

# Copper Isotopic Analysis in Geological and Biological Reference Materials by MC-ICP-MS

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The characterisation of relative copper isotope amount ratios ( $\delta^{65}\text{Cu}$ ) helps constrain a variety of geochemical processes occurring in the geosphere, biosphere and hydrosphere. The accurate and precise determination of  $\delta^{65}\text{Cu}$  in matrix reference materials is crucial in the effort to validate measurement methods. With the goal of expanding the number and variety of available geological and biological materials, we have characterised the  $\delta^{65}\text{Cu}$  values of ten reference materials by MC-ICP-MS using C-SSBIN model for mass bias correction. SGR-1b (Green River shale), DOLT-5 (dogfish liver), DORM-4 (fish protein), TORT-3 (lobster hepatopancreas), MESS-4 (marine sediment) and PACS-3 (marine sediment) have for the first time been characterised for  $\delta^{65}\text{Cu}$ . Additionally, four reference materials (with published  $\delta^{65}\text{Cu}$  values) have been characterised: BHVO-1 (Hawaiian basalt), BIR-1 (Icelandic basalt), W-2a (diabase) and Seronom™ Trace Elements Serum L-1 (human serum). The reference materials measured in this study possess complex and varied matrices with copper mass fractions ranging from  $1.2 \mu\text{g g}^{-1}$  to  $497 \mu\text{g g}^{-1}$  and  $\delta^{65}\text{Cu}$  values ranging from  $-0.20\text{‰}$  to  $0.52\text{‰}$  with a mean expanded uncertainty of  $\pm 0.07\text{‰}$  ( $U, k = 2$ ), covering much of the natural copper isotope variability observed in the environment.

Keywords: non-traditional stable isotopes,  $\delta^{65}\text{Cu}$ , anion exchange chromatography, MC-ICP-MS, mass bias, reference materials.

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The transition metal element copper (Cu) consists of two stable isotopes,  $^{65}\text{Cu}$  and  $^{63}\text{Cu}$ , and is an important element for studying reduction–oxidation (redox) interactions and metal cycling in the geosphere, hydrosphere and biosphere (Moynier *et al.* 2017). It is also an essential trace element for the functioning organs and metabolic processes in plants (Yruela 2005) and animals (Uauy *et al.* 1998). Copper takes part in important redox reactions in the environment, shifting between two oxidation states,  $\text{Cu}^+$  and  $\text{Cu}^{2+}$ , allowing it to act as both an electron donor and acceptor. As an essential trace element in most aerobic organisms, Cu is a useful tool for studying biological processes with their short turnover rates and specific functional roles in biology (Kim *et al.* 2008, Albarède *et al.* 2017). Copper isotope amount ratio measurements were first performed in the 1950s and 1960s, using thermal ionisation mass spectrometry (TIMS), but there was difficulty achieving reproducible data of sufficient precision not to completely obscure natural isotopic variations (Walker *et al.* 1958, Shields *et al.* 1965, Gale

*et al.* 1999). It was only with the development of MC-ICP-MS and anion exchange column chromatography procedures capable of sufficiently purifying low quantities of Cu from complex matrices prior to isotopic analysis that we have been able to resolve subtle changes in Cu isotope amount ratios in the environment (Maréchal *et al.* 1999).

Most recently, the measurement of relative Cu isotope ratios ( $\delta^{65}\text{Cu}$ ) in the human body by MC-ICP-MS has gained increased interest, and research suggests Cu isotopes have potential uses in monitoring age (Jaouen *et al.* 2013), sex (Jaouen *et al.* 2012, Van Heghe *et al.* 2014, Jaouen *et al.* 2017), diet (Van Heghe *et al.* 2014, Jaouen *et al.* 2017), disease pathologies (Aramendía *et al.* 2013, Balter *et al.* 2015, Costas-Rodríguez *et al.* 2015, Télouk *et al.* 2015, Lauwens *et al.* 2016, Sauzéat *et al.* 2018, Miller *et al.* 2019, Moynier *et al.* 2019) and other biological processes (Albarède *et al.* 2011, Jaouen and Balter 2014, Van Heghe *et al.* 2014, Cadiou *et al.* 2017,

Flórez *et al.* 2018). Of the investigated human biological materials, serum is the most extensively studied, being chemically stable, inexpensive and minimally invasive to obtain. Copper in serum has a rapid turnover rate of 6 weeks (Linder and Goode 1991, Milne 1998), making it optimal for monitoring rapidly progressing disease pathologies. This makes it the most convenient material to work with as any relationships discovered with pathologies could be routinely monitored with minimal discomfort to the patients. Copper isotopes in groundwater are also now being explored as potential vectors of covered mineral deposits (Asael *et al.* 2007, Borrok *et al.* 2007, Mathur *et al.* 2013, 2014, Townley *et al.* 2015), and a means of tracking its biological cycling in past and present oceans using seawater and leachable fractions of marine sediments (Bermin *et al.* 2006, Little *et al.* 2014). Copper isotopes may also be used to trace high temperature magmatic processes (Liu *et al.* 2014) and study mantle metasomatism and partial melting in distinct silicate reservoirs on Earth (Liu *et al.* 2015).

Direct comparison of Cu isotope amount ratios in samples with NIST SRM 976 is becoming increasingly difficult due to its limited availability. For this reason, NIST SRM 976 was calibrated against ERM<sup>®</sup>-AE633 and ERM<sup>®</sup>-AE647 that are certified for isotope amount ratios (Moeller *et al.* 2012). While ERM<sup>®</sup>-AE647 is still commercially available, ERM<sup>®</sup>-AE633 has been discontinued. Thus, new Cu isotopic reference materials calibrated against NIST SRM 976 must be continually developed to enable the interlaboratory comparison of results. Recently, the NIST SRM 3114 Cu standard solution was investigated as a candidate Cu isotopic reference material, but disagreeing results within reported uncertainties were obtained by two research groups with Hou *et al.* (2016) reporting a  $\delta^{65}\text{Cu}$  value of  $0.18 \pm 0.04\text{‰}$  ( $2s$ ,  $n = 5$ ) and Baconnais *et al.* (2019) reporting a  $\delta^{65}\text{Cu}$  value of  $-0.06 \pm 0.02\text{‰}$  ( $2s$ ,  $n = 44$ ). This study will examine the isotopic homogeneity of a purified Cu wire (99.999% Cu), henceforth referred to as 'HICU-1', a candidate Cu isotopic CRM produced at National Research Council Canada (NRC, Ottawa, Canada), and determine its Cu isotope amount ratio relative to NIST SRM 976 and ERM-AE647, respectively.

The ability to perform high precision measurements of Cu isotope amount ratios in geological and biological materials is reliant on both the capability of quantitatively separating Cu from complex matrices enriched in elements like sodium (Na) and titanium (Ti) that form prominent polyatomic interferences in the plasma of MC-ICP-MS instruments, and correcting for instrumental mass bias, due to the preferential transmission of higher mass isotopes to detectors (Gillson *et al.* 1988, Heumann *et al.* 1998). This study further adapts methods

developed by Maréchal *et al.* (1999) and Hou *et al.* (2016) to purify Cu from matrix elements using the Bio-Rad AG<sup>®</sup> MP-1M (100-200 mesh) resin. A combined standard-sample bracketing (SSB) and internal normalisation (C-SSBIN) mass bias correction using Ga as an internal standard was selected to correct for instrumental mass bias after successful implementation first by Hou *et al.* (2016) and by Lauwens *et al.* (2018), as it provides a better measurement uncertainty (Yang *et al.* 2018). Other calibrants such as a Zn (Maréchal *et al.* 1999) or Ni (Ehrlich *et al.* 2004) can be used.

