Smart Notes

Q A

How do I measure clumped Δ_{47} from carbonates using the Thermo Scientific portfolio?

What are clumped isotopes?

Clumped isotopes refer to rare isotopologues in natural materials which are characterized by having two or more heavy isotopes (D, ^{13}C, ^{15}N, ^{18}O, etc.)[1]. Clumped isotopologues are naturally very rare and can be used to investigate past climate changes by reconstructing temperatures from diverse archives. Clumped carbonate isotope thermometry (Δ_{47} and Δ_{48})[2-4] is used to tackle paleoclimate reconstruction based on the temperature dependence of the abundance of ^{13}C-^{18}O bonds in the carbonate lattice[5]. Paleo-studies are undertaken on samples such as foraminifera, omnipresent fossil species that precipitate calcite shell. These shells are commonly made of calcium carbonate and therefore offer an excellent source for carbonate clumped isotopes analysis. Due to their limited availability in certain samples and extensive labor work, required to manually pick out individual foraminifera, highest sensitivity at full precision is needed for performing clumped isotope analysis on IRMS systems.

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How can clumped isotopes be measured?

The analysis of clumped isotopes requires Isotope Ratio Mass Spectrometry.

Thermo Scientific solutions (Figure 1) for the analysis of clumped isotopes include:

- Thermo Scientific™ Kiel IV Carbonate Device

- Thermo Scientific™ 253™ Plus 10 kV IRMS

- Thermo Scientific™ Qtegra™ Intelligent Scientific Data Solution (ISDS) Software with Thermo Scientific™ LIDI 2 Software Workflow

The analysis of carbonate clumped isotopes with the Thermo Scientific portfolio is a step-by-step process, from sample weighing to results allowing precise and accurate determination of Δ_{47} from smallest samples.

Figure 1. Kiel IV Carbonate Device (left) coupled to a 253 Plus 10 kV IRMS (right).

The Kiel IV Carbonate Device is an automated sample preparation unit employing acid digestion of carbonates with ~105% H_3PO_4. The released CO_2 is purified from water using a cryogenic trap and cryo-focused in a 50 µL microvolume before measurement. Optionally, a Peltier-cooled Purification Trap can be installed in the Kiel IV Carbonate Device for removal of potential organic contaminants (Figure 2). For measurement of the generated sample CO_2, the 253 Plus 10 kV IRMS is used, equipped with a dedicated collector for isotopologues on masses 44 to 49 and monitoring of the pressure baseline at mass 47.5.
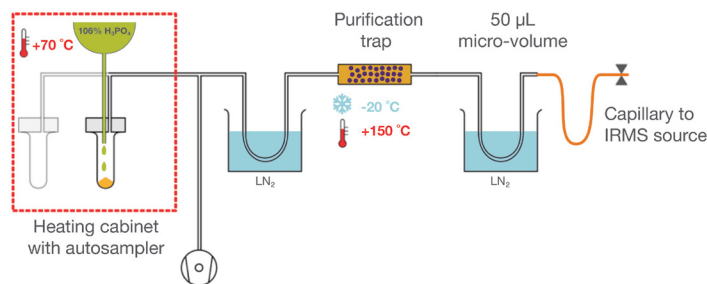
Figure 2. Simplified schematic layout of the Kiel IV Carbonate Device.

Analytical setup

For accurate and precise measurement of clumped Δ_{47} from carbonate minerals, the following prerequisites must be met:

- Capillary crimping (matching of capillary flow rates)

  – Good matching of the sample and reference capillary flow rates is the key to obtaining accurate and precise data. To ensure the same signal depletion rate for both sample and reference signal during a LIDI measurement, crimping should be matched to <50 mV.

