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# COPPER ISOTOPE COMPOSITIONS MEASURED USING THE SAPPHIRE DUAL PATH MC-ICPMS WITH COLLISION/REACTION CELL

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## Abstract

We present a new approach to Cu isotopic measurements using the state-of-the-art Nu Sapphire multi-collector inductively coupled plasma source mass spectrometer equipped with a Collision/Reaction Cell (CRC-MC-ICPMS). We investigate the effects of Na doping and Cu concentration mismatch between bracketing standard and unknown samples, and demonstrate the efficacy of introducing a He-H<sub>2</sub> gas mix into the CRC to efficiently eliminate the sample matrix-based <sup>40</sup>Ar<sup>23</sup>Na<sup>+</sup> isobaric interference on <sup>63</sup>Cu<sup>+</sup>. This capability is crucial when measuring samples with high Na/Cu ratios, such as some biological samples, which have significantly different chemical compositions compared to most geological samples. Moreover, considering the necessity of obtaining large datasets for biological samples to ensure reliable interpretations, the implementation of a CRC for mitigating the <sup>40</sup>Ar<sup>23</sup>Na<sup>+</sup> interference offers the advantage of minimizing the requirement for extensive Cu chemical separation procedure prior to Cu isotopic measurements. Our results demonstrate that the accurate determination of the  $\delta^{65}\text{Cu}$  values is achievable for samples with Na/Cu concentration ratios of up to  $\sim 65$ , even when measuring 100 ppb Cu solutions (equivalent to a signal of  $\sim 3.5$  V to 4 V total Cu). Furthermore, our results showcase a good short-term repeatability on  $\delta^{65}\text{Cu}$  for pure Cu standard solutions (NIST SRM 976 and Cu-IPGP), typically of 0.05 ‰ (2 SD) when measuring  $> 50$  ppb Cu solutions. Our long-term external reproducibility stands at approximately 0.07 ‰ (2 SD). This value accounts for the variable Cu concentrations analysed across the different analytical sequences (from 10 to 100 ppb Cu solutions). To validate the robustness of our analytical method, we first conduct a comparison between datasets from mice brains processed twice through column chemistry using a Thermo Finnigan Neptune MC-ICPMS and a Nu Sapphire CRC-MC-ICPMS in CRC mode. This comparison serves to verify the reliability of our method for measuring Cu isotopic composition using the CRC on samples with low Na/Cu ratio after traditional chemical processing. Then, we compare the datasets obtained for biological standards (tuna fish ERM-CE 464 (IRMM) and human serum Seronorm<sup>TM</sup> Trace Elements Serum L-1) processed either once, or twice, through column

chemistry and demonstrate that the CRC allows accurate Cu isotopic measurements of the samples processed only once and therefore with higher Na/Cu ratio.

## 1. Introduction

Copper, a transition metal, possesses two stable isotopes,  $^{63}\text{Cu}$  and  $^{65}\text{Cu}$ , with natural abundances of 69.17 % and 30.83 %, respectively. The isotopic composition of Cu has served as a valuable tool for tracing volatilization, magmatic and redox processes.<sup>1</sup> In addition to its geological applications, Cu isotopic compositions have found utility in biology as a biomarker, enabling the tracing of changes in Cu homeostasis induced by various diseases, such as liver cirrhosis, colorectal, breast and liver cancer, or neurodegenerative diseases.<sup>2-8</sup> Analysing biological samples, however, presents distinct challenges compared to geological samples, including the need for larger datasets to derive robust interpretations and the complexity of matrices, often featuring elevated sodium contents. Consequently, a more time-consuming chemical separation process is required before measuring Cu isotopic compositions using traditional multi-collector inductively coupled plasma source mass spectrometry (MC-ICPMS) techniques.

Copper isotopic compositions were initially determined using thermal ionisation mass spectrometry (TIMS) in the 1960s.<sup>9-10</sup> However, due to the relatively high first ionisation potential of Cu (7.7 eV) combined with challenges in accurately assessing the instrumental isotopic fractionation (as the double spike technique cannot apply to Cu, which has only 2 isotopes), the analytical uncertainty achieved was poor (no better than 2 ‰ per amu<sup>9-10</sup>). Consequently, there were limited studies on Cu isotopes during that time. The introduction of MC-ICPMS in the late 1990s has revolutionized Cu isotope research by enabling improved analytical capabilities. Thus, MC-ICPMS offers the advantage of correcting for instrumental isotopic fractionation through standard-sample bracketing, either with or without the addition of internal standard elements such as Zn, Ni or Ga.<sup>11-15</sup> As a result, the precision and accuracy of the Cu isotopic data obtained by MC-ICPMS critically depends on two main factors: (i) the accurate correction of instrumental isotopic fractionation, and (ii) the quality of Cu purification, including high column yield, purity of the Cu fraction and low blank compared to the amount of Cu measured. It is important to stress that the standard-sample bracketing procedure assumes a similar matrix between the unknown sample and the bracketing standard, specifically containing only Cu. This assumption underscores the significance of proper Cu purification and handling in ensuring reliable and meaningful Cu isotopic measurements.

The isotopes of masses 63 and 65 of Cu are prone to isobaric interferences from the sample matrix itself, specifically  $^{40}\text{Ar}^{23}\text{Na}^+$  on  $^{63}\text{Cu}^+$  and  $^{40}\text{Ar}^{25}\text{Mg}^+$  on  $^{65}\text{Cu}^+$ . Although most of the Mg and Na are removed during the Cu chemical separation process (see section 2a), a small fraction of Na could still be eluted alongside Cu, especially in Na-rich biological samples like brains or serums. This can pose challenges when accurately measuring these samples using traditional MC-ICPMS techniques. Several studies have highlighted the sensitivity of Cu isotopic compositions to the presence of Na, resulting in significant negative offsets (> 0.1 ‰) in Cu isotopic compositions

when Na contents exceed a few hundreds of ppb only.<sup>14–18</sup> Consequently, accurate determination of Cu isotopic compositions using conventional MC-ICPMS instruments (Thermo Finnigan Neptune Plus, Nu Plasma II, Nu Sapphire in Conventional mode) is only possible when Na/Cu concentrations ratios are approximately  $< 0.5$  for 250 ppb Cu solutions, or  $< 1.5$  for 100 ppb Cu solutions.