The determination of Cu isotope amount ratios in matrix reference materials is crucial in the effort to validate measurements performed on new samples. Commonly, isotope studies only run pure, single-element standards through column chemistry procedures alongside samples to validate the results of their measurements performed on samples with complex matrices. This is insufficient to validate measurements of these new materials as producing expected Cu isotope amount ratios in pure, single-element standards only indicates high quality Cu yield and minimal blank contribution, and not quality of the separation from interfering elements. The measurement of matrix reference materials alongside samples is crucial for verifying that a chromatography procedure can sufficiently purify Cu from a given matrix. For example, a material containing high quantities of Cu with low amounts of interfering elements will have a much lower contaminant-to-Cu ratio than one containing low levels of Cu relative to Na. This is the case for serum, in which Na is typically 3000 times more concentrated than Cu (Harrington *et al.* 2014). For example, if 0.5 g of human serum were digested and put through a chromatography procedure that yielded 100% of Cu and removed 99.9% of Na, 1.5  $\mu\text{g}$  of Na would still remain with the eluted 0.5  $\mu\text{g}$  of Cu. This would give a Na/Cu ratio of 3, which is well within the range where the formation of  $^{23}\text{Na}^{40}\text{Ar}^+$  in the plasma could lead to a falsely lower  $^{65}\text{Cu}/^{63}\text{Cu}$  ratio (Liu *et al.* 2014, Hou *et al.* 2016). Conversely, this would be less of an issue with a basalt such as BHVO-1 which, despite containing much more Na per gram of material, has much lower Na/Cu ratio due to containing significantly higher amounts of Cu (Verma *et al.* 2017).

With the goal of expanding the number and variety of available geological and biological materials with which the isotope geochemistry community can validate their Cu isotopic analysis, we have determined  $\delta^{65}\text{Cu}$  of six commercially available, previously uncharacterised materials, comprising DOLT-5 (dogfish liver), DORM-4 (fish protein), TORT-3 (lobster hepatopancreas), MESS-4 (marine sediment), PACS-3 (marine sediment) and SGR-1b (shale). We also report measurements for two basalts (BHVO-1, BIR-1), diabase (W-2a) and human serum (Seronom<sup>™</sup> Trace Elements Serum L-1).

## Experimental

### Instrumentation

Copper isotope amount ratio measurements were performed on a Thermo Scientific Neptune Plus MC-ICP-MS at National Research Council Canada (NRC). The MC-ICP-MS is equipped with nine Faraday cups, a quartz dual cyclonic spray chamber and a PFA self-aspirating nebuliser (Elemental Scientific, Omaha, NE, USA) with  $\sim 50 \mu\text{l min}^{-1}$  sample uptake rate. All Cu isotope amount ratio measurements were performed at low-resolution mode. The instrument was tuned to obtain flat-top square peaks with high and stable signals. The gain calibration of the Faraday cups was performed weekly, and typical operating conditions are presented in Table 1. In addition, a virtual amplifier function was activated (through software control) during the ratio measurements to eliminate any bias in gain calibration. A Thermo Scientific Element XR High-Resolution ICP-MS was used for semi-quantitative analysis of Cu yields and calibration of the Cu anion exchange column chromatography method.

### Reagents and materials

Reagent-grade hydrochloric (HCl) and nitric acids ( $\text{HNO}_3$ ) were prepared by sub-boiling distillation using a DST-1000 acid purification system (Savillex®). High purity de-ionised water (18 M $\Omega$  cm) was prepared from a Milli-Q

water system (Millipore Corporation). All samples were handled in Savillex® containers that were cleaned in 2 mol l<sup>-1</sup>  $\text{HNO}_3$  and de-ionised water prior to use. Hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) was purchased from Seastar Chemicals Inc.

Copper chromatography was performed using the AG MP-1M Bio-Rad®, 100–200 mesh resin in Eichrom Technologies Inc. columns (Part No: AC-141-AL) with a resin bed length of 1.6 cm, column internal diameter of 0.64 cm and resin bed volume of 0.5 ml.

The production of isotopic certified reference material (CRM) is one of NRC's important activities, and copper isotopic CRM project was initiated in February 2017. A large quantity of 2000 units (last for decades) of the candidate copper isotopic CRM, named HICU-1, were produced from a high purity 2.4 mm diameter copper wire (99.999% Cu), which was cut in 22 mm length and bottled in argon filled glass bottles in Mar. 2017. Each unit contains about 0.9 g of copper wire. The HICU-1 was used as a bracketing calibrator in this study. The surface of each was cleaned by rinsing with 5% v/v nitric acid ( $\text{HNO}_3$ ) before being dissolved in a few millilitres of concentrated  $\text{HNO}_3$  under mild heating and then diluted to 100 g with de-ionised water. An aliquot of each piece was further diluted in 30 g of 2% v/v  $\text{HNO}_3$ , creating twelve  $\sim 100 \mu\text{g g}^{-1}$  Cu solutions. A Cu stock solution (HICU-1) was created by combining 2 g of solution from each of the twelve Cu solutions. An aliquot of this solution was then diluted in 30 g of 2% v/v  $\text{HNO}_3$  to create a  $100 \mu\text{g g}^{-1}$  Cu mixture of all twelve bottles with which to compare the homogeneity of each original piece of Cu wire.

**Table 1.**  
**MC-ICP-MS operating conditions**

Parameter	Instrument settings
Forward power	1250 W
Plasma gas flow rate (Ar)	16 l min <sup>-1</sup>
Auxiliary gas flow rate (Ar)	1 l min <sup>-1</sup>
Carrier gas flow rate (Ar)	1.006 l min <sup>-1</sup>
Sampler cone orifice (Ni)	1.1 mm
Skimmer cone orifice (H, Ni)	0.8 mm
Lens settings	Focus: -674 V; X-deflection: 1.07 V; Y-deflection: 1.60 V; Shape: 177 V; Rot quad 1: 0 V; Source offset: 24; Focus quad 1: -19.89 V; Focus offset: 50 V)
<b>Data acquisition parameters</b>	
Faraday cup configuration	L3 ( <sup>63</sup> Cu), L2 ( <sup>65</sup> Cu), C ( <sup>67</sup> Zn), H2 ( <sup>69</sup> Ga), H3 ( <sup>71</sup> Ga)
Zoom optics	Focus quad: 0 V; Dispersion quad: 0 V
Sensitivity	1 $\mu\text{g g}^{-1}$ Cu = 8.5 V ( <sup>63</sup> Cu); 1 $\mu\text{g g}^{-1}$ Ga = 18 V ( <sup>69</sup> Ga)
Blank signal (2% $\text{HNO}_3$ )	0.008 V <sup>63</sup> Cu; 0.007 V <sup>69</sup> Ga
Signal integration time	2.097 s
No. of integrations, blocks, cycles/block	1, 5, 10

Gallium metal isotopic reference material, NIST SRM 994, with certified value of  $^{69}\text{Ga}/^{71}\text{Ga} = 1.50676 \pm 0.00039$  (95% confidence interval uncertainty) (Machlan *et al.* 1986 and NIST Ga certificate, 1986), was purchased from National Institute of Standards and Technology (Gaithersburg, MD). A 1500  $\mu\text{g g}^{-1}$  stock solution of NIST SRM 994 was prepared by quantitative dissolution of Ga in warm concentrated nitric acid and then diluted with de-ionised water. NIST SRM 994 Ga was added to the purified copper solutions and bracketing calibrator to correct mass bias using C-SSBIN. A 3200  $\mu\text{g g}^{-1}$  stock solution of NIST SRM 976 Cu isotopic material was prepared in 5% v/v  $\text{HNO}_3$ , and a 1300  $\mu\text{g g}^{-1}$  Cu standard solution was prepared by dilution of ERM®-AE647 (obtained from Institute for Reference Materials and Measurements (IRMM), Geel, Belgium), in 2% v/v  $\text{HNO}_3$ .

