$\delta^{13}C$ analysis of bulk organic matter in speleothems using liquid chromatography-isotope ratio mass spectrometry

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ABSTRACT

The determination of $\delta^{13}C$ values in speleothems is of considerable importance in palaeoenvironmental research, but to date has focussed solely on analysis of the carbonate. Here we demonstrate a new method for analysing the $\delta^{13}C$ values of organic matter (OM) trapped in speleothems, utilising flow injection liquid chromatography – isotope ratio mass spectrometry (LC-IRMS). Developmental analysis using a homogenised speleothem powder shows that the method is robust with repeated digests and analyses having an average standard deviation of 0.1‰. Dilution tests with samples of 4 – 23 µg total organic carbon (TOC) show relatively small linearity effects, with the overall standard deviation across a peak response range of 1700 – 9000 mV being 0.2‰.

Keywords

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Speleothem; $\delta^{13}C$; organic matter; TOC; LC-IRMS

1. Introduction

Two principal carbon pools are present in speleothems: carbonate in the calcite and carbon in entrapped organic matter (OM). Conventional $\delta^{13}C$ analysis accesses only the former pool, which is derived from carbonate dissolved from the bedrock, and transported dissolved soil CO_2 (Genty et al., 2001). The second pool, carbon contained in OM, represents compounds derived from the soil and those derived from in situ cave organisms, and has not been studied in speleothems because of methodological difficulties. Investigating the isotopic signal of this OM enables recovery of a new type of $\delta^{13}C$ record in speleothems, as well as helping understand the controls on the calcite signal. Here we propose a simple method for analysing the stable carbon isotope ratio of the acid soluble OM, utilising liquid chromatography – isotope ratio mass spectrometry (LC-IRMS) in flow injection analysis mode. Depending on the acid and oxidant used, the system allows measurement of dissolved inorganic carbon (DIC; e.g. Brandes, 2009) in a liquid sample, or dissolved organic carbon (DOC; more correctly non-purgeable OC, NPOC; e.g. Albéric, 2011). Advantages include direct injection of samples from the acid digest without substantial off line wet chemistry and injection of small sample volume (10 - 20 µl). Combined, these factors mean that the approach uses calcite samples ≤ 200 mg, considerably smaller than the samples needed for other organic analyses (1 – 20 g), allowing for the first time records to be produced at a resolution comparable to inorganic isotope records.

2. Material and Method

49 *2.1. Samples*

50 Bulk calcite powder was obtained by milling cleaned lumps of a large stalagmite to fine
51 powder and mixing to homogeneity (Blyth et al., 2006). A 5 g aliquot of the powder
52 was weighed into a 10 ml vial and further mixed by shaking.

53

54 *2.2. Total OC (TOC) analysis*

55 TOC was measured using an Aurora 1030 wet oxidation TOC analyser (OI Analytical,
56 College Station, TX, USA) which allows measurement of TOC (NPOC) concentration
57 in a sample via high temperature oxidation with sodium persulfate ($Na_2S_2O_8$),
58 following removal of DIC via addition H_3PO_4 and sparging (2 min) in a stream of inert
59 gas. The TOC analyser is attached to a 1088 rotary auto-sampler (OI Analytical,
60 College Station, TX, USA) compatible with 40 ml sample vials and equipped with a 10
61 ml syringe for sample injection. We dissolved 0.5, 1 and 1.5 g aliquots of homogenised
62 powder within the sample vials using 6 - 16 ml sonicated ultrapure HCl and topped up
63 to 40 ml using sonicated MilliQ water. Samples were left overnight to equilibrate before
64 analysis using the standard water sample TOC method described above.

65

66 *2.3. NPOC analysis with LC-IRMS*

67 Samples (200 mg) were digested in 2 ml of 3M H_3PO_4 (Sigma, HPLC grade). H_3PO_4
68 was used in preference to HCl to avoid halide interference with the LC-IRMS oxidant
69 (Albéric, 2011). After complete digestion of the calcite, aliquots of samples were
70 transferred to 1.8 ml LC-MS vials and dissolved CO_2 was removed under vacuum (1 h)
71 in a rotational vacuum concentrator. Tests showed that vacuum treatment removed DIC
72 to below measurement limits, without affecting the NPOC signal (Fig. 1). After

vacuum treatment, samples were sealed with caps and transferred for analysis. Sample vials were not topped up to remove the head space, to avoid diluting the sample. Experiments leaving vials on the bench uncapped for 24 h and measuring DIC before and after showed no detectable redissolution of CO_2.

