$\delta^{18}O$ analysis of phosphate (-PO_4) extracted from bioapatite of skeletal samples

The oxygen isotope values of skeletal samples hold information about the $\delta^{18}O$ value of the drinking water of the animal. This information is useful for geographic tracking of organisms in e.g. archaeological and forensic research, and for palaeoclimatic research. The mineral part of vertebrate skeletal tissue, hydroxyapatite, contains oxygen in two main components, both of which are commonly analyzed for $\delta^{18}O$ values. While the isotopic analysis of the carbonate component is easier, the $\delta^{18}O$ value of the phosphate ($\delta^{18}O_{PO4}$) component is appreciated as more reliable, as the phosphate fraction is much more resistant to possible post depositional alteration in the burial environment. Additionally, much less sample material is needed for a $\delta^{18}O_{PO4}$ analysis while also most calibration equations relating the skeletal $\delta^{18}O$ value to the $\delta^{18}O$ value of the ingested water are for $\delta^{18}O_{PO4}$.

Oxygen is extracted from the bioapatite mineral as silver phosphate (Ag_3PO_4) for IRMS analysis. The extraction procedure involves many stages of precise, time and concentration demanding manual labour, and there are only a handful of laboratories world-wide offering the extraction and IRMS-analysis of Ag_3PO_4 as a service. We have long experience in IRMS-analysis of Ag_3PO_4, and our performance has been validated in an interlaboratory comparison study (Watzinger et al. 2021).

General considerations

Of the different skeletal tissues, the preferred sample material is dental enamel due to its superior preservation and high mineral content. Dentine and bone contain larger amounts of organic matter, making them more susceptible to diagenetic alteration. The amount of material needed for a $\delta^{18}O_{PO4}$ analysis is very small. For enamel, a minimum of 0.300 mg is needed for a single $\delta^{18}O_{PO4}$ analysis, while the corresponding amounts for dentine and bone are 0.500 and 0.600 mg, respectively. For comparison, the $\delta^{18}O$ analysis of structural carbonate oxygen requires a minimum starting sample weight of (3-)5 mg depending on sample condition.

Oxygen isotope analysis is generally more sensitive to disturbances in analytical conditions, and this often results in a larger standard deviation (i.e. less precise) around the $\delta^{18}O$ values. On top of the poorer internal analytical precision, additional uncertainty stems from the isotopic variability intrinsic in all natural materials, degree of sample homogenization as well as the pretreatment and extraction procedure. We strongly 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 from repeated reference Ag_3PO_4 material standard deviation. If extraction and analysis of 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 extracted and analyzed in duplicate or triplicate. However, as prices are charged per extracted and analyzed replicate, the decision of replicate IRMS measurements is left up to the customer's discretion.

Ag_3PO_4 extraction

The procedure of Ag_3PO_4 extraction is based on Widemann-Bidlack et al. (2008) with some modifications, like an extended precipitation time, discussed in Sahlstedt and Arppe (2020). The sample powders are pre-treated for organic contaminants with 2.5% NaOCl, washed to neutrality, dissolved in 2M HNO3 and precipitated as Ag_3PO_4 with an ammoniacal silver ammine solution after precipitation of interfering Ca ions with 2M HF. The precipitation of Ag_3PO_4 takes place in a 50 °C water bath for 30-48 hours. The crystals are washed, transferred to silver capsules, dried at 50°C, weighed, closed and stored in a vacuum oven until IRMS-analysis.

Sample preparation

The Ag_3PO_4 sample is analyzed in high purity silver (Ag) 3.3 x 5 mm (w x h) capsules, closed and crimped for IRMS-measurement.

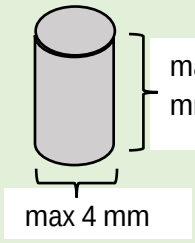
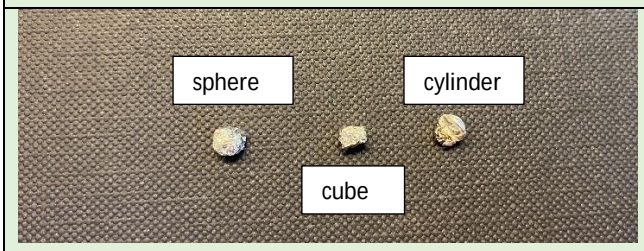
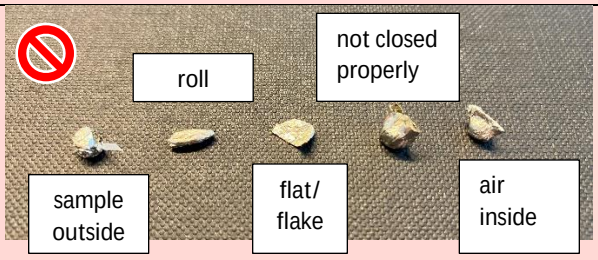
If you're delivering ready-to-measure Ag_3PO_4 samples to us for IRMS-measurement, please see tips for encapsulating samples below, and note that the Wt-% results are dependent on the accuracy of your weighing. You should use a microbalance giving you 3 decimal digits below 1 mg, i.e. 0.001 mg / 1 µg precision.

Manufacturer	Product	ID	Size (mm, W x H)	Common for sample types
IVA Analysentechnik GmbH & Co. KG	Silver capsule (Ag 99,99)	176.9805.36	3.3 x 5	organic solids, Ag3PO4, oils

Table 1: Silver cups commonly used for O isotope analyses at the Laboratory of Chronology. Corresponding capsules are available from many other manufacturers.

Encapsulating samples:

The aim is to have all the sample material inside, all the air squeezed out, nothing leaking out, and have a shape that is bulky (3D) enough that it will not get stuck and wedged in the narrow spaces left between the moving and stationary parts of the autosampler.

YES	NO
☺ compress, squeeze out air from sample+capsule	☹ squeeze/fold only top part of tin cup closed
☺ make bulky shape: spherical, cubical, cylinder…	☹ folded into a flat/ flake-like/ roll-like shape
☺ fold and crimp in the opening	☹ sample is leaking or protruding out of cup
 After closing and compaction, capsule must fit in a cylidrical shape of max 4 x 7 mm	NOTE THAT IF YOU'RE SAMPLES ARE SOMETHING DESCRIBED ABOVE OR PICTURED BELOW, WE WILL HAVE TO REPACK/ RESHAPE THEM AT AN ADDITIONAL CHARGE.
	

IRMS-analysis and data normalization

The samples in Ag capsules are loaded into a Costech Zero Blank autosampler, which is then sealed and flushed with He to minimize the effects of atmospheric moisture and air blanks on analysis. The samples are pyrolyzed at 1400 °C and the isotopic composition of oxygen is measured on a Thermo Scientific Flash IRMS EA coupled to a Thermo Scientific 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 at least *two different* calibration reference materials included in the run. We use matrix matched, i.e. Ag3PO4 reference materials for both result normalization and quality control. The measurement data for samples and QC materials are normalized

using three Ag3PO4 reference materials: Nbs-120c (21.7‰; e.g. Daux et al. 2008; Chenery et al. 2010), AGPO-SCRI (14.58‰, Halas et al. 2011) and SJ1 (5.56 ± 0.1‰). The long-term analytical precision is ± 0.3 ‰.

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

References:

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