Geological reference materials BIR-1, BHVO-1, SGR-1b and W-2a were purchased from the United States

Geological Survey (USGS), and Seronorm™ Trace Elements Serum L-1 (lot 1309438) was purchased from SERO AS (Billingstad, Norway). The remaining reference materials DOLT-5, DORM-4, TORT-3, MESS-4 and PACS-3 were produced at NRC. Two bottles of each NRC reference materials, possessing different lot numbers, were analysed to examine their isotopic homogeneity and subsequent suitability as an isotopic reference material. From each bottle, two to three aliquots were digested, and Cu isotopic analysis performed.

Two synthetic serum standards of different Cu mass fractions ( $9.9 \mu\text{g g}^{-1}$  and  $0.6 \mu\text{g g}^{-1}$ ) were prepared gravimetrically from 99.99% to 99.9999% purity calcium (Ca), iron (Fe), magnesium (Mg), potassium (K), Na and zinc (Zn) single-element standards (SPEX CertiPrep®) and Cu from HICU-1 (Table 2). These high-Na, complex matrix synthetic standards were used to monitor the quality of separation during isotopic analysis by MC-ICP-MS.

### Geological and biological sample preparation and analysis

All samples were prepared in a class-100 clean room laboratory at NRC under laminar flow hoods with HEPA-filtered air. Geological samples (~ 0.25 g) were mixed with 7.5 ml of concentrated  $\text{HNO}_3$  and 1 ml of concentrated HF, whereas biological samples (~ 0.25–0.50 g) were mixed with 8 ml of concentrated  $\text{HNO}_3$  and 0.5 ml of 30%  $\text{H}_2\text{O}_2$  and allowed to stand for 2 h before being microwave digested for 55 min, ramping up to a temperature of 210 °C. After digestion, the contents of the microwave tubes were poured into Savillex® containers and evaporated to dryness on a hotplate at 160 °C. Samples were then re-dissolved in varying volumes of  $7.6 \text{ mol l}^{-1}$  HCl to ensure that 1 ml of solution (load volume) was within less than 6% of the theoretical exchange capacity of the AG MP-1M resin (Sossi *et al.* 2015). Prior to performing anion exchange column chromatography, a volume of 30%  $\text{H}_2\text{O}_2$  was

added to each sample to ensure it made up 0.03% of the solution, keeping Cu in the oxidised state.

Copper was purified from matrix elements using the AG MP-1M resin and a methodology modified after Maréchal *et al.* (1999), who used  $7 \text{ mol l}^{-1}$  HCl, and Hou *et al.* (2016) who used  $8.5 \text{ mol l}^{-1}$  HCl to load samples onto columns. In this study, the most efficient separation from major matrix elements (Ca, K, Mg, Na and Ti) was achieved using  $7.6 \text{ mol l}^{-1}$  HCl to load samples. Columns were filled with 0.5 ml of resin and then cleaned by filling each column to capacity (including the upper reservoir) sequentially with  $2 \text{ mol l}^{-1}$   $\text{HNO}_3$  and  $2 \text{ mol l}^{-1}$  HCl, and then de-ionised water prior to conditioning with 2 ml of  $7.6 \text{ mol l}^{-1}$  HCl + 0.03%  $\text{H}_2\text{O}_2$ . Samples were loaded onto columns in 1 ml  $7.6 \text{ mol l}^{-1}$  HCl + 0.03% v/v  $\text{H}_2\text{O}_2$  and matrix elements removed with a subsequent 2 ml of  $7.6 \text{ mol l}^{-1}$  HCl + 0.03% v/v  $\text{H}_2\text{O}_2$  before Cu was eluted and collected by passing another 15 ml of  $7.6 \text{ mol l}^{-1}$  HCl + 0.03% v/v  $\text{H}_2\text{O}_2$  through columns. The majority of matrix elements (e.g., Na, Mg, K, Ca and Ti) were eluted in the first 3 ml but Fe and Zn were retained on the column throughout the separation procedure. Reference materials were processed through the chromatography procedure twice with the exception of Seronorm™ Trace Elements Serum L-1, which was processed three times due to the high Na/Cu ratio naturally found in human serum. The full chromatography procedure is summarised in Table 3, and the Cu elution profile is visualised in Figure 1.

Copper yield was estimated using two methods. Semi-quantitatively, Cu yield was estimated to be greater than 99% based on elution data (Figure 1), and quantitatively, Cu yield was determined to be  $100.9 \pm 2.3\%$  ( $1s, n = 3$ ) by comparing the Cu intensities of column-processed and unprocessed samples by Element XR High-Resolution ICP-MS. Total procedure blank Cu contributions were no greater than 1.3 ng and represented less than 0.5% of Cu loaded onto columns for all reference materials.

Titanium and Na are the primary matrix elements that can generate polyatomic interferences with  $^{23}\text{Na}^{40}\text{Ar}^+$ ,  $^{23}\text{Na}_2^{16}\text{O}^{1}\text{H}$ ,  $^{23}\text{Na}_2^{18}\text{O}^{1}\text{H}^+$ ,  $^{47}\text{Ti}^{16}\text{O}^+$ ,  $^{46}\text{Ti}^{16}\text{O}^{1}\text{H}^+$ ,  $^{49}\text{Ti}^{16}\text{O}^+$ ,  $^{48}\text{Ti}^{16}\text{O}^{1}\text{H}^+$  on  $^{63}\text{Cu}$  and  $^{65}\text{Cu}$  and cause matrix effects (Hou *et al.* 2016). Titanium and Na signals were monitored by ICP-MS to determine Ti/Cu and Na/Cu ratios and ensure they remained within acceptable levels. The Ti/Cu ratio ranged from 0.003 to 0.011 in high Ti BIR-1 and BHVO, with a mean of 0.007, well below the range where significant polyatomic interferences occur on  $^{63}\text{Cu}$  and  $^{65}\text{Cu}$  (Liu *et al.* 2014, Hou *et al.* 2016). The Na/Cu ratio ranged from 0.011 to 0.110 with a mean of 0.045, also well below

**Table 2.**  
Composition of synthetic serum reference materials

Element	Low Cu synthetic serum ( $\mu\text{g g}^{-1}$ )	High Cu synthetic serum ( $\mu\text{g g}^{-1}$ )
Fe	0.5	0.5
Cu	0.6	9.9
Zn	0.5	0.5
Na	1479.3	1548.7
K	88.8	92.6
Mg	11.1	11
Ca	44.5	47.5

**Table 3.**  
Column procedure using AG MP-1M Bio-Rad resin adapted after Hou *et al.* (2016)

Separation step	Reagent	Volume (ml)
Cleaning 1	2 mol l <sup>-1</sup> HNO <sub>3</sub>	To capacity (about 4 ml)
Cleaning 2	2 mol l <sup>-1</sup> HCl	To capacity (about 4 ml)
Cleaning 3	High purity H <sub>2</sub> O (Milli-Q)	To capacity (about 4 ml)
Conditioning	7.6 mol l <sup>-1</sup> HCl + 0.03% H <sub>2</sub> O <sub>2</sub>	2
Sample load	7.6 mol l <sup>-1</sup> HCl + 0.03% H <sub>2</sub> O <sub>2</sub>	1
Matrix elution	7.6 mol l <sup>-1</sup> HCl + 0.03% H <sub>2</sub> O <sub>2</sub>	2
Cu elution	7.6 mol l <sup>-1</sup> HCl + 0.03% H <sub>2</sub> O <sub>2</sub>	15

the range where significant polyatomic interferences begin to show on <sup>63</sup>Cu and <sup>65</sup>Cu (Liu *et al.* 2014, Hou *et al.* 2016).

