**2H stable isotope analysis of human tooth enamel: a new tool to extract forensic information from human remains?**

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**GOALS**

- Determine the feasibility of obtaining 2H stable isotope abundance data from ground human tooth enamel procured from archaeological and modern tooth samples using continuous-flow isotope ratio mass spectrometry (EMD) coupled on-line to a high-temperature conversion elemental analyzer (TCEA).
- Evaluate the usefulness of the resulting data for determining human provenance in an archaeological and forensic setting.

**INTRODUCTION**

Stable isotope analysis of biogenic tissues such as tooth enamel and bone mineral has become a well-recognized and increasingly important method for determining provenance of human remains, and has been used successfully in both archaeological studies as well as forensic investigations. Both 2H and 23 stable isotopes are well known as proxies for climate conditions and are therefore considered reliable indicators of geographic provenance of human and animal remains. While analysis of 2H signatures in human hair, fingernails, and bone collagen is only used to determine human provenance, i.e. geographic origin and identify possible migration patterns, studies involving the analysis of 2H in tooth enamel appear to be nonexistent in the scientific literature. The apparent lack of research in this area may be associated with the following considerations: (1) Some of the hydroxyl ions in mineral calcium hydroxyapatite, Ca5(PO4)3(OH), by forming bioapatite carbonate ions at a rate of one CO2 per two OH; consequently, there might be not enough hydrogen present for 2H stable isotope analysis. (2) There exists the possibility for hydrogen exchange to render measured 2H-values meaningless even though the latter might be unlikely given the high POC-value of the OH ion.

In this study, feasibility to extract 2H stable isotope abundance data from tooth enamel has been shown in principle. Ground tooth enamel was analysed by continuous-flow isotope ratio mass spectrometry (EMD) coupled on-line to a high-temperature conversion elemental analyzer (TCEA). An array of archaeological and modern tooth enamel was analyzed under different experimental conditions, and results of this proof-of-concept study are presented.

**MATERIALS AND METHODS**

**Tooth sample**

Eleven ground or third premolar crowns from people of North African, Scottish, and Indian origins were all exclusively chosen to remove any surface contamination and adjusted to one of the following pretreatment protocols. Protocol A. 55 – 60 mg of tooth enamel was sampled using a dental drill with a diamond tipped drill bit and the extracted powder was first washed with 1% HCl solution (1 min) using sample for 2H stable isotope analysis. The powder was subsequently washed with 0.1 N HCl solution for 2 min to remove any organic residues from the tooth enamel. Protocol B. The powder was previously washed with 0.1 N HCl solution for 2 min. The tooth enamel powder was digested over phosphoric pyrophosphate (P2O5) in a drying agent. Protocol C. The tooth enamel was washed as described under Protocol B and subsequently sampled using a dental drill with a diamond tipped drill bit and the extracted powder was digested over phosphoric pyrophosphate (P2O5) in a drying agent. Bulk 2H isotope analysis of archaean biominerals.

The working hypothesis, by D.J. Grinsted, T.A. Sirovich, and K. Hecht, was calibrated against VSMOW using the international reference material VMW95. Veizer, J., Eds.), and checked against the international reference material (BIP-1). The BIP-1 was determined on reference H2O with a precision of 30 μg of different sample size and was located in 50 μL. A batch analysis typically comprised 10 samples run in triplicate, grounded and filtered by a set of STM (Schoeller-Cahn 5:12.3:10, USA, Virginia, Austin, and checked against the international reference material (BIP-1)). The BIP-1 was determined on reference H2O with a precision of 30 μg of different sample size and was located in 50 μL. A batch analysis typically comprised 10 samples run in triplicate, grounded and filtered by a set of STM (Schoeller-Cahn 5:12.3:10, USA, Virginia, Austin, and checked against the international reference material (BIP-1)).

**RESULTS**

- Analysis of 2H isotope composition of ground tooth enamel is possible but its low content of hydroxyl groups needed to be compensated for by the use of sample amount of at least 1 mg per silver capsule (cf. Figure 1).
- Adding carbon in the form of graphite to ground enamel samples did not increase 2H yield (cf. Figure 2).
- Exchange experiments carried out on powdered tooth enamel did not show any indication of significant hydrogen exchange as could be expected (see Table 1).
- Variation in cleaning protocols did not affect the observed 2H-values of tooth enamel (see Table 2). Observed differences were not statistically significant (p < 0.13).
- Enamel from molar of different geographic origins yielded the same 2H value of 111.3 ± 2.5% (on average) (sample preparation protocol B).
- The apparent dynamic range (= absolute range / 10) in 2H-values from tooth samples amounted to 4% while the corresponding range for source water at known points of origin was 40%.

**CONCLUSIONS**

- It is possible to measure 2H isotopic composition of the hydroxyl fraction of tooth enamel by TCEA-EMD.
- Given the ratio of proposed proxy range over source range of 0.1 the 2H signature of tooth enamel is not deemed to be an appropriate proxy for provenance.
- However, the seemingly fixed nature of 2H abundance in tooth enamel may hold information of interest to researchers in the field biogeographic minerals.

**REFERENCES**


**ACKNOWLEDGEMENTS**

SCRI gratefully acknowledges the financial support by the Scottish Government Rural and Environment Research and Analysis Directorate (RERAD).