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PAPER

A new separation procedure for Cu prior to stable isotope analysis by MC-ICP-MS

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A novel ion exchange chromatography was developed for the separation of Cu from biological samples prior to stable isotope analyses. In contrast to previous methods, the new technique makes use of the different distribution coefficients of Cu(I) and Cu(II) to anion exchange resin and this helps to significantly improve the purity of the Cu separates obtained from biological samples, whilst maintaining crucial quantitative yields. Careful method validation confirmed that the procedure yields sufficiently pure Cu fractions after a single pass through the anion exchange columns, with a recovery of 100 ± 2%. Subsequent isotopic analyses of the Cu by multi-collector inductively coupled plasma mass spectrometry, using admixed Ni for mass bias correction, produced accurate Cu stable isotope data with a reproducibility of ±0.04‰ for pure standard solutions and of ±0.15‰ for samples of biological origin.

1. Introduction

The measurement of stable $^{65}Cu/^{63}Cu$ isotope ratios in geological, biological and anthropogenic samples is of wide interest for research in Earth,[1–6] environmental[7–11] and life sciences.[12–16] Such Cu isotope studies utilize the technique of multiple collector inductively coupled plasma mass spectrometry (MC-ICP-MS) to obtain Cu isotope data with a precision of about ±0.1 to 0.2‰ (2*s*). These measurements require that Cu is separated from the sample matrix and an admixed element (generally either Ni or Zn) is utilized for correction of the instrumental mass bias by external normalization. A double spike procedure[17–19] cannot be used for mass bias correction of Cu, as this element has only two stable isotopes (^{63}Cu and ^{65}Cu).

Previous work has shown that the separation of Cu from complex sample matrices for isotopic analysis is best accomplished by ion exchange techniques. Carefully optimized methods are required, however, to achieve both the essential quantitative yields and a high purity for the Cu separate. The first requirement follows from the observation of significant Cu isotope fractionation during elution from ion exchange resins[14,20] whilst the second is important to prevent spectral and non-spectral interferences that would be detrimental for the collection of accurate and precise isotopic data.

At present, the most commonly used method for the separation of Cu uses macroporous anion-exchange resin as Cu^{2+} is moderately retained on such resin at higher HCl concentrations. For the separation of Cu from diverse natural samples, the samples are commonly dissolved in ~7 M HCl (containing small amounts of H_2O_2 to ensure that Cu is present as Cu^{2+}) for loading onto the resin columns. The same acid is then used to first remove major matrix elements and then purified Cu^{2+} from the resin column.[21–24] This chromatographic separation is not perfect, however, and variable overlap between the elution of Cu and matrix elements is commonly observed, depending on sample size and type.[21] In order to obtain Cu fractions, which are pure enough for accurate stable isotope analyses, it is therefore often necessary to adopt slight modifications to the elution procedure for different sample matrices and/or to carry out a second column pass.[21,23]

Application of these methods for the separation of Cu from complex biological samples appears to be particularly problematic.[21] This was confirmed by our own work, which showed that the application of these methods leads to a significant overlap of the Cu elution with matrix elements. As a result, Cu isotope analyses of biological samples are readily compromised by significant interferences at mass 63, due to relatively high amounts of Na producing $^{23}Na^{40}Ar^+$ in the plasma.[12,21,25,26] Based on our own experiences, it is often necessary to purify biological samples by at least two stages of ion exchange chromatography to obtain sufficient Cu for isotopic analysis. Such multi-stage procedures, however, are time consuming, suffer from higher blank and are more readily compromised by loss of Cu during elution.

In this study, we have developed a novel ion exchange procedure for the separation of Cu from biological samples prior to stable isotope analyses. This procedure utilizes differences in the affinity of Cu^+ and Cu^{2+} to anion exchange resins, to enable a simple yet efficient separation of Cu from biological samples.