- Determination of the "signal up" parameter

  – The "signal up" refers to the signal increase when the Dual Inlet valve between bellow and Changeover valve is closed to mimic a microvolume. This apparent increase is caused by temporary gas displacement due to physical valve switching and is corrected for by the Qtegra ISDS Software automatically. The "signal up" parameter is determined after the capillaries are matched

- Preparation of phosphoric acid

  – Prepare phosphoric acid with a concentration between 104 and 106% by adding a suitable amount of phosphourous pentoxide to the phosphoric acid. This ensures the acid to be effectively water-free. Do not exceed 106%, otherwise the acid becomes too viscous

- Preparation of the purification trap (optional)

  – Before the first measurement and on a regular basis afterwards, the trapping material must be replaced. The frequency of replacement depends on the type of samples measured. A replacement every 1 to 3 months is sufficient for most applications

- Reference refill

  – Qtegra ISDS Software enables setting a target pressure for the reference gas bellow. Use a pressure which is typically expected from your respective sample weights, and set the bellow position to 80 or 90% to allow for pressure adjustment in both directions

Measurement workflow
Sample weighing
Carbonate samples for clumped isotope measurements must be stored in sealed containers inside a dry cabinet to avoid any impact from moisture.

- Clean all equipment used for weighing (spatulas, tweezers) with analytical grade methanol

- Weigh 80 to 120 µg of sample into aluminum weighing pans using a high precision balance

- Precise weighing is particularly important for LIDI style measurements.

- Transfer samples into the Kiel IV Carbonate Device vials and ensure that all carbonate accumulates at the bottom of the vial

After weighing, the samples are measured immediately or stored in the upper section of the Kiel IV Carbonate Device oven at 70 °C, thereby excluding condensation of moisture.

Long Integration Dual Inlet (LIDI)
The Kiel IV Carbonate device is designed for smallest carbonate sample amounts. To obtain the highest precision optimal sample utilization is required. This is achieved by LIDI (Long Integration Dual Inlet), where sample and reference are measured consecutively rather than alternatingly (Figure 3).

LIDI 2 Software Workflow offers a drift correction by standardization of the sample data with the two enclosing reference data sets ("Bracketing"). Especially when lab temperature fluctuation exceeds 0.1 °C/h, the usage of LIDI 2 Software Workflow is recommended and can significantly improve the analytical precision.[6]

Setup of the method
Setup a method for LIDI using the parameters in Table 1. The pressure for the reference refill and the "signal up" parameters need to be determined as manually. Please note that the following parameters are recommendations only and may differ depending on sample type employed.

Table 1.

Method Parameters	
Interference correction	Enabled for automatic pressure baseline correction at mass 47.5
Acquisition mode	LIDI or LIDI 2 Software Workflow
Integration time	6.71
Reference refill pressure	As determined
Number of cycles	90 cycles
Idle time	30 s
Pressure Adjust mode	Sample (Master Bellow)
Signal up	As determined, typically 2-5%
Cooling temperature*	-20 °C (recommended)
Baking temperature*	150 °C (recommended)
Baking time*	60 min (recommended)
Cool after baking*	Enabled, if next sequence follows within a day

