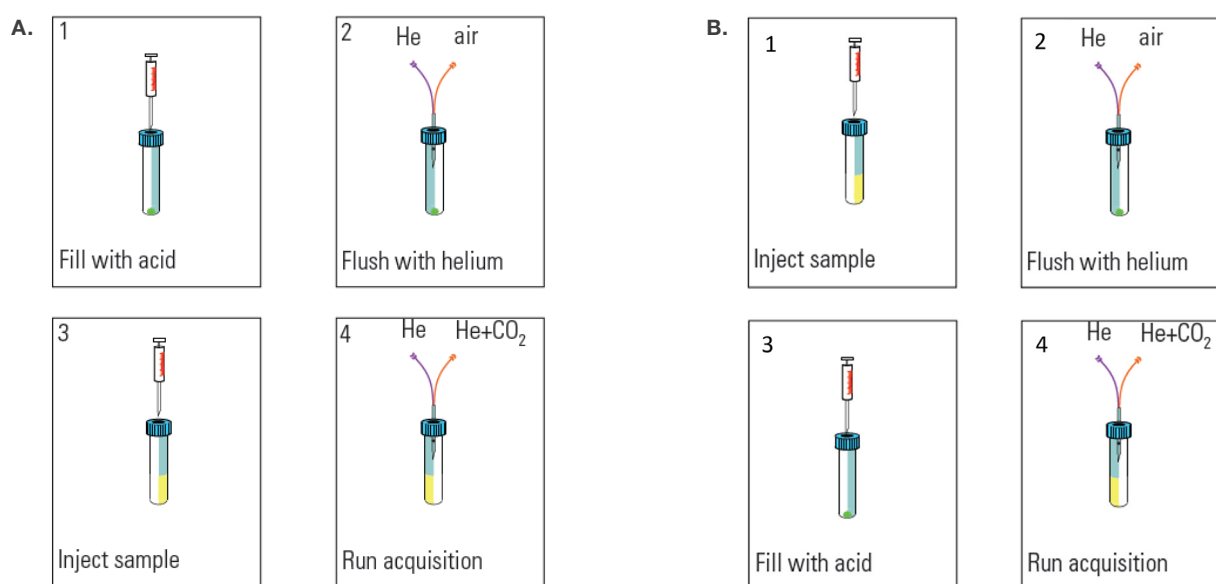
Figure 1. Sample preparation techniques tested on DIC water samples to investigate their effect on the $\delta^{13}C$ results. A) Acid addition, Helium flushing, sample injection (performed for DIC samples). B) Sample injection, Helium flushing, acid addition (typically performed for carbonates samples).

Note: disposable nitrile gloves are of high importance when handling DIC water samples spiked with $HgCl_2$. Nitrile is impermeable to $HgCl_2$ in contrast to other glove materials. If $HgCl_2$ or another hazardous chemical is used for the sterilization of the DIC water samples, safety measures need to be arranged and the waste needs to be appropriately disposed.

During manual injection of sample (method A) or acid (method B) with a syringe in a flushed vial, the septa should be pierced off-centre (close to the rim) and it should be ensured during the removal of the needle that no droplets remain on the inside of the septa. This prevents the sample or acid from entering the GasBench Plus capillary system when the needle of the autosampler is piercing the septa (in the centre) to sample the headspace (CO_2+He mixture). For more information on the preparation of 98% H_3PO_4 see the GasBench Plus Operating Manual 'Preparing Phosphoric Acid' on page 9-13. Here 98% H_3PO_4 was used; in general one can use 98-45% H_3PO_4 for this application.

The equilibration temperature was controlled using a Thermostatted sample tray, which can stabilize the temperature at ±0.1 °C. For an accurate temperature control, it is crucial to ensure that the set value of the tray is always ~2 °C higher than the ambient temperature so that the tray can continuously heat and control the temperature throughout the equilibration time. If the temperature during the sample preparation is not stable, it will impede $\delta^{13}C$ precision and accuracy. The equilibration time is a function of the equilibration temperature and needs to be adapted to it. A lower equilibration temperature might require a longer equilibration time. If the sampling is started before the isotopic equilibrium is reached between the gaseous CO_2 in the headspace and dissolved phase in the liquid, obtained results will be inaccurate, with drifting $\delta^{13}C$ values.