The introduction of the Nu Sapphire MC-ICPMS equipped with Collision/Reaction Cell (CRC-MC-ICPMS) in 2017 has provided a new avenue for studying isotopic systems that were previously inaccessible or technically challenging using traditional MC-ICPMS, notably because of Ar-based interferences. Subsequent studies on Ca, K and Fe isotope systems have demonstrated that employing Collision/Reaction Cell (CRC) with appropriate gases effectively removed interferences such as  $^{40}\text{Ar}^+$  on  $^{40}\text{Ca}^+$ ,  $^{38}\text{ArH}^+$  on  $^{39}\text{K}^+$ ,  $^{40}\text{ArH}^+$  on  $^{41}\text{K}^+$ ,  $^{40}\text{Ar}^{14}\text{N}^+$  on  $^{54}\text{Fe}^+$ ,  $^{40}\text{Ar}^{16}\text{O}^+$  on  $^{56}\text{Fe}^+$ .<sup>19–22</sup> However, to the best of our knowledge, no study has yet been reported on measuring Cu isotope compositions using the CRC of the Nu Sapphire CRC-MC-ICPMS.

In this paper, we demonstrate that utilising a He-H<sub>2</sub> gas mix in the CRC of the Nu Sapphire CRC-MC-ICPMS enables effective removal of the sample matrix-based  $^{40}\text{Ar}^{23}\text{Na}^+$  interference on  $^{63}\text{Cu}^+$ . As a result, Cu isotope compositions can now be accurately determined in biological samples without the need for extensive chemical separation procedures. This advancement represents a significant improvement in the analysis of Cu isotopes, particularly in samples with high Na content.

## 2. Experimental section

### *a. Sample digestion and chemical separation of copper*

Complete digestion of  $\sim 100$  mg of mice brains, tuna fish standard ERM-CE 464 (IRMM) and human serum Seronorm<sup>TM</sup> Trace Elements Serum L-1 was achieved by using a 1:3 mixture of concentrated H<sub>2</sub>O<sub>2</sub> and 16 mol/L HNO<sub>3</sub> acid in a PTFE Teflon beaker. The digestion process took place for 36 hours at room temperature. The samples were then dried down overnight on a hotplate at 120°C, and redissolved in 1 mL of 7 mol/L HCl, making them ready for the chemical purification stage.

For the Cu chemical separation, we implemented a two-step chromatographic procedure adapted from the method published in Maréchal et al.<sup>11</sup> The first step involved an anion-exchange chromatography using 7 mol/L HCl on homemade Teflon columns. These columns had resin reservoirs measuring approximately 0.5 cm in diameter and bed height of 5 cm, filled with 1.6 mL of AG®MP-1 Bio-Rad resin (100-200 mesh). This step, which lasted around 7-8 hours, effectively removed matrix elements (such as Na, K, Mg, Ca, P, S, Cl in the case of a biological sample). However, for samples with high Na/Cu ratio, a fraction of Na could be eluted together with Cu, potentially creating  $^{40}\text{Ar}^{23}\text{Na}^+$  isobaric interferences on the MC-ICPMS. Therefore, this chromatographic procedure was repeated once more to ensure complete removal of Na. This Cu chemical separation procedure allows, for instance when considering a Na-rich and Cu-poor

sample (such as the tuna fish standard ERM-CE 464), to go from a concentration Na/Cu ratio of  $\sim 2500$  before the purification procedure, to Na/Cu = 25 after one purification and down to Na/Cu = 2 at the end of the double column chemistry.

The total procedural blank, spanning from sample dissolution to measurement on the mass spectrometer, was typically of less than 2 ng of Cu, which is negligible ( $< 0.5\%$ ) compared to the amount of Cu measured here ( $\sim 500$  ng for brains of mice). Following the purification process, samples and standards were diluted in 0.5 mol/L HNO<sub>3</sub>, in preparation for Cu isotope measurements. It is worth noting that we consistently used the same batch of freshly prepared 0.5 mol/L HNO<sub>3</sub> acid for both unknown solutions and their bracketing standard. This practice ensures accuracy in determining the Cu isotopic composition, preventing any inaccuracies coming from the HNO<sub>3</sub> acid molarity mismatch. Similar procedures have been reported in Liu et al.<sup>14</sup> using a Thermo Finnigan Neptune Plus MC-ICPMS, Yuan et al.<sup>23</sup> and Hu and Teng<sup>16</sup> using a Nu Plasma II MC-ICPMS, and Wang et al.<sup>18</sup> using a Nu Sapphire CRC-MC-ICPMS in Conventional mode.

