



Environmental Analysis Program

Pomona College
Biogeochemistry Laboratory

SOP-L415

Carbonate Isotope Analysis by GasBench II-IRMS

Standard Operating Procedure

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1. Purpose

This SOP describes the determination of $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ in carbonate minerals and sediments using phosphoric acid digestion and headspace analysis on the GasBench II coupled to the Delta V IRMS (Coplen, 2011; Kim et al., 2007; Swart et al., 1991). It covers sample preparation, vial flushing and acidification, the analytical run, and post-analysis data correction.

For general instrument operation, startup/shutdown, gas checks, tuning, and pre-analysis tests, see [SOP-L208](#).

2. Scope

- **Applies to:** Carbonate-bearing samples (calcite, dolomite, aragonite, shell, foraminifera, soil carbonates, cement, and similar materials) analyzed for $\delta^{13}\text{C}$ (VPDB) and $\delta^{18}\text{O}$ (VPDB or VSMOW) by phosphoric acid digestion and continuous-flow IRMS.
- **Does not apply to:** DIC in water, breath gas, CO_2 at atmospheric concentrations, or water equilibration methods (these use the GasBench but with different sample preparation and Isodat methods).
- **Location:** Sample preparation in SGM Rm 133; analysis in the Oxtoby Isotope Lab, SGM Rm 135.

3. Responsibilities

Role	Responsibility
Lab PI	Approve methods, oversee data quality
Lab Manager	Prepare/verify Isodat methods; maintain GasBench and PAL
Certified User	All steps from sample prep through data reduction
Student Researcher	May perform sample prep and data reduction under supervision

4. Definitions

- **Acid fractionation factor (α^*):** The equilibrium isotope fractionation between carbonate mineral and CO_2 produced by phosphoric acid digestion; temperature-dependent and mineral-specific (Kim et al., 2007).
- $\delta^{13}\text{C}$ (VPDB): Stable carbon isotope ratio in per mil (‰) referenced to Vienna Pee Dee Belemnite (VPDB); the conventional scale for carbonate and organic carbon (Coplen, 2011).
- $\delta^{18}\text{O}$ (VPDB or VSMOW): Stable oxygen isotope ratio in per mil (‰); carbonates are commonly reported on VPDB, which relates to VSMOW by $\delta^{18}\text{O}_{\text{VPDB}} \approx 0.97001 \times \delta^{18}\text{O}_{\text{VSMOW}} - 29.99$ (Coplen, 2011).
- **Exetainer vial:** 12 mL borosilicate glass vial with a septum-capped lid used for sample reaction and headspace analysis.
- **GasBench II:** Continuous-flow peripheral that samples headspace CO_2 from sealed exetainer vials through a flushed needle system and delivers it to the IRMS via the ConFlo IV.

- **PAL autosampler:** CTC Analytics autosampler integrated with the GasBench that positions vials under the injection needle for automated headspace sampling.
- **Phosphoric acid digestion:** Reaction of carbonate with 100% H_3PO_4 at 50 °C to release CO_2 quantitatively from calcite and aragonite (Swart et al., 1991).
- **VPDB (Vienna Pee Dee Belemnite):** The primary international reference scale for $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ in carbonates.
- **VSMOW (Vienna Standard Mean Ocean Water):** The primary international reference scale for $\delta^{18}\text{O}$ and $\delta^2\text{H}$ in water and atmospheric gases.

5. Safety and Hazards

See [SOP-L208, Section 4](#) for the full hazard list. Hazards specific to this application:

Application-Specific Hazards

- **100% phosphoric acid (H_3PO_4):** Corrosive. Handle in the fume hood. Wear nitrile gloves and safety glasses at all times.
- **Pressurized vials:** Flushed vials contain He under slight pressure; use the PAL needle carefully to avoid splashing acid.

5.1. Required PPE

- ☐ Safety glasses
- ☐ Lab coat
- ☐ Nitrile gloves (acid handling and vial preparation)

5.2. Required Training

- [SOP-L002 Laboratory Safety](#)
- [SOP-L003 Hazardous Materials Handling](#) (acid handling)
- [SOP-L208 IRMS General Instrument Use](#) (must be a certified IRMS User)

6. Equipment and Materials

Item	Location	Notes
Exetainer vials (12 mL)	SGM Rm 133	Check septum integrity before use
100% phosphoric acid	Fume hood, SGM Rm 133	Corrosive; store in acid cabinet
He gas (ultra-high purity)	SGM Rm 135	For vial flushing
Flushing manifold / needle	SGM Rm 133	10-port manifold preferred
Micro-spatula	SGM Rm 133	For weighing carbonate powder
Micro-balance	SGM Rm 133	Tare before each vial
GasBench II + PAL autosampler	SGM Rm 135	Confirm needle and trap condition

Item	Location	Notes
Certified reference materials	SGM Rm 133	e.g., NBS-18, NBS-19, IAEA-603
Heating block (50 °C)	SGM Rm 133	For overnight reaction

7. Estimated Time

Task	Time
Vial preparation and flushing	1–2 h (for a 40-vial batch)
Acid addition and reaction	1 h (calcite); overnight (dolomite)
Analytical run (per injection)	~8 min x 11 injections/vial
Data reduction (R script)	30–60 min

8. Procedure

8.1. Vial Preparation

1. Label exetainer vials with sample ID and position number.
2. Weigh 0.1–0.5 mg carbonate powder into each vial (adjust for expected calcite content).
3. Cap vials tightly with septum caps.
4. Flush each vial with He (UHP) for 5–8 min using the flushing manifold to remove atmospheric CO₂.