Reducing the equilibration temperature can provide an advantage if analyzing large sample sets of aqueous DIC samples. The higher the tray temperature, the more water vapor will be in the sample gas which can affect the system performance. This can be improved by choosing a lower tray/equilibration temperature; however, this requires that the room temperature can be readjusted to a lower temperature (the set value of the heated tray has to be always higher than the ambient temperature to ensure a stable tray/equilibration temperature).

At the end of the equilibration time, the automated analysis workflow of the GasBench Plus System was initiated starting with the sampling of the headspace; CO_2+He mixture is transported from the vials by the helium carrier gas into the GasBench Plus unit through a first water-vapor removal trap into the sample loop of an 8-port valve. The sample loop aliquots 100 µL of the sample into an isothermal gas chromatographic oven, where CO_2 is separated from other gases (if present) and then transported through a second water-vapor removal trap for additional cleaning before it is introduced to the IRMS via the ConFlo IV Universal Interface.

Results

Repetitive loop injections were used to analyze the $^{13}C/^{12}C$ ratio for 10 aliquots of each sample together with 3 reference gas injections per sample (Figure 2).
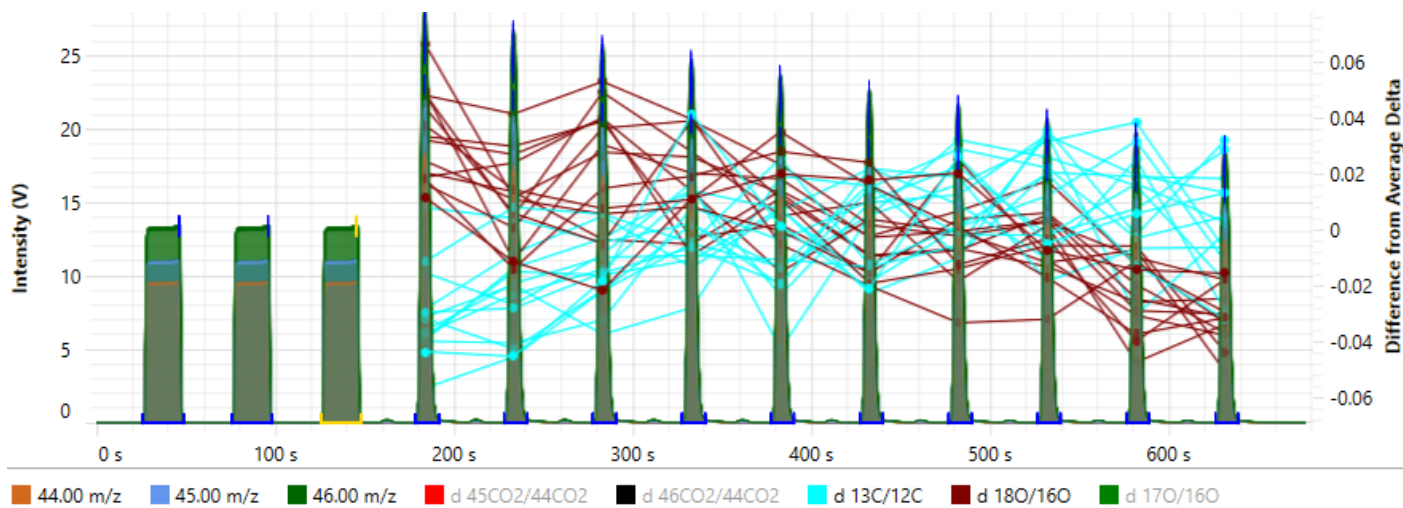
Figure 2: Qtegra ISDS Software data visualization features: overlayed chromatograms of the QC standard ETH-1 (N=14, carbonate). The right y-axis belongs to the line plots which display the variation in $\delta^{13}C$ (light blue) and $\delta^{18}O$ (brown) of the individual pulses from the averaged value for each run. The first sample pulse was conducted for conditioning and discarded from the $\delta^{13}C$ determination.