#### *b. Copper isotope measurements by Sapphire CRC-MC-ICPMS*

A Sapphire from Nu Instruments was installed at the Institut de physique du globe de Paris (IPGP) late 2020 (Serial No. 010). The instrument can be operated either in Conventional mode, which is equivalent to traditional MC-ICPMS, or in Collision/Reaction Cell mode. In this study, both pathways were tested for measuring Cu isotopic compositions. The instrument is equipped with 16 fixed Faraday detectors, that can be dynamically connected to pre-amplifiers with different feedback resistors ( $10^{10}$ ,  $10^{11}$ , and  $10^{13}$   $\Omega$  on our instrument). The fixed Faraday detectors system is compensated by an efficient zoom-lens system located between the magnet and the detector array. This zoom-lens system is made of two lenses: quad 1 primarily focuses the individual beams, while quad 2 controls the dispersion of the beams relative to each other.<sup>24</sup> Copper isotope analyses were performed in low resolution with static data acquisition. The two Faraday cups used (<sup>63</sup>Cu on L3 and <sup>65</sup>Cu on H1) were connected to pre-amplifiers with  $10^{11}$   $\Omega$  feedback resistors. A 30 min gain calibration was performed at the start of each analytical session. Nickel cones for wet plasma were used, and the samples were introduced using a MicroMist glass nebulizer and a Peltier cooled Helix CT spray chamber, with an uptake rate of  $\sim 80$   $\mu\text{L}/\text{min}$ . The sensitivity obtained in Conventional mode is typically around 35-40 V/ppm Cu, comparable to the 35 V/ppm Cu reported by Wang et al.<sup>18</sup> who also used a Nu Sapphire. We obtained a similar sensitivity in CRC mode, with gas flows in the CRC of 2 mL/min for He and 5 mL/min for H<sub>2</sub>. We can note here that a H<sub>2</sub>-He mix is often used in CRC. The reason is that He gas acts as a buffer, increasing ion transmission through collisional focussing, while H<sub>2</sub> gas is used to remove Ar-based interferences using the well-characterized resonant charge exchange and proton transfer reactions between H<sub>2</sub> and Ar<sup>+</sup>.<sup>25</sup> For both modes, analyses were conducted with a standard-sample bracketing procedure to correct for the instrumental isotopic fractionation (internal standard addition using either Zn, Ni or Ga was not applied in this study), and with a total data acquisition time of 200 s per analysis (40 cycles of 5 s integration time). Despite processing the samples only once through column chemistry will lead to the bracketing standard and the sample possibly not having exactly the same matrix (and

although some authors argue that this is also true even with two passes of column chemistry), our results show that a classical standard-sample bracketing procedure actually works (see section 3d for comparison of the data obtained using two different MC-ICPMS on samples with low Na/Cu ratio, and for comparison of the data obtained on biological standards using either one or two passes of column chemistry). The on-peak background was measured at the beginning of an analytical sequence and was then subtracted from the  $^{63}\text{Cu}$  and  $^{65}\text{Cu}$  beam intensities. The Cu isotopic compositions are reported as parts per thousand deviations relative to the NIST SRM 976 Cu isotope standard reference material following:

$$\delta_{\text{Cu}}^{65}(\text{‰}) = \left( \frac{\left( \frac{^{65}\text{Cu}}{^{63}\text{Cu}} \right)_{\text{sample}}}{\left( \frac{^{65}\text{Cu}}{^{63}\text{Cu}} \right)_{\text{SRM 976}}} - 1 \right) \times 1000.$$

### 3. Results and discussion

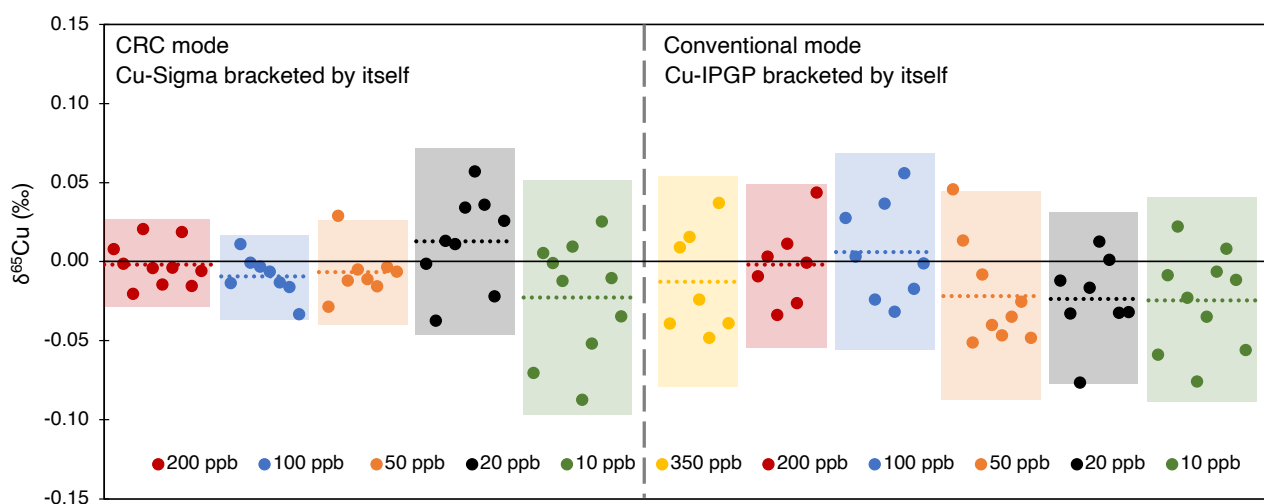
#### *a. Reproducibility and accuracy of pure standard solutions*

In order to evaluate the short-term repeatability of Cu isotopic measurements using the Sapphire CRC-MC-ICPMS, we conducted a series of experiments utilising mono-element Cu solutions bracketed by themselves at different concentrations. In Conventional mode (i.e. without the use of the CRC), we measured the standard solution Cu-IPGP (dilutions of AAS standard Specpure® Cu 1000 ppm in 2 to 5%  $\text{HNO}_3$ ) with concentrations ranging from 350 ppb to 10 ppb. In CRC mode, we examined the Cu-Sigma solution (dilutions of Sigma-Aldrich Cu standard for ICP 1000 ppm in 5%  $\text{HNO}_3$ ) with concentrations varying from 200 ppb to 10 ppb. The results are reported in Table 1 and Fig. 1.

**TABLE 1:** Short-term repeatability reached in both CRC and Conventional modes when running a pure Cu standard solution bracketed by itself at different concentrations.

	CRC mode			Conventional mode		
	$\delta^{65}\text{Cu}$ (‰)	2 SD	n	$\delta^{65}\text{Cu}$ (‰)	2 SD	n
350 ppb				-0.01	0.07	7
200 ppb	0.00	0.03	10	0.00	0.05	7
100 ppb	-0.01	0.03	8	0.01	0.06	8
50 ppb	-0.01	0.03	8	-0.02	0.07	9
20 ppb	0.01	0.06	9	-0.02	0.05	8
10 ppb	-0.02	0.07	10	-0.02	0.06	10

**FIGURE 1:** Pure Cu standard solution bracketed by itself in CRC mode and Conventional mode. The Cu concentration of the standard solution varied from 200 ppb down to 10 ppb in CRC mode, and from 350 ppb down to 10 ppb in Conventional mode. Dashed lines represent the average and the boxes represent the 2 SD uncertainties.



