Gasbench II analysis of dissolved inorganic carbon (DIC)

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Abstract

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Protocol

Consumables

Step 1.

Exetainer vials (Labco 736W or 9RK8W)

Spare caps (Labco VC309 or VC301)

Replacement septa (Labco VW101)

Phosphoric acid (42%, 1:1 dilution with MQ of 85% solution)

27 gauge single-use needles (BD305109)

22 gauge single-use needles (BD305156)

1 ml syringes (BD309659)

Syringe filters, 0.2µm PES

Preparatory Work

Step 2.

Remove caps, wrap in foil, and combust glass exetainers at 460°C for 6 hours in a combustion oven (best to start at the end of the day to run overnight).

Step 3.
Add 100µl 42% phosphoric acid and seal caps so that there is only a slight depression in the center. If a major/conical depression is in the center, the septa will be prone to leaking.

Step 4.
Flush the vials with helium for 3 min using 27 gauge needles. Insert one needle to serve as a flush needle and attach the other needle to the helium gas line.

Step 5.
IF you are interested in DIC CONCENTRATIONS, you must PRE-WEIGH all vials before adding samples. The pre and post-sample weights are used to determine the volume of sample added. Use a balance that is accurate to at least ± 0.2 mgSampling

Sampling

Step 6.
It is best to use a syringe filter to eliminate the possibility of adding solid particles especially carbonate particles to the vial.

Step 7.
For solutions/environmental waters with less than 1 mM DIC (e.g. fresh water systems) use a syringe to inject 2 ml of sample

Step 8.
For solutions/environmental waters with less than 3 mM DIC (e.g. background seawater) use a syringe to inject 1-2 ml of sample

Step 9.
For solutions/environmental waters with more than 3 mM DIC (e.g. methane seep pore waters) use a syringe to inject 0.2 -0.5 ml of sample.

Step 10.
Exact volumes are not important since you will re-weigh vials to determine this later. If you are sample limited, reduce the added volume and adjust the Gasbench II dilution settings or sample loop size as needed.

Step 11.
Prepare a solution of 20mM sodium bicarbonate to use for concentration determination and sample size dependency corrections. Add this solution to additional prepared vials (0-2ml; 2x5 point concentration curve + one for every 10 samples to be analyzed)

Step 12.
Re-weigh all vials and use the difference to determine the added volume.

Step 13.
Allow all samples to react with acid for a minimum of 3 hours. Samples can be stored for several months with no apparent loss of CO2 volume or fractionation.Sample measurement

Sample Measurement

Step 14.
DIC samples are analyzed on a Gasbench II coupled to a Delta V Plus IRMS (Caltech Stable Isotope Facility/Alex Sessions lab). Sample heating block should be set to room temperature. The double-needle headspace sampler injects a continuous flow of He (0.5 ml/min). Following water vapor removal with nafion tubing, samples are transferred to a 50 µl sample loop prior to separation by a PoraPlot Q fused silica column (25m; i.d. 0.32 mm) which was maintained at 72°C. A sample run consists of 3 reference CO2 peaks, 10 replicate sample injections, and 2 final reference CO2 peaks. See Torres et al (2005) for details about this method.

Step 15.
Run a 4-5-point standard curve at the beginning and end of the samples. This can be done using a 20 mM HCO3- solution and adding varying amounts to prepared exetainer vials. For example: 10 µmol – 0.5ml; 8 µmol – 0.4ml; 5 µmol – 0.25ml; 2 µmol - 0.1ml; 1 µmol – 0.05ml.

Step 16.
Using additional vials loaded with a range of volumes of the HCO3- solution, run a standard every 10-15 samples to assess instrument drift and to add additional standard replicates to the concentration vs. area curve.

Step 17.
Arrange samples in the run (as best as possible) from light to heavy such that the lightest (most 13C-depleted) samples are first, and the heaviest (most 13C-enriched) samples are last. In general,

keeping like with like will minimize the impact of potential memory effects in the instrument.

Step 18.

Note about highly 13C-labeled samples:

In highly labeled samples, the mass 45 peak may be the biggest. Due to the amplification settings, dilution over the typical 86% (77% if MS capillary is in) may be necessary. Programs are currently available for 86, 88, 90, 95, and 98% sample dilution. Peaks above 30V are more prone to sample size dependency effects on the δ13C, and peaks above 40V should be avoided by all means available (use less sample, use higher dilution). The subsequent samples will very likely be impacted by carryover and not provide interpretable data.