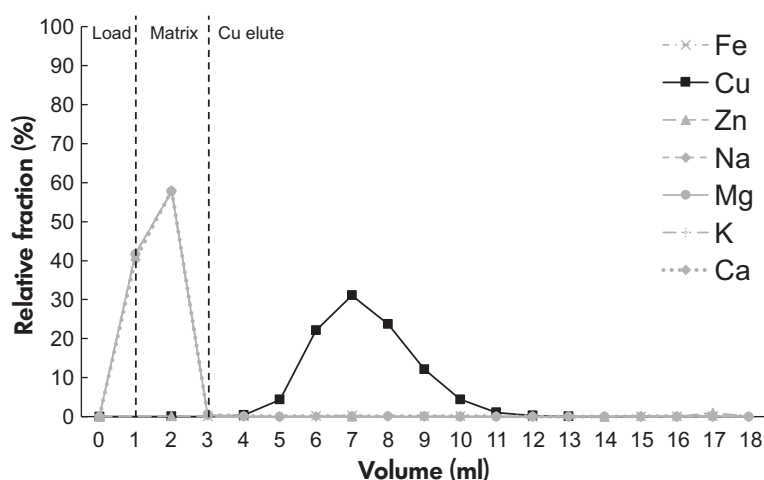
After undergoing chemical separation, purified Cu solutions were evaporated to dryness at 160 °C and refluxed twice in 50 µl of concentrated HNO<sub>3</sub> to remove chloride ions and convert them to the nitrate form for isotopic analysis (Maréchal *et al.* 1999). Samples were diluted in 2% v/v HNO<sub>3</sub>, such that Cu mass fractions in samples and the bracketing calibrator were matched to within 10%, and then doped with NIST SRM 994 such that Ga mass fractions in samples and the bracketing calibrator were also matched to within 10% to ensure accurate isotope amount ratio measurements (Liu *et al.* 2014, Yang *et al.* 2018). Samples and the bracketing calibrator were typically matched at 2 µg g<sup>-1</sup> Ga, and Cu isotope measurements were performed at Cu mass fractions ranging from 0.25 µg g<sup>-1</sup> to 4 µg g<sup>-1</sup>, depending on the amount of Cu in each

reference material. Note that intensities measured in a 2% HNO<sub>3</sub> for all isotopes of interest were subtracted from intensities measured in all bracketing calibrators and samples. Lauwens *et al.* (2018) reported <sup>36</sup>Ar<sup>16</sup>O<sub>2</sub><sup>1</sup>H<sup>+</sup> and <sup>40</sup>Ar<sup>15</sup>N<sup>16</sup>O<sup>+</sup> polyatomic interferences on <sup>69</sup>Ga<sup>+</sup> and <sup>71</sup>Ga<sup>+</sup> isotopes in much lower mass fractions of Ga (< 20 ng g<sup>-1</sup>). But, no significant polyatomic interferences of <sup>36</sup>Ar<sup>16</sup>O<sub>2</sub><sup>1</sup>H<sup>+</sup> and <sup>40</sup>Ar<sup>15</sup>N<sup>16</sup>O<sup>+</sup> on <sup>69</sup>Ga<sup>+</sup> and <sup>71</sup>Ga<sup>+</sup> isotopes, respectively, were observed in the testing samples containing 2 µg g<sup>-1</sup> Ga in this study, as only 0.00036 V and 0.00023 V were measured in the 2% HNO<sub>3</sub> (which were subtracted from all testing samples), compared with 34.44 V and 24.33 V in the testing solutions.

## Results and discussion

### Mass bias correction using C-SSBIN

Combined standard-sample bracketing and internal normalisation (C-SSBIN) using NIST SRM 994 Ga as an internal standard model was used to correct for instrumental mass bias of Cu after Hou *et al.* (2016). Sample measurements were bracketed with measurements of HICU-1, and a <sup>65</sup>Cu/<sup>63</sup>Cu ratio of 0.44561 ( $R_{\text{HICU-1}}^{65/63}$ ) was used to calculate the Cu mass bias factor ( $f^{\text{Cu}}$ ) between it and the <sup>65</sup>Cu/<sup>63</sup>Cu ratio measured ( $r_{\text{HICU-1}}^{65/63}$ ) in each HICU-1 bracketing calibrator with  $m_{\text{xx}}$  being the atomic mass of each isotope of interest (Equation 1). This mass bias correction factor and the measured <sup>71</sup>Ga/<sup>69</sup>Ga ratio ( $r_{\text{HICU-1}}^{71/69}$ ) were used to calculate the mass bias corrected isotope amount ratios of <sup>71</sup>Ga/<sup>69</sup>Ga in each bracketing calibrator ( $R_{\text{HICU-1}}^{71/69}$ ) using Russell's law (Equation 2) (Ingle *et al.* 2003, Pichat *et al.* 2003). The mean of the mass bias corrected <sup>71</sup>Ga/<sup>69</sup>Ga isotope amount ratios in the bracketing calibrators and the



**Figure 1.** Elution profile of Cu-anion exchange column chromatography method employed in this study.

$^{71}\text{Ga}/^{69}\text{Ga}$  isotope amount ratio measured in the sample ( $r_{\text{sample}}^{71/69}$ ) were then used to calculate the Ga mass bias correction factor ( $f^{\text{Ga}}$ , Equation 3), which, along with the  $^{65}\text{Cu}/^{63}\text{Cu}$  isotope amount ratio measured in the sample ( $r_{\text{sample}}^{65/63}$ ), was used to calculate the mass bias corrected  $^{65}\text{Cu}/^{63}\text{Cu}$  isotope amount ratio in the sample ( $R_{\text{sample}}^{65/63}$ , Equation 4).

$$f^{\text{Cu}} = \frac{\ln\left(\frac{R_{\text{HICU-1}}^{65/63}}{r_{\text{HICU-1}}^{65/63}}\right)}{\ln\left(\frac{m_{65}}{m_{63}}\right)} \quad (1)$$

$$R_{\text{HICU-1}}^{71/69} = r_{\text{HICU-1}}^{71/69} \cdot \left(\frac{m_{71}}{m_{69}}\right)^{f^{\text{Cu}}} \quad (2)$$

$$f^{\text{Ga}} = \frac{\ln\left(\frac{R_{\text{HICU-1}}^{71/69}}{r_{\text{sample}}^{71/69}}\right)}{\ln\left(\frac{m_{71}}{m_{69}}\right)} \quad (3)$$

$$R_{\text{sample}}^{65/63} = r_{\text{sample}}^{65/63} \cdot \left(\frac{m_{65}}{m_{63}}\right)^{f^{\text{Ga}}} \quad (4)$$

The parts per thousand or permil (‰) variation in the mass bias corrected  $^{65}\text{Cu}/^{63}\text{Cu}$  isotope amount ratios of the samples relative to the HICU-1 standard are expressed in delta ( $\delta$ ) notation and were calculated using Equation 5) with a  $R_{\text{HICU-1}}^{65/63}$  value of 0.44561. HICU-1 was determined against NIST SRM 976 Cu using C-SSBIN to correct for mass bias, and a  $\delta^{65}\text{Cu}$  value of  $-0.04 \pm 0.03\text{‰}$  ( $U, k = 2$ ) was determined (Table 6). All data were subsequently recalculated and reported relative to NIST SRM 976 (Equation 6).