As expected for a solution bracketed by itself, we obtained a  $\delta^{65}\text{Cu}$  of 0 ‰. Notably, the short-term repeatabilities (2 SD) obtained in both Conventional mode and CRC mode demonstrate good comparability. Furthermore, no significant variation was observed when comparing measurements conducted at 350 ppb and 10 ppb Cu concentrations in Conventional mode, while the short-term repeatabilities seem slightly better at higher Cu concentrations ( $\geq 50$  ppb) in CRC mode. The short-term repeatabilities of the  $\delta^{65}\text{Cu}$  values obtained in this study, typically between 0.03 ‰ and 0.07 ‰ depending on the measured concentration with a clustering around 0.05 ‰, closely align with those routinely obtained using conventional MC-ICPMS instruments.<sup>14,18,23</sup>

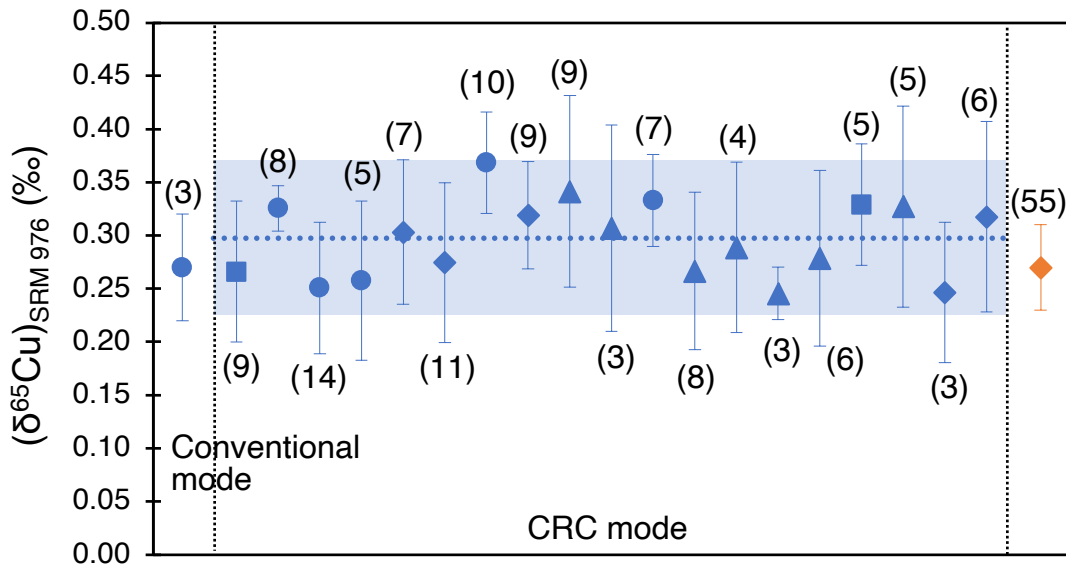
We also measured the Cu-IPGP solution bracketed by NIST SRM 976 as an in-house secondary Cu standard for quality control. The short-term repeatability achieved for  $\delta^{65}\text{Cu}$  was consistently around 0.05 ‰ (2 SD) when analysing 50 ppb to 100 ppb Cu solutions in both Conventional and CRC modes. Specifically, we obtained a  $\delta^{65}\text{Cu}$  value of  $0.27 \pm 0.05$  ‰ (2 SD,  $n = 3$ ) in Conventional mode, and ranging between  $0.25 \pm 0.06$  ‰ (2 SD,  $n = 10$ ) and  $0.37 \pm 0.05$  ‰ (2 SD,  $n = 10$ ) in CRC mode, relative to the NIST SRM 976 (Fig. 2). Considering multiple analyses

conducted over the seven distinct analytical sessions, we assessed a long-term external reproducibility of  $\delta^{65}\text{Cu}$  for Cu-IPGP in CRC mode resulting in  $0.30 \pm 0.07 \text{ ‰}$  (2 SD,  $n = 19$ ) (Table 2 and Fig. 2). This mean accounts for the variable Cu concentrations analysed across the different analytical sequences, ranging from  $\sim 100$  ppb Cu down to  $\sim 10$  ppb Cu solutions.

**TABLE 2:** Long-term reproducibility obtained in CRC mode for the Cu-IPGP in house standard bracketed by the NIST SRM 976 international standard.

Date	$\delta^{65}\text{Cu}$ (‰)	2 SD	n
March 22 <sup>nd</sup> , 2023	0.27	0.07	9
March 23 <sup>rd</sup> , 2023	0.33	0.02	8
March 24 <sup>th</sup> , 2023	0.25	0.06	14
March 25 <sup>th</sup> , 2023	0.26	0.07	5
April 24 <sup>th</sup> , 2023	0.30	0.07	7
April 25 <sup>th</sup> , 2023	0.27	0.07	11
April 27 <sup>th</sup> , 2023	0.37	0.05	10
May 10 <sup>th</sup> , 2023	0.32	0.05	9
May 11 <sup>th</sup> , 2023	0.34	0.09	9
May 12 <sup>th</sup> , 2023	0.31	0.10	3
June 12 <sup>th</sup> , 2023	0.33	0.04	7
June 13 <sup>th</sup> , 2023	0.27	0.07	8
June 14 <sup>th</sup> , 2023	0.29	0.08	4
June 16 <sup>th</sup> , 2023	0.25	0.02	3
September 15 <sup>th</sup> , 2023	0.28	0.08	6
September 28 <sup>th</sup> , 2023	0.33	0.06	5
October 24 <sup>th</sup> , 2023	0.33	0.09	5
October 25 <sup>th</sup> , 2023	0.25	0.07	3
October 27 <sup>th</sup> , 2023	0.32	0.09	6
<b>Weighted mean</b>	<b>0.30</b>	<b>0.07</b>	<b>19</b>