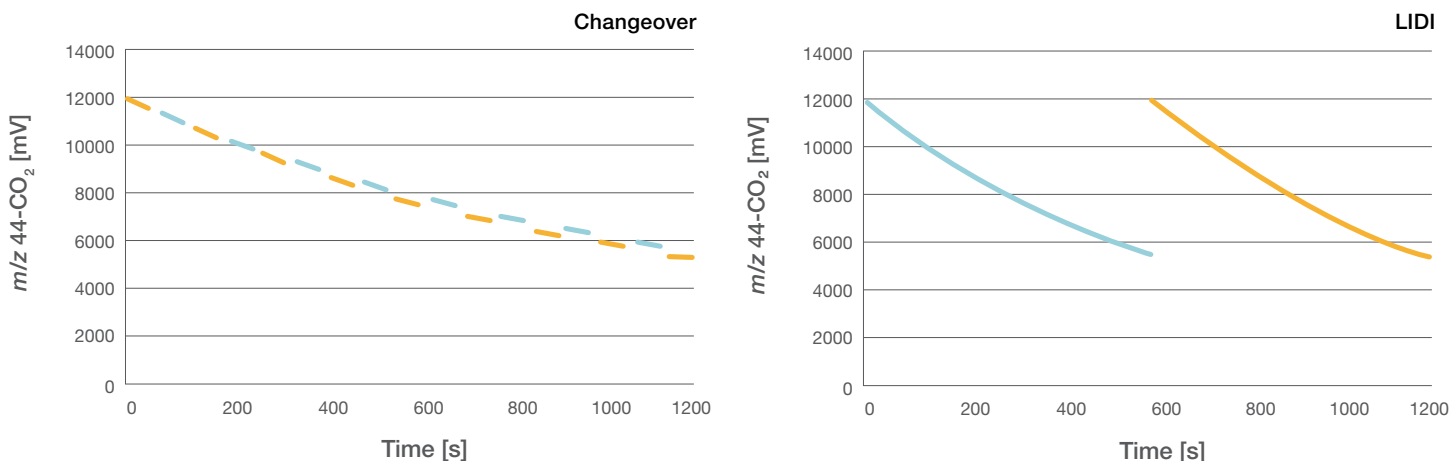* only available with optional Purification Trap

Figure 3. Signal sequence for one conventional Dual Inlet measurement (left) and one LIDI measurement (right). Blue: Sample, Yellow: Reference data.

Figure 4. Layout of a typical sample list. In between sets of samples with unknown isotopic composition (3) two different standard types can be placed; (1) Internationally referenced standard materials ("Delta Standard"), which are used for external referencing of the unknowns. Qtegra ISDS Software will automatically perform an external 1-, 2-, or multi-point referencing, depending on how many Delta Standards are analyzed in the run. (2) In-house carbonate standards ("QC Standard", cf. Figure 6) which are used to monitor the quality of the analyses throughout the sample list. If a measured QC Standard deviates more than a user-defined value from the expected number, Qtegra ISDS Software assumes a major problem with the system (e.g. a leak) and can automatically stop the acquisition to prevent loss of precious samples.

Typically, a sample list consists of several standards of known isotopic composition and unknown samples (Figure 4). While the sorting of samples and standards inside the sequence is solely dependent on the standardization approach, it is recommended to measure each standard on both sample lines. This facilitates the identification of a possible leak or contamination affecting one of the sample lines.

Baseline interference correction

The Clumped-CO_2 collector of the 253 Plus 10 kV IRMS is equipped with a cup at mass 47.5, dedicated to baseline monitoring. The beam intensity on $m/z=47$ is corrected by using the baseline signal and k-factor, which is a coefficient describing the proportionality between the baseline at mass 47.5 and the actual signal at mass 47 (Figure 5, Formula 1). Determination of the k-factor using a mass scan (Figure 5) is recommended on a daily basis.

The k-factor for mass 47 is determined using the following procedure:

- Adjust the focussing in the TuneBook as for the measurement

- Use CO_2 from the reference bellow and adjust the beam intensity at mass 44 to 15000 – 20000 mV. It is good practice to use the same intensity each time the k-factor is determined

- Perform a peak shape scan across the whole mass 47 peak +/-20 V on both sides (cf. Figure 5)

- Visually determine the pressure baseline on the mass 47 peak as shown in Figure 5. Alternatively, export the scan data and, use a linear fit of a spreadsheet software (e.g., Microsoft™ Excel)

- The relevant baseline values ^{47}I and $^{47.5}I$ are obtained from this pressure baseline (PBL) and the baseline signal on the monitoring cup at the position of the peak center