$$\delta^{65}\text{Cu}_{\text{sample vs. HICU-1}} = \left(\frac{R_{\text{sample}}^{65/63}}{R_{\text{HICU-1}}^{65/63}} - 1\right) \quad (5)$$

$$\delta^{65}\text{Cu}_{\text{sample vs. SRM976}} = \left[\left(\frac{\delta^{65}\text{Cu}_{\text{sample vs. HICU-1}}}{1000} + 1\right) \cdot \left(\frac{\delta^{65}\text{Cu}_{\text{HICU-1 vs. SRM976}}}{1000} + 1\right) - 1\right] \quad (6)$$

It should be noted that the true isotope amount ratios of Cu in HICU-1 ( $R_{\text{HICU-1}}^{65/63}$ ) and Ga in NIST SRM 994 are not required for this correction because the Ga internal standard element serves as an isotopic fractionation correction proxy (Yang *et al.* 2018). The value obtained for the Ga isotope amount ratio may be biased due to the limitations of the isotopic fractionation correction model (e.g.,  $f_{\text{Cu}} = f_{\text{Ga}}$ ), but this

is mostly negated in the second step of the calibration from Ga→Cu (Yang *et al.* 2018). The main advantage of the C-SSBIN as compared with SSB is that irregular changes in the instrumental isotopic fractionation during the measurement sequence are corrected for (Yang *et al.* 2018) since the internal standard is added to both the bracketing calibrator and sample, and is measured in both solutions. As a result, C-SSBIN produces better measurement precision in general.

### Uncertainty estimation for $\delta^{65}\text{Cu}$

The combined uncertainty associated with the reported  $\delta^{65}\text{Cu}$  was estimated in accordance with JCGM 2008 ‘Guide to the Expression of Uncertainty in Measurement’, using the following law of propagation of uncertainty:

$$u^2(y) = \sum_{i=1}^N \left(\frac{\partial f}{\partial x_i}\right) \cdot u^2(x_i) + 2 \cdot \sum_{i=1}^{N-1} \sum_{j=i+1}^N \left(\frac{\partial f}{\partial x_i}\right) \cdot \left(\frac{\partial f}{\partial x_j}\right) \cdot u(x_i, x_j) \quad (7)$$

where  $y = f(x_1, x_2, \dots, x_N)$ ,  $\partial f/\partial x_i$  are partial derivatives,  $u(x_i)$  is the standard uncertainty associated with the input  $x_i$ , and  $u(x_i, x_j)$  is the estimated covariance associated with  $x_i$  and  $x_j$ . For a simplicity, the Equation (8) can be used to calculate the uncertainty of  $\delta^{65}\text{Cu}$ , since Equation (5) can be simplified to  $\delta^{65}\text{Cu} = r_{\text{sample}}^{65/63}/r_{\text{std}}^{65/63} - 1$ . Detailed information and an example of the calculation of  $u(\delta^{65}\text{Cu})$  are provided in online supporting information Appendix S1.

$$u^2(\delta^{65}\text{Cu}) = \left(\frac{1}{r_{\text{std}}^{65/63}}\right)^2 \cdot u^2(r_{\text{sample}}^{65/63}) + \left(-\frac{r_{\text{sample}}^{65/63}}{(r_{\text{std}}^{65/63})^2}\right)^2 \cdot u^2(r_{\text{std}}^{65/63}) \quad (8)$$

### Copper isotopic analysis in the candidate isotopic reference material HICU-1

The results of the Cu isotopic analysis of the twelve individual Cu wire pieces compared with the mixture of Cu solutions (HICU-1) are summarised in Table 4. Using SSB, a  $\delta^{65}\text{Cu}_{\text{HICU-1}}$  value of  $-0.02 \pm 0.17\text{‰}$  ( $U, k = 2$ ) was determined, whereas with C-SSBIN, the  $\delta^{65}\text{Cu}_{\text{HICU-1}}$  value was  $-0.01 \pm 0.04\text{‰}$  ( $U, k = 2$ ). It is evident that the uncertainty of measurements was improved ~ fourfold using C-SSBIN compared with SSB alone, demonstrating its superiority. The  $\delta^{65}\text{Cu}_{\text{HICU-1}}$  value of the Cu wire pieces is within the uncertainty of measurements of the candidate HICU-1 reference material, demonstrating sufficient

**Table 4.**  
**Results of Cu wire homogeneity test**

Material	<i>n</i>	SSB $\delta^{65}\text{Cu}_{\text{HICU-1}}$ (‰)	C-SSBIN $\delta^{65}\text{Cu}_{\text{HICU-1}}$ (‰)
Cu-1	5	-0.01 ± 0.10	-0.01 ± 0.01
Cu-2	5	-0.01 ± 0.05	-0.01 ± 0.01
Cu-3	5	0.01 ± 0.06	-0.01 ± 0.01
Cu-4	5	0.01 ± 0.04	-0.01 ± 0.01
Cu-5	5	0.01 ± 0.09	0.00 ± 0.00
Cu-6	5	0.02 ± 0.14	-0.01 ± 0.01
Cu-7	5	-0.01 ± 0.03	-0.01 ± 0.01
Cu-8	5	-0.02 ± 0.03	-0.01 ± 0.01
Cu-9	5	-0.16 ± 0.22	-0.01 ± 0.01
Cu-10	5	-0.10 ± 0.06	-0.01 ± 0.01
Cu-11	5	0.06 ± 0.26	-0.02 ± 0.02
Cu-12	5	-0.02 ± 0.29	-0.01 ± 0.01
Mean		-0.02	-0.01
2s		0.12	0.01
<i>U, k = 2</i>		0.17	0.04

*n* = total number of Cu isotope ratio measurements performed on each dissolved pure Cu wire piece by MC-ICP-MS.

homogeneity and the suitability of this material as a Cu isotopic reference material.

Subsequent experiments were performed, and a  $\delta^{65}\text{Cu}$  value of  $-0.25 \pm 0.05\text{‰}$  ( $U, k = 2$ ) was obtained in HICU-1 relative to ERM<sup>®</sup>-AE647, whereas a  $\delta^{65}\text{Cu}$  value of  $-0.04 \pm 0.03\text{‰}$  ( $U, k = 2$ ) was found in HICU-1 relative to NIST SRM 976. As stated in the ERM<sup>®</sup>-AE647 certificate (2013), its Cu isotope amount ratio was calibrated with NIST SRM 976, and Moeller *et al.* (2012) reported a value of  $-0.21 \pm 0.05\text{‰}$  ( $U, k = 2$ ) for NIST SRM 976 relative to the ERM<sup>®</sup>-AE647; however, this is in disagreement with the mean  $\delta^{65}\text{Cu}$  value ( $(0.44563/0.44560-1) \times 1000 = 0.067\text{‰}$ ) calculated from the certified values in NIST SRM 976 ( $R_{65/63} = 1/R_{63/65} = 1/2.2440 = 0.44563$ , NIST Cu certificate 1994) and ERM<sup>®</sup>-AE647 ( $R_{65/63} = 0.44560$ , IRMM Cu certificate, 2013), respectively. Thus in this study, NIST SRM 976 was measured against ERM<sup>®</sup>-AE647, and a  $\delta^{65}\text{Cu}$  value of  $-0.21 \pm 0.06\text{‰}$  ( $U, k = 2$ ) was obtained, in excellent agreement with the result reported by Moeller *et al.* (2012).