**FIGURE 2:** Short-term repeatability and long-term reproducibility of the Cu-IPGP in-house standard bracketed by the NIST SRM 976 standard obtained in this study for both Conventional and CRC mode (blue symbols). Each data point corresponds to the average of  $n$  analyses ( $n$  being represented by the number in brackets) within one single analytical sequence, with the associated 2 SD uncertainties. The short-term repeatability is typically of  $0.05 \text{ ‰}$  (2 SD) while the long-term reproducibility is of  $0.07 \text{ ‰}$  (2 SD,  $n = 19$ , blue box). This mean accounts for the variable Cu concentrations analysed across different analytical sequences ( $> 75$  ppb Cu: dots, 50-75 ppb Cu: squares, 25-50 ppb Cu: diamonds,  $< 25$  ppb Cu: triangles). Data and associated 2 SD uncertainty obtained for the same solution on a Thermo Finnigan Neptune Plus<sup>27</sup> is also shown for comparison (orange diamond).



Nevertheless, this level of reproducibility remains comparable to previously published values obtained through standard-sample bracketing procedure using either Thermo Finnigan Neptune Plus or Nu Plasma II MC-ICPMS.<sup>16,26</sup> The data obtained in this study for the Cu-IPGP secondary standard are consistent with the value of  $0.271 \pm 0.044$  ‰ (2 SD,  $n = 55$ ) from Moynier et al.<sup>27</sup> who conducted the analyses on a Thermo Finnigan Neptune Plus MC-ICPMS at IPGP.

It is important to highlight here that being able to get precise and accurate Cu isotopic compositions for low Cu concentration samples (down to  $\sim 15$  ppb) is crucial in the case of biological samples, which contain very low amounts of copper compared to geological samples ( $< 5$  ppm Cu in human brains, or  $< 1$  ppm Cu in blood serums, plasma or red blood cells<sup>3,8,28</sup>, versus 120-130 ppm Cu in the BHVO-2 basalt for instance (from GeoReM database)). The analytical precision reported in this study allows for resolving the variability in  $\delta^{65}\text{Cu}$  values of biological samples of interest, e.g., with  $\delta^{65}\text{Cu}$  varying by almost 3 ‰, from  $\sim -0.9$  ‰ in the serum to  $\sim 1$  ‰ in the brains and up to  $\sim 1.5$  ‰ in the kidney of mice.<sup>8,29,30</sup>

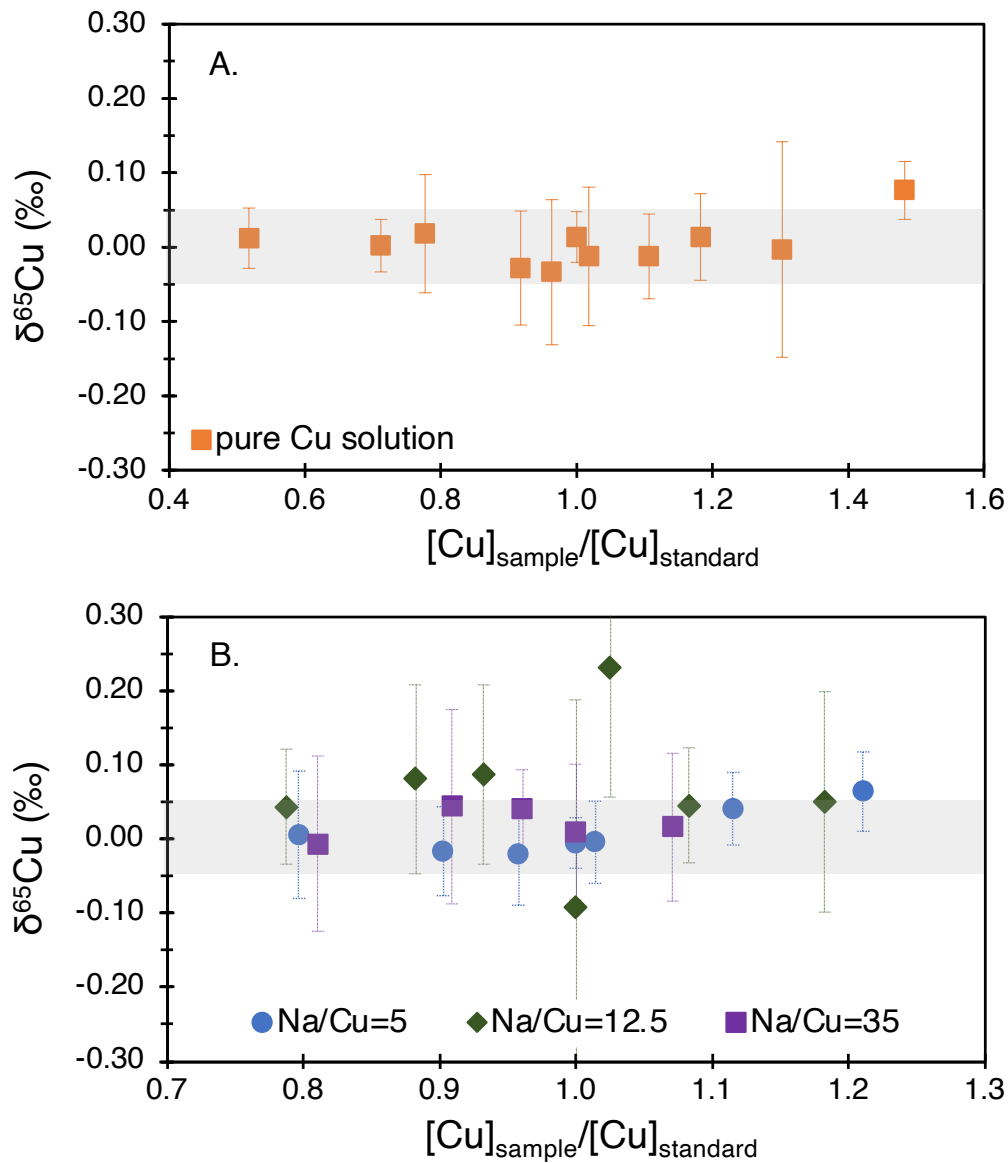
#### *b. Tests of concentration mismatch*