Step 19.
Concentrations of DIC were determined based on comparison of the average total peak area (masses 44, 45, 46) of replicate sample injections to a standard curve generated from a laboratory solution of HCO3-.

Step 20.

δ13C values were corrected for sample size dependency and then normalized to the VPDB scale with a two-point calibration (Coplen et al., 2006) using NBS-19 and a previously calibrated laboratory carbonate as internal standards. Accuracy was determined by analyzing independent standards as samples and precision was determined from NBS-19.

Step 21.
If necessary, use the tools associated with the mass chromatogram window to adjust peak integration. This should not be done wantonly. The computer is much better at consistently interpreting the beginning and end of peaks than a user. However, sometimes the computer fails at accurately integrating a peak and should be corrected.

Processing data from the Gasbench II for DIC concentration and δ13C: Processing data from the Gasbench II for DIC concentration and δ13C: Viewing and exporting data

Step 22.
On the Delta V computer, individual samples runs can be viewed using the Data Analysis window (other windows are Instrument Control and Data Acquisition). Samples are identified by a three part naming scheme: Gasbench run #_Sample Identifier 1_Sample Identifier 2, where the Identifier 1 is a sample name, and the Identifier 2 is an exetainer vial number (e.g. 15345_std1_127). The second identifier is not strictly necessary; however it does allow sorting by both sample names and pre-weighed vial numbers.a. Opening a sample will give you a three paneled window. The upper window shows the ratio traces for the CO2 masses measured (45/44 and 46/44). The middle window shows the amplitudes of the mass 44, 45, and 46 peaks as an overlaid chromatogram. The amplification at the faraday cups is increases from low to high mass (44 < 45 < 46), which means that in a natural sample the trace for mass 46 should be the highest peak followed by mass 45 and mass 44. The bottom window provides a summary of peak retention times, amplitudes, areas, and calculated values.

Step 23.
If necessary, adjust the standard values associated with the method. This may include the reference gas values, or which two of the five reference gas peaks to use for sample evaluation. Again, this should not be done without good reason. After making these changes, re-evaluate the chromatogram with the new values.

Step 24.
Save any changes made to sample chromatograms that are desired on closing the chromatogram. To cancel any changes, say no to save on closing.

Step 25.
Highlight to select all chromatograms to be exported. Right click and select ‘Reprocess’. In the pop-up window, select add template and choose the Orphan lab DIC export template. Other templates may not provide all of the needed columns. New templates can be made if other exported values are needed. Back in the main pop-up, adjust the name of the excel file to be generated.a. Reprocessing may take 20-30 minutes for 200 samples.b. This excel file will be located in the same folder as the raw data, which is anchored to the desktop. Gasbench data year month dd-mm-yyyyProcessing exported data

Processing data from the Gasbench II for DIC concentration and δ13C: Processing exported data

Step 26.
Use the ‘Gasbench DIC worksheet 2017 ksd’ excel file as a template for processing new data.

Step 27.
Copy and paste the data exported into the tab labeled ‘exported data’.

Step 28.
From ‘exported data’ copy and paste the ‘Date’, ‘Time’, ‘Row’, ‘Identifier 1’, ‘Identifier 2’, ‘Method’, ‘Sample Dilution’, and ‘Area All’ columns onto the existing spaces for them in the ‘raw’ tab. Next copy and paste the ‘rArea All’, ‘Area 44’, and ‘Ampl 44’ columns. Repeat for ‘Area 45’ and ‘Ampl 45’; ‘Area 46’ and ‘Ampl 46’; ‘R 45CO2/44CO2’, ‘R 46CO2/22CO2’, and ‘d 13C/12C’; ‘R 13C/12C’, ‘AT% 13C/12C’, and ‘d 18O/16O’; and finally ‘R18O/16O’ and ‘AT% 18O/16O’.a. Do not overwrite the top three rows. These are necessary for the transfer of data from this tab to the next one.

