# $\delta^{13}C$ and $\delta^{15}N$ analysis of collagen extracted from bones, teeth, skin or scales

The $\delta^{13}C$ and/or $\delta^{15}N$ values of vertebrate skeletal tissues are usually measured from its most common protein, i.e. collagen. In addition to bones and teeth, collagen can also be extracted from e.g. skins and fish scales. Please note, that analyses of bulk total $\delta^{13}C$ and $\delta^{15}N$ values in bones and teeth (directly from bone powder) are charged as analyses of "difficult materials" due to their high total mineral content.

## General considerations

Collagen extraction and subsequent analysis of its $\delta^{13}C$ and $\delta^{15}N$ values are possible only from unburnt samples. Typically, 500 mg of bone or dentine is needed for extraction depending on material condition. For skin or scales, less is needed. A starting dry weight of 100-150 mg (skin) to 250 mg (scales) is sufficient in most cases. For very well preserved skeletal samples (nitrogen Wt-% 4.5-5%), collagen extraction can be easily perfomed with a starting weight of only 100 mg. We have also developed a custom procedure for very very small sample sizes, with a starting weight of only 2 mg (Sahlstedt and Arppe, 2020) which can be considered in cases of extreme sample constraints.

A low yield of collagen from extraction is a sign of bad preservation of the sample, often indicating that the quality of the collagen has suffered. Samples showing yields lower than 1 Wt-% are not submitted for $\delta^{13}C$ and $\delta^{15}N$ analysis. We'll contact you to let you know if this turns out to be the case for some of your samples. Like most animal tissues in general, collagen has a relatively low C/N ratio allowing the determination of both $\delta^{13}C$ and $\delta^{15}N$ values from one sample during the same IRMS measurement.

Collagens usually show very good precision for both δ values, and the typical external reproducibility will be estimated based on in-house quality control bone materials included in each run. However, we recommend selecting a few samples for duplicate measurement, especially if **a)** your samples are not bone (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 materials). However, as prices are charged per analyzed replicate, the decision of replicate IRMS measurements is left up to the customer's discretion.

## Extraction procedure

Collagen extraction at the Laboratory of Chronology follows the methods described in Bocherens et al. 1997. The sample powder is demineralized for 20 minutes with 1M HCl. The HCl is removed by filtration, and after MilliQ rinses, humic substances are removed with a 20 hour treatment in 0.125 M NaOH (room T). NaOH is then removed by filtration, the sample is washed with MilliQ and pre-conditioned with HCl before dissolution to pH=2 HCl at 100 °C. Any remaining undissolved particles are filtrated out of the solution after 17 hours, and the gelatin containing solution is frozen overnight and subsequently freeze-dried for a minimum of 40 hours. The total mass of collagen obtained is weighed and recorded, and collagen yield is calculated to evaluate sample preservation. Extracted collagens are stored in a desiccator cabinet until weighed for analysis.

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

## Sample preparation

Typically, ca. 0.35 mg samples of freeze-dried collagen are weighed into tin (Sn) 3.2 x 4 mm (w x h) capsules, closed and crimped for measurement.

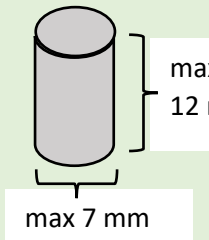
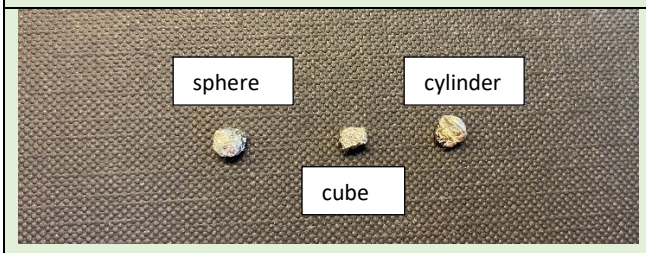
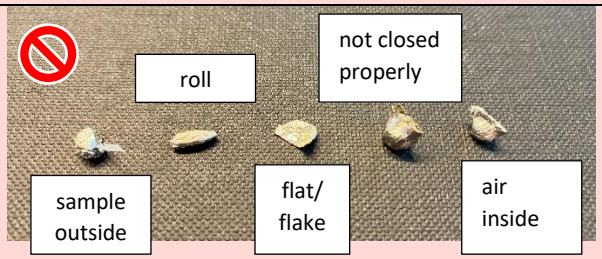
If you're weighing and closing your samples yourself, please see tips for encapsulating samples below, and note that the Wt-% results are dependent on the accuracy of your weighing. You should use a microbalance giving you 3 decimal digits below 1 mg, i.e. 0.001 mg / 1 µg precision.

| Manufacturer | Product | ID | Size (mm) | Common for sample types |
|---|---|---|---|---|
| IVA Analysentechnik GmbH & Co. KG | Tin capsules for solids | SA76980002 | 3.2 x 4 | animal tissues, collagen, plants, fungi… |
| IVA Analysentechnik GmbH & Co. KG | Tin capsules for solids, light | SA76980702L | 4 x 6 | plant N, soils, sediments, food crust (arch) |
| IVA Analysentechnik GmbH & Co. KG | Tin capsules for solids | SA76980902 | 5 x 8 | plant N, soils, sediments, food crust (arch) |

*Table 1: Tin cups commonly used for C and N 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 7 x 12 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 isotopic composition of carbon and nitrogen is measured on a Carlo Erba NC2500 elemental analyzer 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 *two different* calibration reference materials included in the run. For collagen samples, we use USGS-40 and USGS-41 for result normalization. As QC

materials we include a caffeine reference material, and replicates of in-house bone powder quality standards (elk bone, camel bone, alpine ibex bone) that have been extracted alongside 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 ± 0.2 for both $\delta^{13}C$ and $\delta^{15}N$ values.

In addition to the requested isotope values, the report of analysis also includes the Weight-% of the element(s) in the samples, the yield and atomic C/N ratio of the extracted collagen, 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:


Bocherens, H., Fizet, M., Mariotti, A., Lange-Badre, B., Vandermeersch, B., Borel, J.P., Bellon, G., 1991. Isotopic biogeochemistry ($^{13}C$,$^{15}N$) of fossil vertebrate collagen: application to the study of a past food web including Neandertal man. Journal of Human Evolution 20, 481-492. https://doi.org/10.1016/0047-2484(91)90021-M

Sahlstedt, E. and Arppe, L. 2020. Sequential extraction of phosphate and collagen fractions of small bone samples for analysis of multiple isotope systems ($\delta^{18}O_{po4}$, $\delta^{13}C$, $\delta^{15}N$). Rapid Communications in Mass Spectrometry 34, e8877. https://doi.org/10.1002/rcm.8877