The impact of the concentration mismatch between unknown samples and the bracketing standard was tested in CRC mode. In the first set of experiments, the Cu-Sigma solution was measured at various concentrations, while keeping the Cu-Sigma bracketing standard at a concentration of  $\sim 100$  ppb Cu. Despite a variation of  $\pm 50$  % in the  $[\text{Cu}]_{\text{sample}}/[\text{Cu}]_{\text{standard}}$ , no effect was observed on the Cu isotopic composition as the  $\delta^{65}\text{Cu}$  values remained within error of the value of  $0.00 \pm 0.05$  ‰ (Fig. 3a). Subsequently, we measured three Na-doped Cu-IPGP solutions (with Na/Cu = 5, Na/Cu = 12.5 and Na/Cu = 35, respectively) bracketed by the Na-doped Cu-IPGP solution with the appropriate Na/Cu ratio kept at  $\sim 100$  ppb Cu. Again, despite a variation of  $\pm 20$

% in the  $[Cu]_{\text{sample}}/[Cu]_{\text{standard}}$  and the presence of Na in the solutions, no significant effect was observed on the Cu isotopic composition. All  $\delta^{65}\text{Cu}$  values remained within error of 0 (Fig. 3b).

**FIGURE 3:**

Influence of the concentration mismatch on the accuracy of the  $\delta^{65}\text{Cu}$  for (a) a self-bracketed pure Cu-Sigma solution, and (b) three self-bracketed Na-doped Cu-IPGP solutions with Na/Cu = 5 (blue dots), Na/Cu = 12.5 (green diamonds) and Na/Cu = 35 (purple squares). No effect is seen in both figures despite  $[Cu]_{\text{sample}}/[Cu]_{\text{standard}}$  varying up to  $\pm 50\%$  (a) or  $\pm 20\%$  (b). The grey box represents the 2 SD uncertainty of 0.05 ‰ typically obtained in this study.



These tests and setup show the remarkable tolerance of the Cu isotope system to concentration mismatch. In contrast, isotope systems such as Ca or K can be very sensitive to concentration mismatching when using the Collision/Reaction Cell with suboptimal tuning of key cell parameters

such as the RF alternating current applied to the rods of the collision cell.<sup>19,20,21,31,32</sup> This enhanced tolerance of the Cu isotope system to concentration mismatch is likely due to the relatively lower abundance of interfering species. Similar observations regarding the tolerance of the Cu systematics to concentration mismatch was also reported by e.g., Wang et al.<sup>18</sup> using a Nu Sapphire CRC-MC-ICPMS in Conventional mode, but also Liu et al.<sup>14</sup> with a Thermo Finnigan Neptune Plus MC-ICPMS when applying an on-peak zero correction, or Hu and Teng<sup>16</sup> using a Nu Plasma II MC-ICPMS. Overall, when analysing natural samples (geological or biological) on the Nu Sapphire (in both CRC and Conventional modes), it is recommended to achieve a concentration match better than  $\pm 10\%$  between the unknown sample and the bracketing standard.

### *c. Tests of Na doping*

One crucial assumption in the standard-sample bracketing procedure, which corrects for instrumental isotopic fractionation, is that the unknown sample and the bracketing standard share the same matrix, specifically containing only Cu. As previously mentioned, the higher amount of Na and the lower Cu content in biological samples compared to geological samples ( $\text{Na}/\text{Cu}_{\text{serum}} \sim 3000$ ,  $\text{Na}/\text{Cu}_{\text{brain}} \sim 500$  versus  $\text{Na}/\text{Cu}_{\text{BHVO-2 or BIR-1}} \sim 120$ ) necessitate either a more time-consuming Cu chemical separation process before Cu isotopic measurements, or the efficient removal of the sample matrix-based  $^{40}\text{Ar}^{23}\text{Na}^+$  interference through application of a Collision/Reaction Cell.