Stable isotope analysis was carried out with a Thermo Scientific LC-IRMS instrument (consisting of an Accela autosampler and Accela 600 pump attached to a Delta V plus isotope ratio mass spectrometer via an LC-Isolink). Reagents and mobile phase were made with MilliQ water, degassed (1h) under vacuum with sonication and then constantly sparged with He. The analytical method was similar to that described by Albéric (2011). Analysis was flow injection mode using a mobile phase of dilute H_2SO_4 (pH 4.0-4.2; 100 µl 1:50 H_2SO_4 in 1 l of MilliQ water) at 300 µl min^{-1} and maintained at 20 °C using the column oven. For each run, 10 µl of sample were injected using the autosampler and oxidation of the OC was achieved using a catalyst (1.28 M H_3PO_4 at 20 µl min^{-1}) and oxidant (0.13 M $Na_2S_2O_8$ at 20 µl min^{-1}). The oxidation reactor in the LC-Isolink was maintained at 99.9 °C. Run time was 5 min, with measurements made relative to the second of two 20 s reference gas pulses at the start of the run; three more reference gas pulses were used after the analyte peak had appeared, to check for drift over the run. The reference gas was calibrated to -22.92‰ VPDB (Vienna Peedee Belemnite) using USGS-41 glutamic acid (+37.626‰ VPDB) as standard. As this is an enriched standard to which we were restricted due to availability, the calibration was also checked against in-house amino acid standards in the range of -7.6 ‰ to -31.6 ‰ which gave satisfactory results. Between analytical runs H_3PO_4 blanks were run to help clean the sample loop and reduce sample carry over. To prevent build-up of calcium phosphate solids in the needle, flushing used non-degassed

mobile phase. Due to the presence of two in-line filters in the system, providing areas for salts to nucleate, build-up of calcium phosphate solids within the instrument itself was not observed. However, as a precaution, in addition to the blank runs, we maintained water flow through the system at all times, and also subjected it to intermittent runs of sulphuric acid.

Tests for possible DIC interference in the degassed phosphate solution and samples utilised the same methodology as above with the exception that only the acid catalyst reagent was used (no oxidant) and the oxidation reactor was 60 °C (similar to the method of Brandes, 2009).

3. Results and discussion

The samples had a mean (n = 6) TOC concentration of 114 µg/g calcite or 0.011%, demonstrating the generally low abundance of OM in stalagmites, and equating to a TOC of 23 µg in our 200 mg calcite samples. However LC-IRMS showed a good measurable peak response of 1500 – 9000 mV, even at 1/6 dilution of the original concentration (a TOC of approximately 4 µg), indicating that the method can easily handle low abundance samples. Blanks showed no measurable contamination.

To test the repeatability of the technique, four separate digests of powder (test stal a-d) were each analysed 5 x (6 x for test stal d). This tests both instrumental repeatability and the influence on repeatability of the wet chemistry process. The consistency of the method (Table 1) is excellent, with a mean $\delta^{13}C$ value of -19.9‰ and standard deviation (SD) of 0.1‰ across the 21 runs. These injections showed a peak response of 5000 – 9000 mV, the variation indicating that although robust for isotopes, this method should not be used for measuring abundance of NPOC. The consistency of the isotopic values

121 indicates that linearity is not an issue for samples within this range, although as it is
122 known that both precision and accuracy are affected by changing sample abundance, to
123 test the effect of this, the original digest of test stal b was diluted into two new samples
124 1/3rd and 1/6th of the original concentration, and analysed 5 x and 6 x respectively. The
125 1/3rd dilution show a peak amplitude of 2200 - 4200 mV, a mean $\delta^{13}C$ value of – 19.7‰
126 (SD of 0.4‰). The SD was affected by the second injection, which showed an
127 abnormally high value of -19.1‰. For the 1/6th dilution, the peak amplitudes were
128 1700 – 2100 mV, with an isotopic mean of -19.5‰ and SD 0.2‰. This indicates that
129 lower sample size did not have a significant effect on the precision of the technique, but
130 there was a slight trend towards slightly heavier values with lower peak amplitude (Fig.
131 2). However, when the analyses were considered across the whole amplitude range (n =
132 32; peak amplitude 1700 – 9000 mV), the mean was -19.8‰ and the SD 0.2‰. This is
133 an acceptable level of error and indicates that the method is sufficiently robust to apply
134 to time series samples, provided analytical repeats are run.
135 A major issue for future work in the field is the identification of the precise OM fraction
136 being measured by this technique. It is established that it is the NPOC, not the TOC
137 that is measured, accepting loss of volatile purgeable compounds during preparation.
138 Equally, any interaction of the OM with the H_3PO_4, or any precipitation of solids during
139 the wet chemistry process could introduce bias. In the case of interaction with the acid,
140 we observed during early method development that samples rerun on later days showed
141 an increase in isotopic value. This did not occur consistently, but was seen frequently
142 enough to be of concern. As redissolution of CO_2 from the atmosphere had been
143 experimentally tested and ruled out, we hypothesise that the changes were due to
144 prolonged exposure of the OM to H_3PO_4. However, these problems did not occur