- The k-factor is given by the equation (Formula 1):

$$K = -\frac{^{47}I}{^{47.5}I}$$

Attention: The above equation considers both ^{47}I and $^{47.5}I$ to be negative.
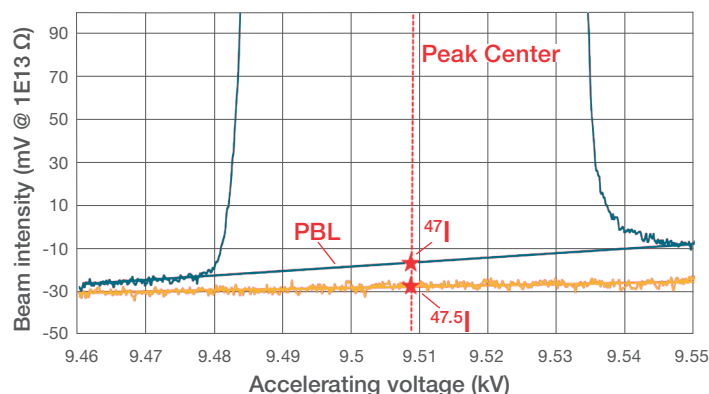
Figure 5. Determination of the k-factor. The blue line is the visually determined pressure baseline of the peak at mass 47 (linear fit).

Entering the k-factor in Qtegra ISDS Software

1. Open the LabBook and navigate to Method Parameters -> Delta Calculation.

2. To correct mass 47 with the baseline at mass 47.5, select mass 47.5 as trace and enter the previously determined k-factor in the section "Interference Correction" (Figure 6, point 4).

3. Save the LabBook. Note that in a successfully ran LabBook, the interference correction can be switched on and off and the data is immediately re-evaluated.

Figure 6. Delta Calculation parameters in a Clumped Carbonate LabBook. (1) Select automated delta calculation approach and (2) select the in-house bottle CO_2, define the ratios of interest, and define the outlier threshold. (3) Define the warning and failure limits of the QC standards, if applicable. (4) Define the baseline interference correction.

Running the LabBook

Before starting the LabBook perform the following cleaning procedures on the Kiel IV Carbonate Device to ensure smooth hardware operation and highest quality data:

- Wipe off any particles from the O-rings which seal off the vials. Occasionally, a tiny amount of grease, suitable for high temperature and high vacuum, should be put on the O-rings

- Use lint-free paper, carefully remove droplets of phosphoric acid which may be present at the tip of the acid capillary or the drop detection wire

- Run an automated Acid Drop Test and release at least 20 acid drops into an empty vial to remove "old" acid in the acid line. Generally, the drop test is successful, when the time period in between two acid drops has stabilized to a value around 20 seconds.

Start the LabBook in the Qtegra ISDS Software. The following steps will now be executed automatically:

- Setting the purification trap to the desired cooling temperature (if purification trap is installed)

- Pre-reference measurement (when LIDI 2 Software Workflow is used)

- First sample preparation and subsequent measurement

- Further reference and sample measurements, and concurrent sample preparations (Figure 7)

- Baking of the purification trap at the end of the sequence (if purification trap is installed)
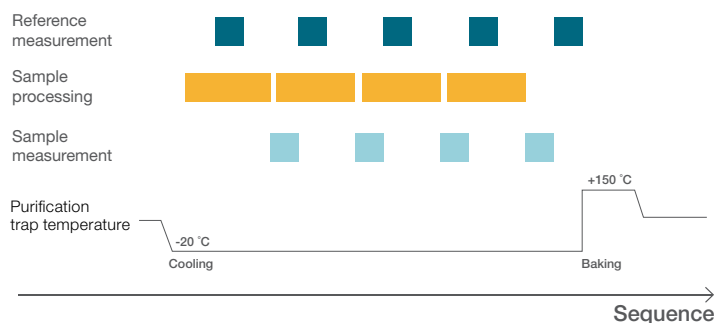
Figure 7. Graphical visualization of a LabBook run with 4 samples.

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4. Fiebig, J., Daëron, M., Bernecker, M., Guo, W., Schneider, G., Boch, R., Bernasconi, S.M., Jautzy, J., Dietzel, M. (2021), Geochimica et Cosmochimica Acta, 312, 235-256.

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6. Thermo Scientific Technical Note TN0668 (2019) LIDI 2 Software Workflow: Overcoming challenges for clumped isotopes CO2 analysis on carbonate samples.

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