### Monitoring of in-house standards and reference material matrix doped with HICU-1

Synthetic serum standards doped with HICU-1 and a HICU-1 single-element standard were processed alongside samples through the entire anion exchange column chromatography procedure to monitor the efficacy of the separation method. The synthetic serum standards and single-element standard were prepared with HICU-1,

meaning that when measured against the HICU-1 bracketing calibrator during C-SSBIN, they should return  $\delta^{65}\text{Cu}_{\text{HICU-1}}$  values of 0‰, with any variation being accounted for by measurement uncertainty. The results of this monitoring returned  $\delta^{65}\text{Cu}_{\text{HICU-1}}$  values of synthetic serum standards and pure HICU-1 from  $-0.02 \pm 0.08\text{‰}$  ( $U, k = 2$ ) to  $0.00 \pm 0.05\text{‰}$  ( $U, k = 2$ ) throughout the study period (Table 5). As a further means of validating results, elements removed in the first 3 ml of the chromatography procedure (Figure 1) were collected from several reference materials after both the first and the second passes through columns and combined. This matrix solution contained all elements except Cu, which was eluted in the subsequent 15 ml of  $7.6 \text{ mol l}^{-1}$  HCl, and Fe and Zn, which remained on the column throughout Cu elution. This Cu-free solution of matrix elements was then doped with HICU-1 to replace sample Cu with the same mass of Cu that would have been in the original processed aliquot of sample and then dried down and processed through the entire two-pass chromatography procedure before isotopic analysis. As with the synthetic serum standards and pure HICU-1 standard, reference material matrix doped with HICU-1 should produce  $\delta^{65}\text{Cu}_{\text{HICU-1}}$  values of 0‰ within uncertainty of measurements. If any original Cu or matrix/interference elements remain after reprocessing, they would impart a portion of the  $\delta^{65}\text{Cu}$  value from the original sample on HICU-1 and cause matrix/interference effects, respectively. DOLT-5, DORM-4, TORT-3 and BIR-1 underwent this novel validation method, and despite the differences in the matrices and original  $\delta^{65}\text{Cu}$  values,  $\delta^{65}\text{Cu}_{\text{HICU-1}}$  values of

**Table 5.**  
**Synthetic standards and reference materials matrix doped with HICU-1 (mean, *U, k = 2*)**

Material	<i>n</i> (l)	$\delta^{65}\text{Cu}_{\text{HICU-1}}$ (‰) [Doped with HICU-1]
Low Cu synthetic serum (0.6 µg Cu)	19 (7)	-0.02 ± 0.08 (0.05) <sup>a</sup>
High Cu synthetic serum (9.9 µg Cu)	13 (4)	-0.02 ± 0.07 (0.06)
HICU-1 (10 µg Cu)	11 (3)	0.00 ± 0.05 (0.03)
DOLT-5 Cu-doped matrix (3.1 µg Cu)	8 (3)	-0.01 ± 0.03 (0.02)
DORM-4 Cu-doped matrix (1.6 µg Cu)	9 (3)	0.02 ± 0.06 (0.04)
TORT-3 Cu-doped matrix (30.9 µg Cu)	3 (1)	0.02 ± 0.05 (0.03)
BIR-1 Cu-doped matrix (3.0 µg Cu)	3 (1)	-0.02 ± 0.06 (0.04)

l = number of reference material aliquots digested and processed through chromatography procedure, *n* = total number of Cu isotope ratio measurements performed on each material by MC-ICP-MS.

<sup>a</sup>) values in parenthesis are 2s.

**Table 6.**  
Results (mean,  $U$ ,  $k = 2$ ) for reference materials

Material	Type	Cu ( $\mu\text{g g}^{-1}$ )	$n$ ( $l$ )	$\delta^{65}\text{Cu}_{\text{HICU-1}}$ (‰)	$\delta^{65}\text{Cu}_{\text{SRM 976}}$ (‰)	Reported <sup>a</sup>	Source
HICU-1	Copper wire		18		$-0.04 \pm 0.03$ (0.02) <sup>c</sup>		
DOLT-5	Dogfish liver	35	23 (6)	$0.02 \pm 0.07$ (0.04) <sup>c</sup>	$-0.02 \pm 0.08$ (0.04)		
DORM-4	Fish protein	15.7	17 (6)	$0.57 \pm 0.07$ (0.05)	$0.52 \pm 0.08$ (0.05)		
TORT-3	Lobster hepatopancreas	497	18 (6)	$0.41 \pm 0.04$ (0.03)	$0.36 \pm 0.05$ (0.03)		
MESS-4	Marine sediment	32.9	21 (6)	$-0.05 \pm 0.06$ (0.04)	$-0.09 \pm 0.07$ (0.04)		
PACS 3	Marine sediment	326	15 (5)	$-0.05 \pm 0.08$ (0.05)	$-0.10 \pm 0.09$ (0.05)		
BHVO-1	Hawaiian basalt	136	9 (3)	$0.04 \pm 0.07$ (0.05)	$-0.01 \pm 0.08$ (0.05)	$-0.23 \pm 0.14$ ( $n = 3$ )	Makishima (2014)
BIR-1	Icelandic basalt	120	9 (3)	$0.04 \pm 0.07$ (0.04)	$-0.01 \pm 0.08$ (0.04)	$-0.01 \pm 0.04$ ( $n = 5$ )*	Liu <i>et al.</i> (2014)
						$-0.02 \pm 0.10$ ( $n = 31$ )	Li <i>et al.</i> (2009)
						$0.00 \pm 0.03$ ( $n = 2$ )	Sossi <i>et al.</i> (2015)
						$0.08 \pm 0.07$ ( $n = 6$ )	Moeller <i>et al.</i> (2012)
W-2a	Diabase	110	11 (3)	$0.09 \pm 0.08$ (0.06)	$0.04 \pm 0.09$ (0.06)	$0.11 \pm 0.02$ ( $m = 2$ )*	Liu <i>et al.</i> (2014)
SGR-1b	Green River Shale	53.3	11 (3)	$0.34 \pm 0.08$ (0.06)	$0.29 \pm 0.09$ (0.06)		
Seronorm <sup>b</sup>	Human serum	1.2	5 (2)	$-0.15 \pm 0.05$ (0.03)	$-0.20 \pm 0.06$ (0.03)	$-0.20 \pm 0.04$ ( $m = 3$ )*	Lauwens <i>et al.</i> (2018)
						$-0.19 \pm 0.05$ ( $m = 4$ )*	Lauwens <i>et al.</i> (2017)
						$-0.24 \pm 0.14$ ( $m = 4$ )*	Lauwens <i>et al.</i> (2016)
						$-0.07 \pm 0.07$ ( $m = 2$ )*	Costas-Rodriguez <i>et al.</i> (2015)

<sup>a</sup> The previously reported Cu isotopic compositions were calculated relative to NIST SRM 976 (mean, 2s).

<sup>b</sup> Seronorm™ Trace Elements Serum L-1,  $l$  = number of reference material aliquots digested and passed through column chemistry,  $n$  = total number of Cu isotope ratio measurements performed on each reference material by MC-ICP-MS.

<sup>c</sup> () values in parenthesis are 2s, \* mean  $\delta^{65}\text{Cu} \pm 2s$  of mean after  $m$  isolation and measurement sessions.

$-0.02 \pm 0.08\text{‰}$  ( $U$ ,  $k = 2$ ) to  $0.02 \pm 0.06\text{‰}$  ( $U$ ,  $k = 2$ ) (Table 5) were measured. For example, DORM-4 has a  $\delta^{65}\text{Cu}_{\text{HICU-1}}$  value of  $0.57 \pm 0.07\text{‰}$  ( $U$ ,  $k = 2$  in Table 6), but DORM-4 matrix doped with HICU-1 Cu and reprocessed through the chromatography procedure produced a  $\delta^{65}\text{Cu}_{\text{HICU-1}}$  value of  $0.02 \pm 0.06\text{‰}$  ( $U$ ,  $k = 2$  in Table 5). These results further confirm that the proposed method is accurate within measurement uncertainties reported.