The DIC water samples were prepared for CO_2 extraction using method A (Acid-Flushing-Sample) and method B (Sample-Flushing-Acid), as described in the Analytical setup section. Two to three triplicates of each option were analyzed in one continuous acquisition to facilitate a direct comparison between both sample preparation techniques. The presented $\delta^{13}C_{VPDB}$ data were linearity and scale corrected using an in-house Solenhofen carbonate standard (SHK-2020, $\delta^{13}C_{VPDB}$=-1.164 ‰; N=17, distributed as three blocks in the sequence). The four seawater samples varied only slightly in DIC concentration resulting in very similar sample peak amplitudes hence the linearity correction had only a small effect, but the DIC concentration in a sample set can vary extensively (e.g., lacustrine or river water) and in these cases a linearity correction based on the analyses of different standard amounts is important.

Creating a DIC in-house standard for testing or as quality control requires only ultra clean, decarbonized water and a sodium hydrogen carbonate, which must be isotopically calibrated against other solid carbonate standards before dissolving it in water to match a certain DIC concentration. However, it is quite common that solid carbonate standards are used as standards for the scale correction or/and as quality control during DIC water analyses because it is challenging to maintain a DIC in-house standard which is stable in its isotopic composition (uncontaminated by atmospheric CO_2). This approach was also chosen for this study. ETH-1 (carbonate standard, $\delta^{13}C_{VPDB}$=2.02 ‰) was analyzed as quality control 18 times during the sequence, with average $\delta^{13}C_{VPDB}$= 2.07±0.03 ‰. The measurement precision and accuracy are within system specifications (Table 1 and Figure 3).

Table 1: $\delta^{13}C$ results of the carbonate standards and the DIC samples (S1-S4) analyzed with the two different sample preparation techniques A and B. The target values for the carbonate standards are noted in brackets; SHK-2020, in-house standard used for the scale correction and ETH-1, certified isotope standard used as quality control, treated identically as samples.

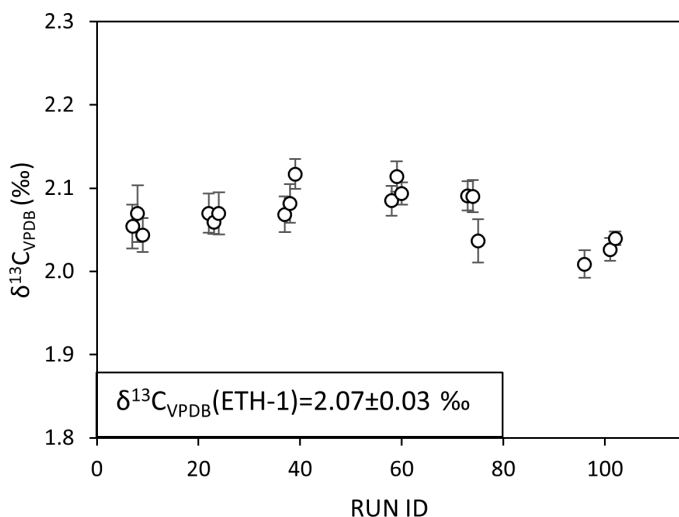| Sample	Mean ($\delta^{13}C_{VPDB}$) ‰	SD ($\delta^{13}C_{VPDB}$) ‰	N
SHK-2020	-1.16 (-1.16)	0.04	17
ETH-1	2.07 (2.02)	0.03	18
A) Sample preparation: Acid, flushing, sample			
S1	-0.71	0.04	9
S2	-0.41	0.02	6
S3	0.05	0.05	9
S4	-0.58	0.02	6
B) Sample preparation: Sample, flushing, acid			
S1	-1.56	0.07	9
S2	-1.26	0.06	6
S3	-0.75	0.06	8
S4	-1.23	0.08	6