during the first 24 h after digestion, and we therefore consider that as long as samples are prepared and run in small batches, with the analysis taking place within 24 h of digestion, this should not be an issue. The question of precipitation bias is more serious, and more difficult to investigate. It is well established that humic acids in particular will precipitate in acidic solutions, a fact that is exploited in their extraction (e.g. Van Beynen et al. 2001). We would therefore expect humic rich samples to precipitate some compounds in this context. The extent to which this is a problem for the technique depends on whether the precipitation of compounds is consistent between heterogeneous samples, and establishing this should be a focus of future work. If it is consistent, then the bias would not affect the results of this technique, as long as it is noted that what is being measured in each case is only the acid soluble NPOC, not total bulk OM.

Conclusions

The study demonstrates a simple and robust new method for the study of acid soluble OM in small (≤200 mg) calcite samples from speleothems (or indeed any other $CaCO_3$ medium). Work now needs to focus on understanding the controls on the signal (vegetation, microbial degradation, soil turnover, in situ and external inputs, chemical biases) in order to ensure the utility of the palaeoenvironmental $\delta^{13}C$ records which can now be recovered.

Acknowledgements

The study was funded by an AINSE Research Fellowship to A.J.B., ARC Future Fellowship to C.S. (FT0992258), and NERC Research Grant NE/G016925/1 to A.J.B.

169 The stalagmite sample was collected on the Ethiopia Venture 2000 expedition. Stefan
170 Schouten and an anonymous reviewer are thanked for their constructive comments to
171 improve the manuscript.
172

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175 dissolved organic carbon in stream and soil waters. Rapid Communications in Mass
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188

189 **Table and figure captions**

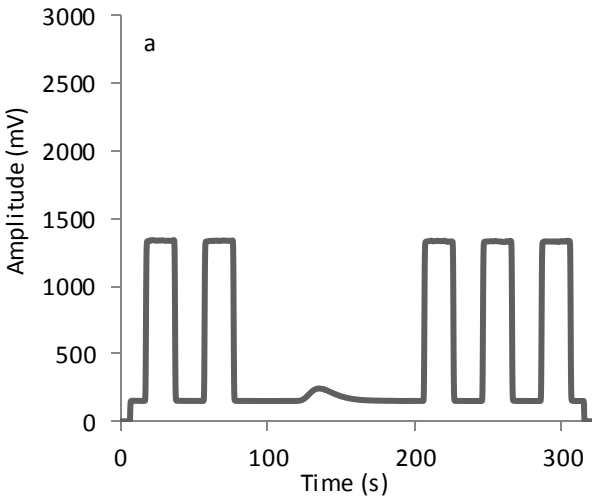
190 **Table 1**

191 $\delta^{13}C$ results with mean and standard deviation for all stalagmite digests.

192 **Fig. 1.** Chromatogram (*m/z* 44) showing analyte peak for a) DIC run and b) NPOC run
193 on a method development test sample. Square peaks represent reference gas. The
194 samples had been vacuum purged for 30 mins, and the DIC peak is equivalent to that
195 seen for the reagent blanks. Prior to preparation of the test stal samples reported in this
196 study, vacuum purge time was increased to 1 h, to ensure maximum removal of DIC.
197 **Fig. 2.** Scatter plot showing the change in $\delta^{13}C$ of the NPOC with peak amplitude
198 response.