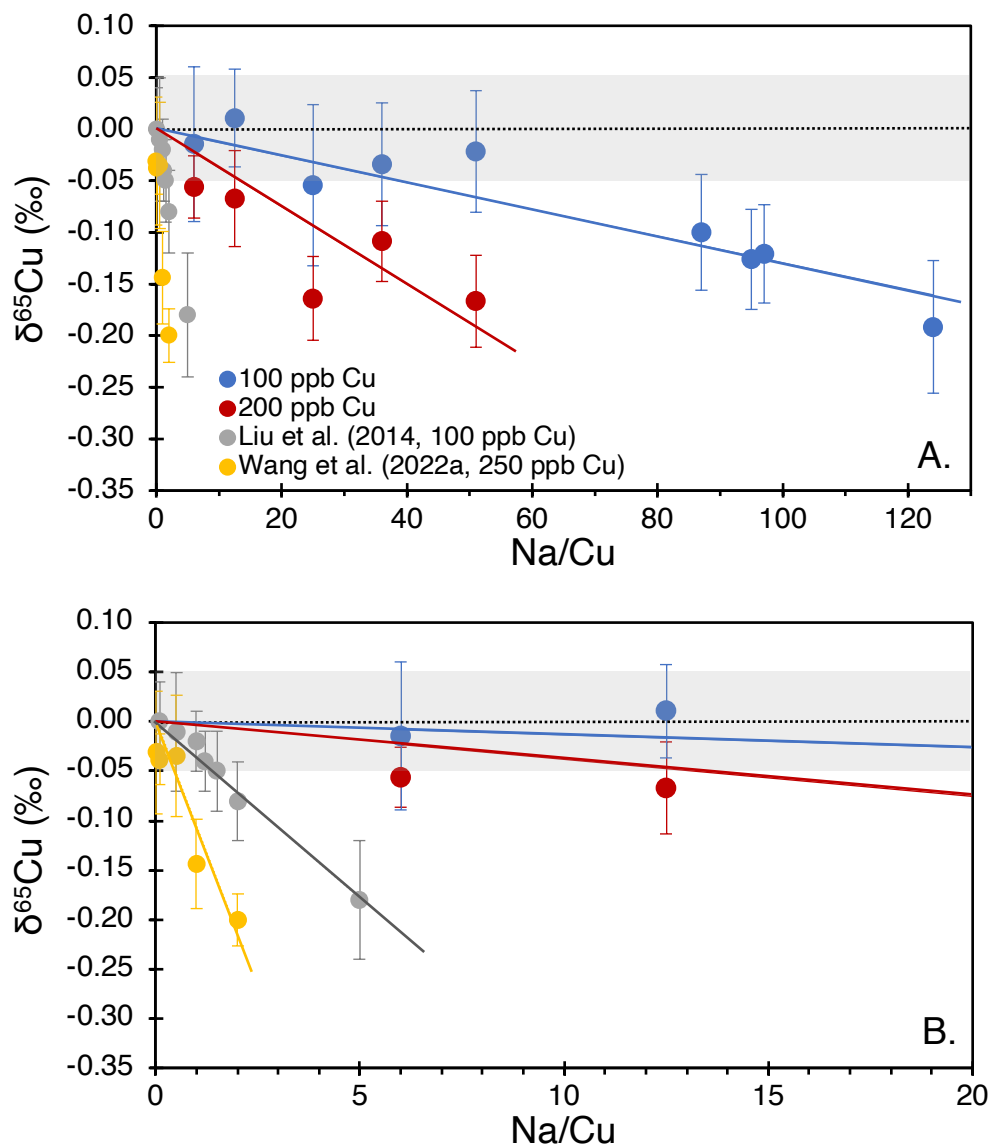
To assess the effect caused by the presence of Na in Cu isotopic measurements, we conducted experiments by adding various quantities of Na (dilutions of Chem-Lab Na 1000 ppm (Plasma HIQU) standard in 2 to 5%  $\text{HNO}_3$ ) to the pure Cu-IPGP solution. The Cu isotopic composition was measured in CRC mode, using a pure Cu-IPGP solution as the bracketing standard. Our data (Table 3 and Fig. 4) demonstrate that there is no significant influence of the  $^{40}\text{Ar}^{23}\text{Na}^+$  interference on the precision and accuracy of  $\delta^{65}\text{Cu}$  for concentration Na/Cu ratios up to  $\sim 65$  when analysing 100 ppb Cu solutions. This corresponds to an absolute Na amount of  $\sim 6.5$  ppm. However, when analysing 200 ppb Cu solutions, the influence of the interferences becomes noticeable for Na/Cu ratio of approximately 15 only (3 ppm of Na). This observation suggests higher Cu concentrations may limit the capacity to accommodate larger Na amounts, presumably limited by the plasma load capacity and the higher ionization energy for Cu.

**TABLE 3:** Impact of the Na content on the Cu isotope fractionation when using a He-H<sub>2</sub> gas mix in the Collision/Reaction Cell.

Na/Cu	100 ppb Cu			200 ppb Cu		
	$\delta^{65}\text{Cu}$ (‰)	2 SD	n	$\delta^{65}\text{Cu}$ (‰)	2 SD	n
6	-0.01	0.07	10	-0.06	0.03	10
12.5	0.01	0.05	9	-0.07	0.05	10
25	-0.05	0.08	9	-0.16	0.04	10
36	-0.03	0.06	10	-0.11	0.04	10
51	-0.02	0.06	9	-0.17	0.04	10
87	-0.10	0.06	10			
95	-0.13	0.05	10			
97	-0.12	0.05	10			
124	-0.19	0.06	10			

**FIGURE 4:**

Effect of the Na doping on the accuracy of  $\delta^{65}\text{Cu}$  for Na-doped 100 ppb Cu-IPGP solution (blue dots) and 200 ppb Cu-IPGP solution (brown dots), with the use of a He-H<sub>2</sub> gas mix in the Collision/Reaction Cell (a and b, with b being a close-up). The influence of the  $^{40}\text{Ar}^{23}\text{Na}^+$  interference on the  $\delta^{65}\text{Cu}$  is very limited for concentration Na/Cu ratio up to  $\sim 65$  when measuring  $< 100$  ppb Cu solutions, while the tolerance to Na is restricted for more concentrated Cu solutions. Typical uncertainties on the concentration ratio of Na/Cu determined using an Agilent 7900 single quadrupole ICP-MS at IPGP are better than 2 % (2 SD). The grey box represents the 2 SD uncertainty of 0.05 ‰ typically obtained in this study. Data from Liu et al.<sup>14</sup> (Thermo Finnigan Neptune Plus MC-ICPMS, grey dots) and Wang et al.<sup>18</sup> (Nu Sapphire CRC-MC-ICPMS in Conventional mode, yellow dots) are shown for comparison.



