

NOTICE: this is the author's version of a work that was accepted for publication in Organic Geochemistry. Changes resulting from the publishing process, such as peer review, editing, corrections, structural formatting, and other quality control mechanisms may not be reflected in this document. Changes may have been made to this work since it was submitted for publication. A definitive version was subsequently published in Organic Geochemistry, Vol. 55 (2013). DOI: 10.1016/j.orggeochem.2012.11.003

1 $\delta^{13}\text{C}$ analysis of bulk organic matter in speleothems using liquid
2 chromatography-isotope ratio mass spectrometry

3
4 Alison J Blyth ^{a*}, Yulia Shutova ^b, Colin Smith ^c

5
6 ^aWA-OIGC, Department of Chemistry, Chemistry and Resources Precinct, Curtin
7 University, GPO Box U1987, Perth, WA 6845, Australia

8 ^bWater Research Centre, School of Civil and Environmental Engineering, University of
9 New South Wales, Australia

10 ^cArchaeology Program, La Trobe University, Victoria 3086, Australia

11
12 **ABSTRACT**

13 The determination of $\delta^{13}\text{C}$ values in speleothems is of considerable importance in
14 palaeoenvironmental research, but to date has focussed solely on analysis of the
15 carbonate. Here we demonstrate a new method for analysing the $\delta^{13}\text{C}$ values of organic
16 matter (OM) trapped in speleothems, utilising flow injection liquid chromatography –
17 isotope ratio mass spectrometry (LC-IRMS). Developmental analysis using a
18 homogenised speleothem powder shows that the method is robust with repeated digests
19 and analyses having an average standard deviation of 0.1‰. Dilution tests with samples
20 of 4 – 23 μg total organic carbon (TOC) show relatively small linearity effects, with the
21 overall standard deviation across a peak response range of 1700 – 9000 mV being
22 0.2‰.

23
24 **Keywords**

*Corresponding author. Tel.: +61(0)892669388; fax: +61(0)892662300.
Email address: alison.blyth@curtin.edu.au (A.J. Blyth).

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

26

27 **1. Introduction**

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

47

48 **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 ($\text{Na}_2\text{S}_2\text{O}_8$),
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

73 vacuum treatment, samples were sealed with caps and transferred for analysis. Sample
74 vials were not topped up to remove the head space, to avoid diluting the sample.
75 Experiments leaving vials on the bench uncapped for 24 h and measuring DIC before
76 and after showed no detectable redissolution of CO₂.
77 Stable isotope analysis was carried out with a Thermo Scientific LC-IRMS instrument
78 (consisting of an Accela autosampler and Accela 600 pump attached to a Delta V plus
79 isotope ratio mass spectrometer via an LC-Isolink). Reagents and mobile phase were
80 made with MilliQ water, degassed (1h) under vacuum with sonication and then
81 constantly sparged with He. The analytical method was similar to that described by
82 Albéric (2011). Analysis was flow injection mode using a mobile phase of dilute
83 H₂SO₄ (pH 4.0-4.2; 100 µl 1:50 H₂SO₄ in 1 l of MilliQ water) at 300 µl min⁻¹ and
84 maintained at 20 °C using the column oven. For each run, 10 µl of sample were
85 injected using the autosampler and oxidation of the OC was achieved using a catalyst
86 (1.28 M H₃PO₄ at 20 µl min⁻¹) and oxidant (0.13 M Na₂S₂O₈ at 20 µl min⁻¹). The
87 oxidation reactor in the LC-Isolink was maintained at 99.9 °C. Run time was 5 min,
88 with measurements made relative to the second of two 20 s reference gas pulses at the
89 start of the run; three more reference gas pulses were used after the analyte peak had
90 appeared, to check for drift over the run. The reference gas was calibrated to -22.92‰
91 VPDB (Vienna Peedee Belemnite) using USGS-41 glutamic acid (+37.626‰ VPDB) as
92 standard. As this is an enriched standard to which we were restricted due to availability,
93 the calibration was also checked against in-house amino acid standards in the range of -
94 7.6 ‰ to -31.6 ‰ which gave satisfactory results. Between analytical runs H₃PO₄
95 blanks were run to help clean the sample loop and reduce sample carry over. To
96 prevent build-up of calcium phosphate solids in the needle, flushing used non-degassed