Stalagmite digest	Amplitude (mV) of m/z 44 peak	$\delta^{13}C$ (‰)	Digest mean (‰)	Digest SD (‰)
Test stal a	6777 6823 6732 6824 6711	-19.8 -19.9 -20.0 -19.9 -19.9	-19.9	0.1
Test stal b	9068 9054 5785 5074 8087	-20.0 -20.0 -19.7 -19.8 -19.8	-19.9	0.1
Test stal c	5302 5176 5144 5063 5054	-19.9 -19.9 -20.0 -20.0 -19.9	-20.0	0.1
Test stal d	6941 7093 6968 6947 6993 6592	-19.8 -19.9 -19.9 -20.0 -20.0 -19.9	-19.9	0.1
Test stal b 1/3	4064 4219 2218 3968 3796	-19.8 -19.1 -19.9 -19.8 -20.1	-19.7	0.4
Test stal b 1/6	2104 2001 2061 2030 1968 1747	-19.5 -19.6 -19.5 -19.3 -19.5 -19.8	-19.5	0.2
All digests (avg.)			-19.8	0.2

Figure 1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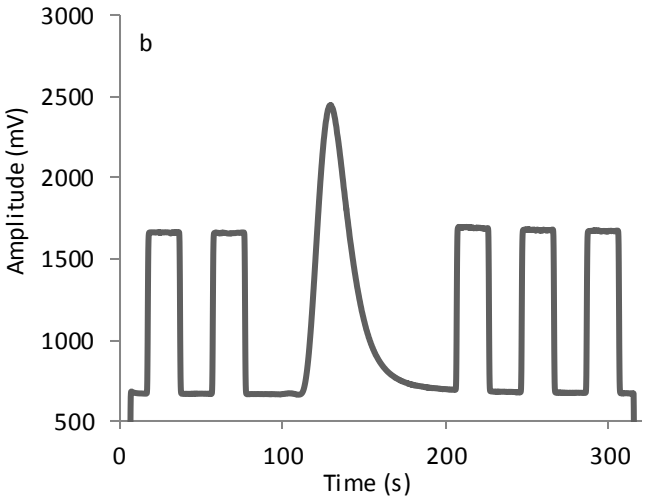
2500
2000
1500
1000
500
0
Amplitude (mV)
0
100
200
300
Time (s)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3000
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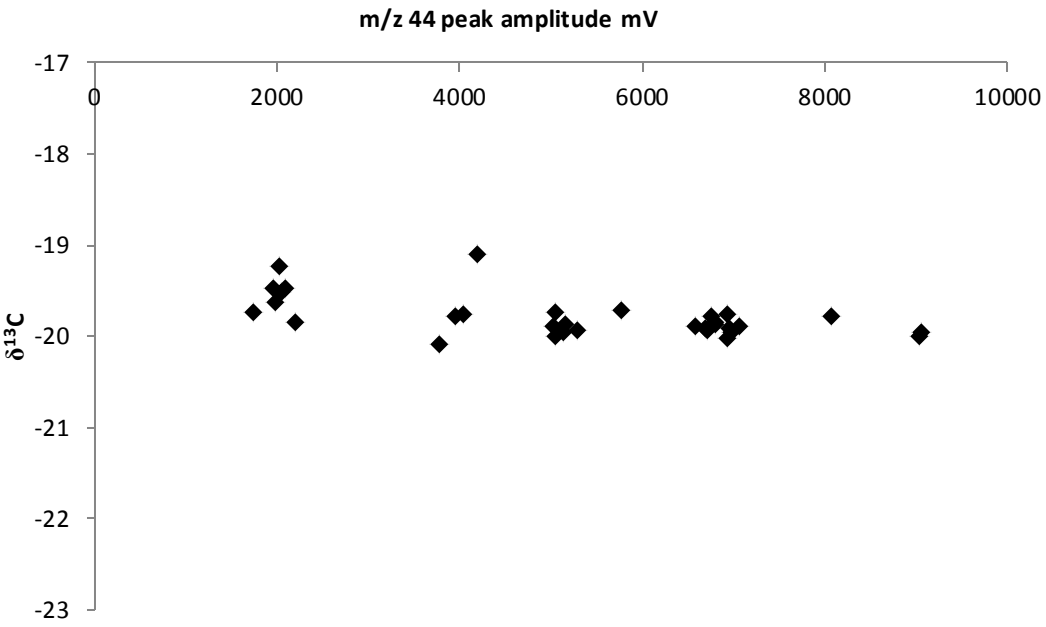
1000
500
Amplitude (mV)
0
100
200
300
Time (s)

Figure 2


m/z 44 peak amplitude mV
-17
-18
-19
-20
-21
-22
-23
δ13C
0
2000
4000
6000
8000
10000