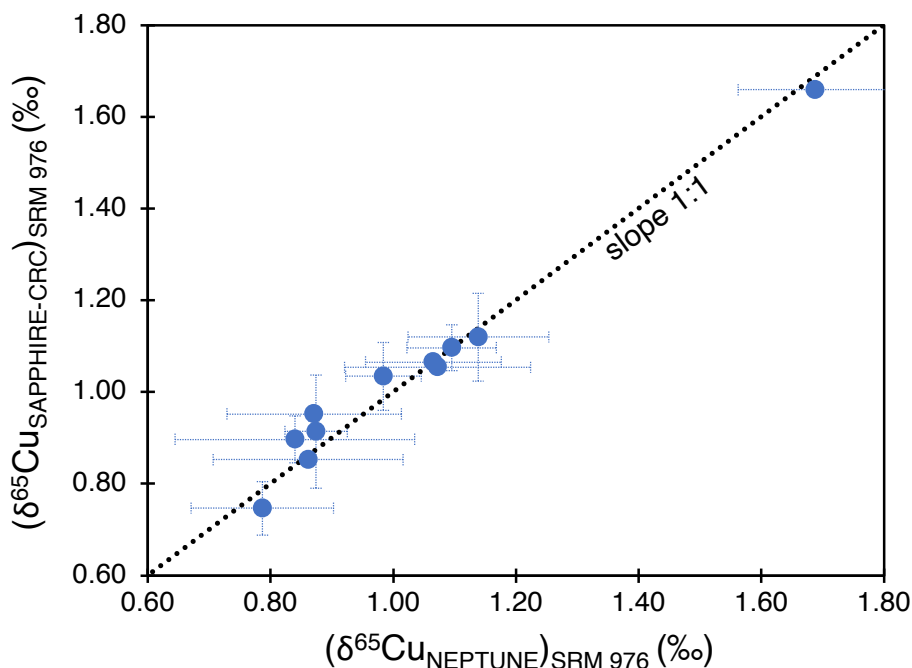
The larger absolute amount of Na tolerated in this study compared to what has previously been reported by e.g., Hu and Teng<sup>16</sup> on a Nu Plasma II MC-ICPMS, Wang et al.<sup>18</sup> on a Nu Sapphire CRC-MC-ICPMS in Conventional mode, and Liu et al.<sup>14</sup>, Hou et al.<sup>15</sup> and Wang et al.<sup>17</sup> on a Thermo Finnigan Neptune Plus MC-ICPMS (see section Introduction) underscores the significant advantages offered by the Collision/Reaction Cell through the efficient removal of sample matrix-based  $^{40}\text{Ar}^{23}\text{Na}^+$  interference on  $^{63}\text{Cu}^+$ . This has been reported recently also by Télouk et al.<sup>33</sup> using a Thermo Scientific Neoma MC-ICPMS/MS. Therefore, the CRC capabilities play a crucial role in enabling accurate measurements of Cu isotope compositions for samples with high Na/Cu ratio, such as some biological samples, which have undergone a simplified chemical processing procedure. This improvement is crucial for generating larger datasets critical for reliable interpretations in biochemical and medical sciences.

*d. Test with biological samples*

To validate and assess the effectiveness of our technical approach on natural samples, we conducted Cu isotopic composition measurements using the Nu Sapphire in CRC mode. Specifically, we first analysed brain samples obtained from healthy male and female mice of 6, 9 and 12 months. These samples had undergone the Cu chemical separation procedure twice on column chemistry. Notably, the Cu isotopic compositions of these samples had been previously measured using a Thermo Finnigan Neptune at IPGP two months prior.<sup>34</sup> Figure 5 illustrates the remarkable agreement between the dataset obtained on the two different instruments.

**FIGURE 5:**

Comparison of the Cu isotopic compositions for a set of brains of mice measured at the Institut de physique du globe de Paris (IPGP) using either the Thermo Finnigan Neptune<sup>34</sup> or the Nu Sapphire in CRC mode (this study). We can see the remarkable agreement between the dataset obtained on the two different instruments. Error bars associated are 2 SD uncertainties calculated on n replicates (n between 2 and 4).



We have also measured another set of brains from the same population of healthy male and female mice of 6, 9 and 12 months old using our Nu Sapphire in Conventional mode and the data obtained are in line with previously published data measured on a Thermo Finnigan Neptune Plus.<sup>8,34</sup>

Furthermore, we measured the Cu isotopic composition of the tuna fish ERM-CE 464 (IRMM) and the human serum Seronorm<sup>TM</sup> Trace Elements Serum L-1 standards, using our Nu Sapphire in CRC mode. These standards were first processed through column chemistry twice, and we obtained

a  $\delta^{65}\text{Cu}$  of  $0.09 \pm 0.02$  ‰ (2 SD, n = 4) for ERM-CE 464 and of  $-0.26 \pm 0.09$  ‰ (2 SD, n = 10) for Seronorm Trace Elements Serum L-1. These results are consistent with the value of  $0.11 \pm 0.05$  ‰ (2 SD, n = 17) previously reported by Sauzéat et al.<sup>35</sup> for the tuna fish, and of  $-0.20 \pm 0.06$  ‰ (2 SD, n = 5) previously reported by Sullivan et al.<sup>36</sup> for the human serum. These two biological standards were then processed only once through column chemistry to determine whether the CRC could remove the left-over sample matrix-based  $^{40}\text{Ar}^{23}\text{Na}^+$  interference. We obtained a  $\delta^{65}\text{Cu}$  of  $0.18 \pm 0.06$  ‰ (2 SD, n = 3) for ERM-CE 464 and of  $-0.24 \pm 0.07$  ‰ (2 SD, n = 7) for Seronorm Trace Elements Serum L-1, consistent with the results obtained after two passes on the column chemistry.

This demonstrates the Nu Sapphire ability to accurately measure Cu isotope compositions in biological samples, through a streamlined chemical processing procedure.

#### 4. Conclusions

This study reports for the first time the effect of Cu concentration mismatch and Na doping on the precision and accuracy of standard-sample bracketed Cu isotopic measurements using a Nu Sapphire CRC-MC-ICPMS in CRC mode. Our data demonstrate that when measuring < 100 ppb Cu solutions, the Nu Sapphire CRC-MC-ICPMS allows for efficient removal of the sample matrix-based  $^{40}\text{Ar}^{23}\text{Na}^+$  interference, with accurate and precise determination of  $\delta^{65}\text{Cu}$  for concentration Na/Cu ratios up to  $\sim 65$ . This Na/Cu threshold (and the absolute Na content tolerated) is much higher than previously reported tolerated Na/Cu, due to the use of the Collision/Reaction Cell. This makes the Sapphire a tool of choice to accurately measure Cu isotope compositions in Na-rich samples, such as seawater or some biological samples, through a streamlined chemical processing procedure. Therefore, future applications of this method can be extended to a variety of geological Na-rich and Cu-poor samples, such as seawater or groundwater samples.

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