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2. Experimental

2.1. General procedures and materials

Sample preparation was performed in Class 10 laminar flow hoods in the Class 1000 MAGIC Clean Room Laboratory at Imperial College London. For sample handling, Teflon Savillex vials were used throughout, unless otherwise stated. AnalaR grade HCl (6 M) and HNO_3 (15.4 M) were purified by sub-boiling distillation in quartz stills. These distilled acids, Omnipure 9 M $HClO_4$ (Fisher Scientific) and 18 MΩ H_2O (MilliPore) were used throughout this study. Care was taken when handling $HClO_4$ due to the harmful and potentially explosive nature of this acid. All handling was performed in an extraction hood, whilst evaporation of $HClO_4$ was carried out in a specially adapted hood with an exhaust wash down system. Acid solutions that contained Suprapur H_2O_2 (stored below 5 °C) and L(+)-ascorbic acid (Acros Organics) were made freshly on day of use, due to degradation of such solutions. Commercial solutions of Cu (Romil) and Ni (BDH) (dissolved in HNO_3) were used as an in-house zero-delta Cu isotope reference material and for instrumental mass bias correction, respectively.

Foetal bovine serum (FBS) is used for preparing matrix-matched standards for trace element concentration measurements by quadrupole inductively coupled plasma mass spectrometry in health institutions. In this study, FBS from Selbourne Biological Services Ltd (UK) was applied to validate the performance of the Cu isotope analyses, using a stock matrix that was stored below −40 °C and which was defrosted at ~5 °C 12 h prior to digestion. The Cu concentration of this serum was determined in the Trace Element Laboratory at Charing Cross Hospital.

2.2. Sample digestion

Foetal bovine serum samples of 0.5 ml were digested with 4.5 ml 15.4 M HNO_3 and 3 ml 30% H_2O_2 at 210 °C, 250 psi for 90 min using a MARS Microwave Digestion System (CEM Corp., UK) with XP-1500 Plus™ (PTFE) vessels. A blank sample consisting of 0.5 ml 0.1 M HNO_3 was digested with every batch of samples.

2.3. Anion exchange separation

The anion exchange procedure for the separation of Cu from samples is summarized in Table 1. BioRad *PolyPrep* columns (i.d. = 8 mm) were first filled with 2 ml pre-cleaned BioRad AG 1 X8, 200–400 mesh resin and the resin was then cleaned with 0.1 M HNO_3, conditioned with 2 M HCl and finally equilibrated with 0.01 M HCl–0.02 M L(+)-ascorbic acid. Two hours prior to loading, dried sample digests were dissolved in 1 ml 0.01 M HCl–0.02 M L(+)-ascorbic acid at 50 °C for 1 h to ensure all Cu was present in the +1 oxidation state and then allowed to cool. The samples were then loaded and matrix elements eluted with 30 ml HCl–ascorbic acid, and 5 ml 6 M HCl. Following oxidation of Cu^+ to Cu^{2+} with 6 M HCl–0.01% H_2O_2, clean Cu fractions were eluted in 0.1 M HNO_3.

The Cu separates were collected, evaporated to dryness, re-dissolved in 1 ml 15.4 M HNO_3 and then refluxed at 160 °C for 3 h prior to drying at this temperature to remove easily oxidised organic material from remaining ascorbic acid and its degradation products. After this, within a specially adapted hood with an exhaust wash down system, the samples were refluxed with 1 ml 9 M $HClO_4$ at 140 °C for 3 h, then 225 °C for 24 h and evaporated to dryness at 225 °C to destroy more resistant organic material. These temperature steps were used to prevent over-reaction of the organic material with $HClO_4$, due to the potentially explosive nature of the acid. Finally, the samples were refluxed and dried at 225 °C with 1 ml and then 2 drops of 15.4 M HNO_3 to remove any remaining chloride ions. Prior to isotopic analysis, the samples were dissolved in an appropriate volume of 0.1 M HNO_3 and doped with a dilute solution of Ni (~10 ppm) in 0.1 M HNO_3, to obtain final concentrations of 50 ppb Cu and 250 ppb Ni.