### Cu isotope amount ratio measurements of reference materials

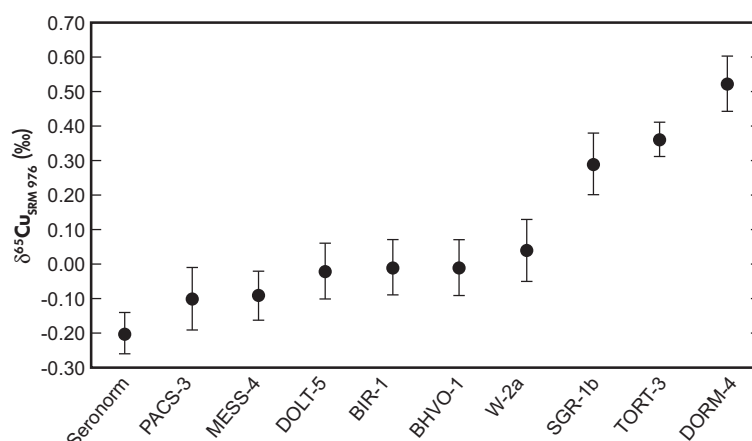
The reference materials measured in this study possess complex and varied matrices with Cu mass fractions ranging from  $1.2 \mu\text{g g}^{-1}$  to  $497 \mu\text{g g}^{-1}$ , and the  $\delta^{65}\text{Cu}_{\text{SRM976}}$  values range from  $-0.20\text{‰}$  to  $0.52\text{‰}$  (Figure 2, Table 6), covering much of the natural Cu isotope variability observed in the environment.

DOLT-5, DORM-4 and TORT-3 are intended for use in the calibration of procedures and the development of methods for the determination of trace and matrix constituents in marine fauna and materials of similar matrix. For the first time, these materials were analysed for the  $\delta^{65}\text{Cu}$ . The Cu isotopic homogeneity of two separate bottles for each material was examined with three 0.25 g aliquots digested from each for a total of six aliquots analysed from

each material. These materials were found to possess  $\delta^{65}\text{Cu}_{\text{SRM976}}$  values ranging from  $-0.02$  to  $0.54\text{‰}$ , with  $^{65}\text{Cu}$  enrichment increasing from DOLT-5 (dogfish liver) with a  $\delta^{65}\text{Cu}_{\text{SRM976}}$  value of  $-0.02\text{‰}$  to TORT-3 (lobster hepatopancreas) with a  $\delta^{65}\text{Cu}_{\text{SRM976}}$  value of  $0.36\text{‰}$  to DORM-4 (fish protein) with a  $\delta^{65}\text{Cu}_{\text{SRM976}}$  value of  $0.52\text{‰}$ . Cadmium (Cd), Zinc (Zn) and lead (Pb) isotope amount ratios have been used to distinguish between natural and anthropogenic sources and fingerprint sources of metal contamination in bivalves off the coasts of France, Western Canada and the United States (Shiel *et al.* 2012, 2013). Copper abundances in bivalve mussels from the United States, Hong Kong, Canada, Turkey, Italy and Chile range from  $1.4$  to  $744 \mu\text{g g}^{-1}$  (De Gregori *et al.* 1994), which is similar to the Cu mass fractions found in DOLT-5, DORM-4 and TORT-3, making them suitable candidates for being measured alongside bivalve mussels being analysed for  $\delta^{65}\text{Cu}$  values. The characterisation of these marine fauna reference materials with varying Cu abundances as isotopic standards may enable Cu isotopes to be used in conjunction with Cd, Zn and Pb isotopes for the definitive fingerprinting of pollution sources and distinguishing of natural and anthropogenic metal sources.

Wilson's disease (WD) is an inherited Cu metabolism disorder that leads to excess Cu accumulation in the liver. The Cu mass fraction in liver biopsies is currently used as one





**Figure 2. Visualisation of reference material  $\delta^{65}\text{Cu}$  values. Bars represent  $U$ ,  $k = 2$  (see text for details).**

of the parameters to diagnose WD, but a combination of clinical and biochemical tests is required to diagnose WD and exclude other diseases (Ferenci *et al.* 2005). A comprehensive study found liver biopsy Cu mass fractions in ‘healthy’, non-WD subjects ranged from  $12.5 \mu\text{g g}^{-1}$  to  $80.8 \mu\text{g g}^{-1}$  with a mean of  $34.9 \mu\text{g g}^{-1}$ , whereas patients with WD had liver Cu mass fractions that went as high as  $5000 \mu\text{g g}^{-1}$  (Ferenci *et al.* 2005). Given the invasive nature of the surgery, gaining additional diagnostic information from liver biopsy samples should be a priority to justify the procedure. Copper mass fractions alone are insufficient for a WD diagnosis, but it is possible that a  $\delta^{65}\text{Cu}$  could complement this data by tracing the functional deficiency of Cu-transporting adenosinetriphosphatase, ATP7B, that is highly active in hepatocytes and leads to the gradual accumulation of Cu in the liver (Bull *et al.* 1993, Tanzi *et al.* 1993). The combination of Cu mass fraction and isotopic data may provide greater sensitivity and specificity in the diagnosis of WD, and the validity of these measurements could be ensured through the analysis of high Cu DOLT-5 and TORT-3 alongside liver biopsy samples.

MESS-4 and PACS-3 are primarily intended for use in the calibration of procedures and development of methods for the determination of trace and matrix constituents in marine sediments and materials with similar matrices. For the first time, we have determined the  $\delta^{65}\text{Cu}$  of NRC marine sediment certified reference materials MESS-4 and PACS-3. Two bottles of each material were used in this study, and three 0.25 g aliquots (following the recommendation of the certification report) were digested from both MESS-4 bottles whereas three were digested from the first bottle of PACS-3 and two from the second. MESS-4 was found to have a  $\delta^{65}\text{Cu}_{\text{SRM976}}$  value of  $-0.09 \pm 0.07\text{‰}$  ( $U$ ,  $k = 2$ ) whereas PACS-3 was found to have a  $\delta^{65}\text{Cu}_{\text{SRM976}}$  value of

$-0.10 \pm 0.09\text{‰}$  ( $U$ ,  $k = 2$ ). Older reference materials, MESS-3 and PACS-2, were analysed in a recent paper (Araújo *et al.* 2019) and found to possess slightly different  $\delta^{65}\text{Cu}_{\text{SRM976}}$  values of  $0.03 \pm 0.10\text{‰}$  ( $2s$ ,  $n = 9$ ) and  $0.05 \pm 0.06\text{‰}$  ( $2s$ ,  $n = 3$ ), respectively. This might be due to the difference in the production of reference materials in different time. It is interesting that both marine sediment reference materials were found to possess similar  $\delta^{65}\text{Cu}_{\text{SRM976}}$  despite MESS-4 being taken from the Beaufort Sea and PACS-3 being taken from the harbour Esquimalt off the southern tip of Vancouver Island in British Columbia, Canada. However, this is unsurprising given the similar origin of the island arcs that eventually became the accreted terranes that comprise the rocks of both British Columbia and Alaska and eroded to form these marine sediments.