Figure 3: Performance Plot ($\delta^{13}C$ vs. RUN ID/time) based on the results of the quality control standard ETH-1 (certified value: $\delta^{13}C_{VPDB}$= 2.02 ‰).
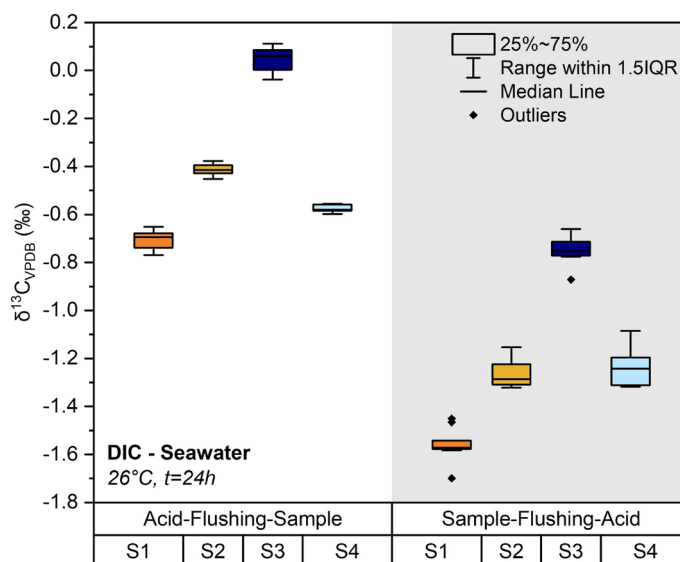
Figure 4: Boxplot visualization of the $\delta^{13}C$ results for the different samples (S1-S4) for the two different sample preparation techniques (A: Acid-Flushing-Sample; B: Sample-Flushing-Acid). The equilibration temperature was 26 °C and the equilibration time 24h. The length of the box is defined by the upper and lower quartile of the respective data set (lower quartile: 25% of the data points are smaller or identical to this value; upper quartile: 75% of the data points are smaller or identical to this value). Hence, the length of the box represents the range in which the central 50% of the data points are located. The line within the box represents the median of this data set. The end of the lower whisker represents the minimum value, the end of the upper whisker represents the maximum value of the respective data set. However, both are restricted to 1.5 times the interquartile range. The diamonds on top and below the boxes represent outliers.

There is a systematic difference in the $\delta^{13}C_{VPDB}$ data connected to the sample preparation technique (A vs. B; see Figure 4) with respect to accuracy and precision; highlighting the importance of this analytical step. If the sample preparation technique A is used, the external reproducibility is between 0.02-0.05 ‰; this is the standard deviation (SD) over 6-9 replicates distributed as triplicates in an acquisition with 100 sample measurements (Figure 4 and Table 1). The GasBench Plus IRMS System specification is ≤0.08 ‰ for 5 consecutive DIC seawater samples.

If the sample preparation technique B is used for DIC water samples, which is the established approach for solid carbonates, the $\delta^{13}C_{VPDB}$ data has a lower precision (SD on 3 replicates = data from 1 triplicate) and external reproducibility (SD over 6-9 replicates = data from 2-3 triplicates) due to drift in $\delta^{13}C_{VPDB}$ (Figure 4 and Table 1). This results in systematically lower $\delta^{13}C_{VPDB}$ values; the offset in this study is on average -0.8 ‰. The offset and the drifting $\delta^{13}C_{VPDB}$ values can be explained by the contamination of the fragile carbonate balance in the DIC water sample by atmospheric CO_2 during sample preparation. If the sample is added first, it will start interacting directly with the CO_2 (from air) in the headspace of the vial. In our experiment this results in shifting sample composition to lower $\delta^{13}C_{VPDB}$ (the $\delta^{13}C_{VPDB}$ of atmospheric CO_2 is ~-8.5 ‰ (status 2015, Graven et al., 2017)). This exchange is a function of temperature, time and the volume ratio between liquid and gas phase. The exchange is stopped as soon as the headspace in the vials is replaced by pure helium. However, we can demonstrate with this example that in a relatively short period of time (a few hours), at ambient temperature using a typical sample volume, contamination can be significant. And as vials are being flushed with the TriPlus RSH SMART Autosampler (flush time: 6 min), the exchange time is not constant resulting in the observed drift towards more negative $\delta^{13}C_{VPDB}$ over time.

Conclusion

$\delta^{13}C_{VPDB}$ analysis were conducted with the GasBench Plus IRMS System on DIC water samples investigating the importance of a thoughtful sample preparation to prevent atmospheric contamination. CO_2 from DIC water samples need to be extracted differently to solid carbonates by adding the acid first into the vial, closing the vial, flushing the headspace with helium and then sample injection with a syringe through the septum. If this is considered and applied, a $\delta^{13}C_{VPDB}$ precision of ≤0.08 ‰ for 5 continuously measured seawater samples can be expected.

References

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