8.2. Acid Addition

1. In the fume hood, add 3–5 drops (~100 µL) of 100% H₃PO₄ to each vial through the septum using a syringe.
2. Incubate at 50 °C for 1 h (calcite and aragonite) or overnight (dolomite) in the heating block.
3. Allow vials to equilibrate to room temperature before loading into the PAL tray.

8.3. Sequence Design

1. Open Isodat Sequence Editor and load the GasBench carbonate method.
2. Build the sequence: 3 blank vials (flushed, no sample), reference materials, unknowns (1 RM per 8 unknowns), check standards.
3. Set the PAL to collect 11 injections per vial; the first 2–3 are discarded in post-processing.
4. Save the sequence file as YYYYMMDD_Project_SeqID_User.seq.

8.4. Analytical Run

1. Complete the pre-analysis checklist in SOP-L208.
2. Load the PAL tray with vials in correct order.
3. Press **Start** in Isodat and monitor the first 5 injections.

8.5. Post-Analysis Data Correction

Apply corrections using the R script in the project repository:

```
library(tidyverse)

raw <- read_csv("data/raw/YYYYMMDD_Project_SeqID_User_raw.csv")

# Each vial has 11 injections; discard injections 1-3
clean <- raw |>
  filter(injection >= 4) |>
  group_by(vial_id) |>
  summarise(
    d13C_mean = mean(d13C_raw),
    d18O_mean = mean(d18O_raw),
    d13C_sd   = sd(d13C_raw),
    d18O_sd   = sd(d18O_raw),
    n_inj     = n()
  )

# Scale normalization (two-point) using certified RMs
# Drift correction across run order
# QC: flag vials where within-vial SD > 0.15 permil
```

9. Quality Control and Acceptance Criteria

- Reference materials must recover within $\pm 0.2\text{‰}$ ($\delta^{13}\text{C}$) and $\pm 0.3\text{‰}$ ($\delta^{18}\text{O}$) of certified values after corrections.
- Within-vial SD (injections 4–11) must be $< 0.15\text{‰}$ for both isotopes.
- Blank vials must produce no CO_2 peak above noise.

10. Data Management

1. Export raw Isodat data immediately after the run as `.csv`.
2. Save as `data/raw/YYYYMMDD_Project_SeqID_User_raw.csv` in the project repository.
3. Commit raw data and the correction script per [SOP-L801](#).

11. Waste Disposal

Waste Type	Disposal Method
Used exetainer vials and acid	Acid waste carboy per SOP-L006
Gloves, Kimwipes	Standard trash unless acid-contaminated

12. References

- Coplen, T. B. (2011). Guidelines and recommended terms for expression of stable-isotope-ratio and gas-ratio measurement results. *Rapid Communications in Mass Spectrometry*, 25(17), 2538–2560. <https://doi.org/10.1002/rcm.5129>
- Kim, S.-T., Mucci, A., & Taylor, B. E. (2007). Phosphoric acid fractionation factors for calcite and aragonite between 25 and 75 °C: revisited. *Chemical Geology*, 246(3–4), 135–146. <https://doi.org/10.1016/j.chemgeo.2007.08.005>
- Swart, P. K., Burns, S. J., & Leder, J. J. (1991). Fractionation of the stable isotopes of oxygen and carbon in carbon dioxide during the reaction of calcite with phosphoric acid as a function of temperature and technique. *Chemical Geology: Isotope Geoscience Section*, 86(2), 89–96. [https://doi.org/10.1016/0168-9622\(91\)90055-2](https://doi.org/10.1016/0168-9622(91)90055-2)

13. Related SOPs

SOP	Title	Relationship
L002	Laboratory Safety	Required prerequisite
L003	Hazardous Materials Handling	Acid handling
L208	IRMS General Instrument Use	Instrument-level procedures
L414	Soil CN by Flash EA-IRMS	Companion application SOP
L802	Quality Control and Quality Assurance	General QC framework

14. Training Record

Before performing carbonate isotope analysis independently, personnel must:

1. Read this SOP and [SOP-L208](#) in full
2. Be a certified IRMS User (SOP-L208, Training Record)
3. Complete [SOP-L003 Hazardous Materials Handling](#) (acid handling)
4. Observe a GasBench carbonate run from vial preparation through data export
5. Perform a supervised run with acceptable QC results
6. Complete a supervised data reduction session
7. Be signed off by the lab manager or PI