The Hawaiian basalt reference material, BHVO-1, was selected because to our knowledge, its  $\delta^{65}\text{Cu}_{\text{SRM976}}$  value is reported in just one study (Makishima 2014). In that study, a  $\delta^{65}\text{Cu}_{\text{SRM976}}$  value of  $-0.23 \pm 0.14\text{‰}$  ( $2s$ ) was determined, which is approximately 0.33‰ lighter than that reported for the later BHVO-2 and the mean precision of the measurements of silicate reference materials during the study was  $\pm 0.25\text{‰}$  ( $1s$ ). We report a  $\delta^{65}\text{Cu}_{\text{SRM976}}$  value of  $-0.01 \pm 0.08\text{‰}$  ( $U$ ,  $k = 2$ ) in three separate digests of BHVO-1, bringing its  $\delta^{65}\text{Cu}_{\text{SRM976}}$  more in-line with a weighted mean of results previously reported for BHVO-2 ( $\delta^{65}\text{Cu}_{\text{SRM976}} = 0.10\text{‰}$ ,  $n = 49$ ) (Moynier *et al.* 2010, Moeller *et al.* 2012, Liu *et al.* 2014, Sossi *et al.* 2015, Hou *et al.* 2016) and values typically observed in basalts. Despite the  $\sim 0.10\text{‰}$  difference between our measurements and the BHVO-2 weighted mean, Sossi *et al.* (2015) reported a BHVO-2  $\delta^{65}\text{Cu}_{\text{SRM976}}$  value of  $0.01 \pm 0.04\text{‰}$  ( $2s$ ,  $n = 12$ ), which is within the uncertainty of our measurements.

The Icelandic basalt reference material, BIR-1, is commercially available and widely reported in Cu isotopic studies (Li *et al.* 2009, Moeller *et al.* 2012, Liu *et al.* 2014, Sossi *et al.* 2015). Here, we report a  $\delta^{65}\text{Cu}_{\text{SRM976}}$  value of  $-0.01 \pm 0.08\text{‰}$  ( $U, k = 2$ ) in three separate digests of BIR-1, which is in agreement with the weighted mean BIR-1  $\delta^{65}\text{Cu}_{\text{SRM976}}$  value reported in past studies ( $0.00\text{‰}$ ,  $n = 44$ ). The agreement between our BIR-1 results and past published results supports the validity of the results reported in this study for BHVO-1 due to their highly similar matrix and geologic origin. The diabase, W-2a, is commercially available and has been reported in one past study published by Liu *et al.* (2014). Here, we report a  $\delta^{65}\text{Cu}_{\text{SRM976}}$  value of  $0.04 \pm 0.09\text{‰}$  ( $U, k = 2$ ) in three separate digests of W-2a, which is within the uncertainty of the reported W-2a  $\delta^{65}\text{Cu}_{\text{SRM976}}$  value ( $0.11\text{‰}$ ,  $n = 12$ ).

For the first time, we report a  $\delta^{65}\text{Cu}_{\text{SRM976}}$  value for the Green River shale reference material, SGR-1b, of  $0.29 \pm 0.09\text{‰}$  ( $U, k = 2$ ). To our knowledge, this is the first report of  $\delta^{65}\text{Cu}$  values for a commercially available shale reference material, and its measurement will provide researchers investigating redox processes and metallic cycling in shales with a matrix-matched RM to support the accuracy and validity of their measurements. Of a sampling of four studies that investigated Cu isotopic fractionation in shales (Asael *et al.* 2007, Mathur *et al.* 2012, Chi Fru *et al.* 2016, Lv *et al.* 2016), just one reported the measurement of Cu reference materials alongside their samples (Lv *et al.* 2016). However, these reference materials are igneous rocks, which possess a different matrix from shales. Basalts and other igneous rocks tend to contain greater mass fractions of most elements, including Na, Ti, Ca and Fe, but SGR-1b contains over 3 and 66 times more K than BHVO-1 and BIR-1, respectively, which chromatography procedures may not be calibrated to completely remove from samples, potentially causing matrix effects.

Seronorm™ Trace Elements Serum L-1 has been measured for  $\delta^{65}\text{Cu}$  in four previous studies (Costas-Rodríguez *et al.* 2015, Lauwens *et al.* 2016, 2017, Lauwens *et al.* 2018), and bottles with at least three different lot numbers were used (unreported in Costas-Rodríguez *et al.* (2015)). A weighted mean  $\delta^{65}\text{Cu}_{\text{SRM976}}$  value of  $-0.19 \pm 0.05\text{‰}$  ( $2s, n = 33$ ) was obtained in the two most recent publications using Seronorm™ as a reference material (Lauwens *et al.* 2017, Lauwens *et al.* 2018). A study published the year before (Lauwens *et al.* 2016) reported a  $\delta^{65}\text{Cu}_{\text{SRM976}}$  value of  $-0.24 \pm 0.14\text{‰}$  ( $2s$ ), which is also within the uncertainty. However, the measurements performed by Costas-Rodríguez *et al.* (2015) are outliers with reported  $\delta^{65}\text{Cu}_{\text{SRM976}}$  values of  $-0.09 \pm 0.05\text{‰}$  ( $2s$ ) and  $-0.04 \pm 0.05\text{‰}$  ( $2s$ ) over

an unknown number of measurements on a bottle of undeclared lot number. Our result of  $-0.20 \pm 0.06\text{‰}$  ( $U, k = 2$ ) is in agreement with those reported in the three Lauwens *et al.* studies but additional bottles of different lot numbers should be analysed by different research groups to determine its suitability as the first serum Cu isotopic reference material.

## Conclusions

The robust characterisation of  $\delta^{65}\text{Cu}$  values in a wide variety of geological and biological reference materials is a crucial step in the effort to study the processes that govern Cu distribution in new media and environments. Six reference materials comprising DOLT-5 (dogfish liver), DORM-4 (fish protein), TORT-3 (lobster hepatopancreas), MESS-4 (marine sediment), PACS-3 (marine sediment) and SGR-1b (Green River shale) have for the first time been characterised for  $\delta^{65}\text{Cu}$ . Additionally, four reference materials with published  $\delta^{65}\text{Cu}$  values have been measured, comprising BHVO-1 (Hawaiian basalt), BIR-1 (Icelandic basalt), W-2a (diabase) and Seronorm™ Trace Elements Serum L-1 (human serum).

The determination of  $\delta^{65}\text{Cu}$  values was achieved using the C-SSBIN mass bias correction model with Ga as an internal standard after Hou *et al.* (2016) and validated by (a) monitoring Ti/Cu and Na/Cu ratios of column-processed materials to ensure contaminant levels remained negligible, (b) determining Cu yield and separation quality using quantitative and semi-quantitative methods, (c) consistently achieving procedural blank Cu contributions that were negligible ( $< 0.5\%$ ) relative to the amount of Cu processed in all study materials, (d) preparing and processing two synthetic standards doped with HICU-1 Cu through the chromatography procedure and consistently producing  $\delta^{65}\text{Cu}$  values of  $0\text{‰}$  relative to bracketing calibrator HICU-1, (e) doping matrix elements collected during Cu chromatography with HICU-1 Cu and reprocessing doped materials through the chromatography procedure to verify that  $\delta^{65}\text{Cu}$  values of  $0\text{‰}$  were produced when bracketed with HICU-1 and (f) measuring similar  $\delta^{65}\text{Cu}$  values in previously characterised reference materials using our methodologies. We recommend the reference materials characterised by this study to be analysed alongside samples with similar matrices in future Cu isotopic studies and expect them to help researchers investigating biological processes in marine fauna, high Cu tissues and serum, high temperature geological processes in igneous rocks, anthropogenic processes in marine sediments, and redox processes and metallic cycling in shale.

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## Conflict of interest

The authors declare no competing financial interests to influence the work reported in this paper.

## Data availability statement

Data available on request due to privacy/ethical restrictions.

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## Supporting information

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The following supporting information may be found in the online version of this article:

Appendix S1. Uncertainty estimation for  $^{65}\text{Cu}$ .

This material is available from: <http://onlinelibrary.wiley.com/doi/10.1111/ggr.12315/abstract> (This link will take you to the article abstract).