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

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

106

107 **3. Results and discussion**

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

114 To test the repeatability of the technique, four separate digests of powder (test stal a-d)
115 were each analysed 5 x (6 x for test stal d). This tests both instrumental repeatability
116 and the influence on repeatability of the wet chemistry process. The consistency of the
117 method (Table 1) is excellent, with a mean $\delta^{13}\text{C}$ value of -19.9‰ and standard deviation
118 (SD) of 0.1‰ across the 21 runs. These injections showed a peak response of 5000 –
119 9000 mV, the variation indicating that although robust for isotopes, this method should
120 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 $1/3^{\text{rd}}$ and $1/6^{\text{th}}$ of the original concentration, and analysed 5 x and 6 x respectively. The
125 $1/3^{\text{rd}}$ dilution show a peak amplitude of 2200 - 4200 mV, a mean $\delta^{13}\text{C}$ 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 $1/6^{\text{th}}$ 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

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

157

158 **Conclusions**

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

165

166 **Acknowledgements**

167 The study was funded by an AINSE Research Fellowship to A.J.B., ARC Future
168 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

173 **References**

174 Albéric, P. 2011. Liquid chromatography/mass spectrometry stable isotope analysis of
175 dissolved organic carbon in stream and soil waters. *Rapid Communications in Mass*
176 *Spectrometry* 25, 3012-3018.

177 Blyth, A.J., Farrimond, P., Jones, M., 2006. An optimised method for the extraction
178 and analysis of lipid biomarkers from stalagmites. *Organic Geochemistry* 37, 882-890.

179 Brandes, J.A., 2009. Rapid and precise $\delta^{13}\text{C}$ measurement of dissolved inorganic
180 carbon in natural waters using liquid chromatography coupled to an isotope-ratio mass
181 spectrometer. *Limnology and Oceanography: Methods* 7, 730-739.

182 Genty, D., Baker, A., Massault, M., Proctor, C., Gilmour, M., Pons-Branchu, E.,

183 Hamelin, B., 2001. Dead carbon in stalagmites: carbonate bedrock palaeodissolution
184 vs. ageing of soil organic matter. Implications for ^{13}C variations in speleothems.

185 *Geochimica et Cosmochimica Acta* 65, 3443-3457.

186 van Beynen, P., Bourbonniere, R., Ford, D., Schwarcz, H., 2001. Causes of colour and
187 fluorescence in speleothems. *Chemical Geology* 175, 319-341.

188

189 **Table and figure captions**

190 **Table 1**

191 $\delta^{13}\text{C}$ 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 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 $\delta^{13}\text{C}$ of the NPOC with peak amplitude
198 response.

Stalagmite digest	Amplitude (mV) of m/z 44 peak	$\delta^{13}\text{C}$ (‰)	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

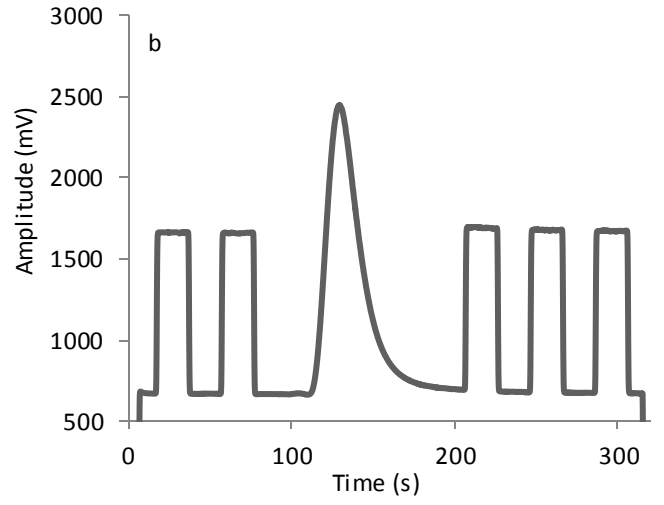
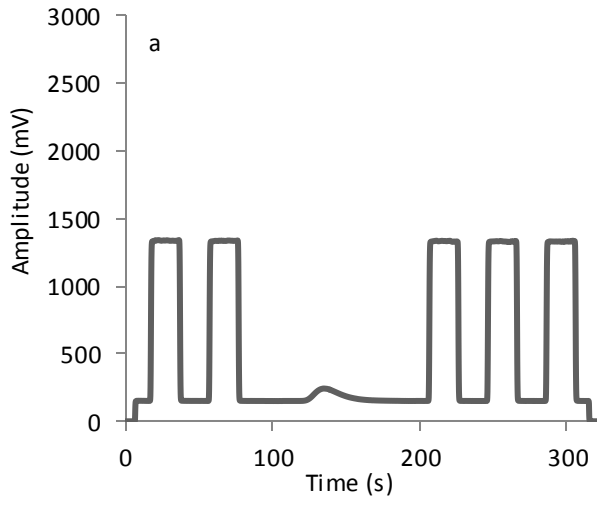


Figure 2