Step 29.
Scroll over to columns W and X. After every 15 rows, the average (col W) and standard deviation (col X) are calculated for the sample peaks. Manually, check the data by examining the standard deviation. It should remain less than 0.5‰ for natural abundance samples, and as low as possible for 13C-enriched samples.a. High standard deviations indicate a problem in the sample run that will require manual adjustment of the range selected for this calculation.i. This can happen for a number of reasons. Carry-over due to incomplete venting of the previously injected sample (typically 1-3 peaks, values that clearly belong to the previous sample followed by a higher amplitude and a new value), carry-over due to the presence of an abnormally high positive and large peak in the previous injection (high value that trends to a lower more consistent value after a few peaks), and clogs in the transfer line that result in missing peaks or poorly measured peaks.b. In addition to adjusting the calculation to account for injection problems, occasionally a sample may not have 10 replicate injections. This could be due to a clog, a blank sample, or a low concentration sample that became below detection.i. Each sample MUST have 10 lines for the transfer of data to the next tab. For blanks, delete the entire sample. For missing peaks, insert lines at the start of the sample.c. Once the average and standard deviation for all samples have been checked, copy the W and X columns onto the AB and AC columns, and then copy the W column onto the I, M, P, and S columns.

Step 30.
The ‘extracted’ tab collects data from every 15th row and compiles it. Check that the rows are filled in with values from the ‘raw’ tab.a. There may be blanks that will indicate a location that requires inserted lines. Fix this as necessary.

Step 31.
Copy and paste special the values from ‘extracted’ into ‘just values’. This can now be sorted by the vial number in ‘Identifier 2’, which will make it easier to insert a columne for and transfer the sample

volumes determined from the difference in the initial and final vial weights (’12 ml exetainer vial for DIC’ tab)

Step 32.
After adding the volume data, ‘just values’ can be sorted to examine the standard values. These can then be pasted into the appropriate columns in the ‘DIC standards’ tab.

Step 33.
For the lab HCO3- standards, update the concentration in the ‘HCO3 mM’ column for the mixture used. Then generate a µmol HCO3 vs Area All curve. The R2 of the linear fit should be greater than 0.9.a. If there are outliers consider whether they must be included.i. Was this standard injected multiple times?ii. Was this standard injected during an carry-over or a clog event?iii. Is this standard clearly misidentified?b. Copy and paste the slope and intercept in the box next to the graph, and update the n value for the number of standards used to generate the curve.

Step 34.
Next determine the sample size dependency by plotting δ13C vs 1/area. This will have a logarithmic fit. Again, if there are outliers, consider if they must be included.a. In the box below the plot, update the slope and intercept. Examine the difference between the pre-correction and post-correction average value for δ13C. There will likely be a slight difference, but it should not be greater than 0.5‰.

Step 35.
Finally, the Hydrate Ridge (δ13C -45.93), NBS-19 (δ13C 1.95), and the lab HCO3 (δ13C -2.9) can be used to perform a correction for any offset that may have occurred with the instrument during the run. Compile the average measured value for each of these from the lin corr d13C column.a. If there is an obviously spurious value in these standards, DO NOT include it here.b. Update the slope and intercept and examine the 2-pt (really 3-pt) corr average and standard deviation.

Step 36.
8-10 can be repeated for the oxygen data if desired.

Step 37.
Transfer the lin corr standard values from the ‘DIC standards’ tab to the appropriate locations in the ‘Longterm precision and accuracy’ tab.a. Make sure that the avg, n, and stdev calculations include the updated data.b. This precision and accuracy should be reported with publication. A run specific calculation should also be made.

Step 38.
Transfer the slopes and intercepts to the top of the ‘Samples all’ tab.

Step 39.
Copy and paste sample data from ‘just values’ into the appropriate locations.

Step 40.

Compile the corrected data for specific projects into individual new tabs if desired.After two decades a second anchor for the VPDB δ13C scale. Rapid Communications in Mass Spectrometry 20, 3165-3166.

Useful references

Step 41.

Torres, M.E., Mix, A.C., and Rugh, W.D. (2005). Precise δ13C analysis of dissolved inorganic carbon in natural waters using automated headspace sampling and continuous-flow mass spectrometry. Limnol. Oceanogr.: Methods 3, 349-360.

Coplen, T.B., Brand, W.A., Gehre, M., Gröning, M., Meijer, H.A.J., Toman, B., and Verkouteren, R.M. (2006).