A solution of Romil Cu (5 μg) dissolved in 1 ml 0.01 M HCl–0.02 M L(+)-ascorbic acid and a pure solution of 1 ml 0.01 M HCl–0.02 M L(+)-ascorbic acid were also loaded onto the columns and processed alongside each batch of samples. For these solutions, the Cu fractions were also collected but this was achieved using 45 ml (rather than the "normal" 15 ml, Table 1) 0.1 M HNO_3 + 0.01% H_2O_2. Furthermore, the subsequent acid treatments also used volumes that were three times larger than those applied for "normal" samples. After completion of the last evaporation step, BDH Ni (25 μg) was added to the Romil Cu, and both fractions were diluted to 100 ml with 0.1 M HNO_3. This procedure yielded (i) a solution of Romil Cu doped with Ni (50 ppb Romil Cu–250 ppb BDH Ni) that was the zero-delta reference material for the Cu isotope analyses of samples and (ii) a wash solution that was applied for rinsing the MC-ICP-MS sample introduction system between analyses of different

Table 1 Anion exchange chromatography for the separation of copper from biological samples[a]

Process	Medium	Volume/ml	Cu valency	D_V (Cu)
Resin loading	AG 1 X8, 200–400 mesh	2		
Cleaning	0.1 M HNO_3, H_2O	10, 2		
Conditioning	2 M HCl	10		
Equilibration	0.01 M HCl–0.02 M L-AA	4 × 2	+1	>480
Sample loading	0.01 M HCl–0.02 M L-AA	1	+1	>480
Matrix elution	0.01 M HCl–0.02 M L-AA	30	+1	>480
	6 M HCl	5	+1, +2	32, ~11
Cu oxidation	6 M HCl–0.01% H_2O_2	2	+2	~11
Cu elution	0.1 M HNO_3–0.01% H_2O_2	15	+2	<1

[a] L-AA = L(+)-ascorbic Acid. D_V (Cu) = volume distribution coefficient for Cu between the anion-exchange resin and acid (ml ml^{-1}); data from ref. 28.

samples and standards. The procedure that was applied for the preparation of these solutions ensured that differences in the matrix between samples, standards and wash solutions were minimized as far as possible, with the objective of obtaining stable mass bias behaviour for accurate and precise collection of Cu stable isotope data.

2.4. Mass spectrometry

All isotopic measurements were performed with a Nu Instruments NuPlasma HR MC-ICP-MS instrument at the MAGIC Laboratories of Imperial College London using operating conditions summarized in Table 2. Sample introduction utilized a Nu Instruments DSN desolvator system that was used with glass MicroMist nebulisers at flow rates of $\sim$100 μl min^{-1} (Table 2).

The Cu isotope analyses were carried out by static multiple collection with five Faraday cups to monitor masses 60, 61, 62 (for Ni), and 63, 65 (for Cu). Data acquisition involved three blocks of 20 integrations of 5 s each and electronic baselines of 15 s were measured prior to each block, whilst the ion beam was deflected in the electrostatic analyzer. Instrumental sensitivities of about 90–100 V ppm^{-1} were routinely achieved for Cu and Ni (Table 2).

