# $\delta^{13}C$ and/or $\delta^{18}O$ analysis of calcium carbonate, including bioapatite carbonate (structural carbonate in bioapatite of bones and teeth)

## General considerations

The $\delta^{13}C$ and $\delta^{18}O$ values of calcium carbonates ($CaCO_3$; calcite, aragonite), including the structural carbonate in skeletal bioapatite, can be determined simultaneously.

For isotopic analysis of pure CaCO3, less than 1 mg of material is enough for several replicate analyses. We usually weigh ca. 180 µg CaCO3 for analysis.

For skeletal apatites, i.e. for the isotopic analysis of the structural carbonate component of bioapatite, more sample material is needed, because the bioapatite mineral contains only ca. 10% structural carbonate by weight. A minimum of 5 mg is weighed in for pretreatments, but if possible, a starting weight of 10 mg is preferred. Bone, dentine and dental enamel contain different amounts of bioapatite and organic components, which also affects the sample weights needed for analysis. For dentine and bone, which have higher contents of organics and structural water, higher starting weights are preferred. Especially in cases where the skeletal specimens are not in pristine condition, a larger proportion of sample is lost during pretreatment steps, and a higher starting weight of 15 mg is recommended. Contact us at stableisotopes@helsinki.fi for the recommended exact weighing amounts.

Natural materials always contains a certain degree of isotopic variability on top of which come possible effects of sample preparation, and we **recommend analyzing more than one replicate per sample** to get a realistic estimate of the uncertainty for the isotope value. Note that this is a different thing than analytical uncertainty quoted in the analytical report, which only measures the precision of the instrument. If analyzing the whole set of samples in duplicate/triplicate is not feasible, we recommend choosing a representative set of samples, e.g. 10-30% of total sample number, to be analyzed in duplicate or triplicate.

For skeletal bioapatites, the typical external reproducibility, i.e. including effects of pretreatment, will be estimated based on replicates of an in-house quality control enamel material included in each run. Nonetheless, we recommend selecting a few of your own samples for duplicate measurement, especially if **a)** your samples are not tooth enamel (like the lab's quality control material), **b)** your samples are in variable condition and don't all show pristine preservation (like the lab's quality control material material).

However, as prices are charged per analyzed replicate, the decision of replicate IRMS measurements is left up to the customer's discretion.

## Pretreatment of skeletal carbonates

The chemical pretreatment follows Bocherens et al. 1996 with modified reaction times. Organic substances are oxidized with a 17 hour treatment in 2.5% NaOCl in room temperature, after which the powders are treated with a 1 M Ca acetate+CH3COOH buffer solution for 30 minutes to remove possible secondary carbonate phases. After MilliQ washes, the powders are dried at 40-50°C over 48 hours. The total mass of sample retained is weighed and recorded, and loss in pretreatment is calculated to help evaluate sample preservation. Poorly preserved samples show larger pretreatment losses. Sample powders are stored in a desiccator cabinet until weighed for analysis.

$$Pretreatment\ loss\ \% = \frac{sample\ dry\ weight\ into\ analysis}{collagen\ dry\ weight} * 100$$

## Sample preparation

Completely dry and homogenized sample powders, ca. 0.100-0.150 mg of pure CaCO3, or 2.5 mg of pretreated bioapatite powder is weighed in for IRMS analysis. The samples are weighed into Labco Exetainer 12 ml borosilicate glass vials (see details below), after which they are dried in a vacuum oven at room temperature for a minimum of 48 hours. The vial caps are then closed, the vials flushed with He gas, and 8 drops of >100% $H_3PO_4$ added to release $CO_2$ from the carbonate. The reaction takes place for 18 hours at an isothermal heating block set at 25°C.

If you're weighing your samples yourself, make sure that the material is finely ground (no grits or chunks) and focus at getting all the material to the bottom of the vial. Sample powders remaining on the walls of the vial will not react with H3PO4, preventing complete conversion of carbonate to CO2 for analysis. You should use a microbalance giving you 3 decimal digits below 1 mg, i.e. 0.001 mg (/ 1 µg) precision. Aim at a constant weight with a precision of ± 10% throughout your sample series. For example, aiming at a mean weight of 3.00 mg for a bioapatite, you would accept sample weights from 2.70 to 3.30 mg.

| Manufacturer | Product | ID | Size (mm, W x H) | Used for sample types |
|---|---|---|---|---|
| Labco Ltd | Exetainer 12ml Vial - Round Bottom | 938W, 939W | 15.5 x 101 (with cap) | carbonates |

*Table 1: Vials used for carbonate isotope analyses at the Laboratory of Chronology.* ***No other vial type can be accommodated.***

## IRMS-analysis and data normalization

The isotopic composition of carbon and oxygen is measured using a Thermo Scientific GasBenchII coupled to a Delta V series isotope ratio mass spectrometer in continuous flow mode. Alongside samples, each analytical run contains ca. 30% reference materials that are used for calibration (i.e. normalization) of isotope values and for quality control. The isotope values of the samples and QC materials are normalized using the known isotope values of IAEA-611, Nbs-18 and Nbs-19 calcite materials. As QC materials we include an in-house calcite powder, and for bioapatites, an in-house enamel powder treated identically with your samples. Data normalization is performed using the 'LIMS for Light Stable Isotopes' software developed by Tyler Coplen of the Reston Stable Isotope Laboratory at the US Geological Survey. The long-term analytical precision is ± 0.15 for both $\delta^{13}C$ and $\delta^{18}O$ values.

In addition to the requested isotope values, the report of analysis a method description and standard deviations of the reference materials to evaluate precision and external reproducibility. If the customer wishes to also receive a copy of the uncorrected, 'raw' data, this can be made available upon prior agreement.

## References:

Bocherens H., Koch P., Mariotti A., Geraads D., Jaeger J.-J., 1996. Isotopic biogeochemistry (13C, 18O) of mammalian enamel from African Pleistocene hominid sites. Palaios 11, 306-318.