Instrumental mass bias corrections for the measured ^{65}Cu/^{63}Cu isotope ratios were performed using admixed Ni and a combination of the exponential law and standard sample bracketing, as described by Nielsen *et al.*[27] To this end, the exponential law was used to mass bias correct the ^{65}Cu/^{63}Cu ratios that were acquired in each measurement, relative to ^{62}Ni/^{60}Ni. The corrected Cu isotope data obtained for a sample were then compared with the average ^{65}Cu/^{63}Cu ratio obtained for the Romil Cu solution, based on at least one measurement conducted immediately before and after the sample analysis. A δ^{65}Cu value was then calculated to denote the relative difference of the results in ‰:

$$\partial^{65}\mathrm{Cu} = \left[\frac{(^{65}\mathrm{Cu}/^{63}\mathrm{Cu})_{\mathrm{Sample}}}{(^{65}\mathrm{Cu}/^{63}\mathrm{Cu})_{\mathrm{Romil}}} - 1\right] \times 1000 \qquad (1)$$

3. Results and discussion

The following sections describe the efforts undertaken to ensure that the new Cu separation method is suitable for the acquisition of accurate and precise Cu isotope data for biological samples.

Table 2 Operating conditions for the Nu Plasma MC-ICPMS and the DSN desolvating nebulizer system

Nu plasma MC-ICP-MS	
Acceleration voltage	$\sim$6000 V
Mass resolution ($M/\Delta M$)	$\sim$400
Rf power	1300 W
Coolant Ar	13 l min^{-1}
Auxiliary Ar	1 l min^{-1}
Faraday cup resistors	10^{11} Ω
Sensitivity for Cu and Ni	$\sim$100 V ppm^{-1}
Transmission efficiency for Cu, Ni	$\sim$0.05%
DSN nebulizer system	
Argon sweep gas	$\sim$3 l min^{-1}
Hot gas	$\sim$0.2 l min^{-1}
Nebulizer pressure	$\sim$30 psi
Sample uptake rate	$\sim$100 μl min^{-1}

3.1. Anion exchange separation

A particular advantage of the new separation is that cationic elements that can produce problematic molecular interferences (Table 3) and/or which occur at high concentrations in biological materials (*e.g.*, K, Mg, Ca, Ti, Fe) all have distribution coefficients (D) of <1 for the AG-1 anion exchange resin when present in 0.01 M HCl. In contrast, Cu(I) displays a D value of >480[28] for this system. This large difference in distribution coefficients allows complete elution of the sample matrix whilst Cu is strongly retained on the resin, as long as a holding reductant ensures complete reduction to Cu^+. As such, the new procedure allows a better and more straightforward separation of Cu from biological samples than previously published ion exchange methods, which do not utilize the high D values of Cu(I) but only feature Cu(II).

The disadvantage of the new approach is that complete reduction of aqueous Cu^{2+} to Cu^+ must be achieved using reagents that are compatible with the ion exchange technique. The choice of holding reductant is not straightforward as (i) quantitative yields must be achieved for Cu; (ii) the reductant must be of sufficient purity so as not to significantly increase the Cu blank; and (iii) the reagent must be readily separable from Cu so that it does not generate spectral interferences or matrix effects in the isotopic analyses.

The application of gaseous SO_2 dissolved in acid was investigated for reduction, as this was used previously with success to reduce Tl(III) to Tl(I) on anion exchange resin.[27,29] Application of SO_2, however, was associated with low Cu yields following ion exchange separation, presumably due to incomplete reduction of Cu^{2+} to Cu^+ in the sample solutions. In contrast, the use of L (+)-ascorbic acid was found to be straightforward in application and effective in terms of Cu yield.

The main problem in the application of ascorbic acid as reductant is the separation of the reagent from Cu, so that the ion exchange procedure produces a highly pure Cu fraction that does not produce erratic matrix effects during isotopic analyses. To achieve this goal, our preferred elution protocol involves matrix elution with 30 ml of 0.01 M HCl to which ascorbic acid is added as holding reductant. Following this, the resin is rinsed with 5 ml 6 M HCl and 2 ml 6 M HCl–H_2O_2 (Table 1). A number of calibration experiments showed that these were the maximum rinse volumes that could be safely used, without causing premature elution of Cu. The addition of H_2O_2 to the 6 M HCl and 0.1 M HNO_3 that are used prior to and for the elution of Cu (Table 1) ensures that all Cu^+ is oxidised to Cu^{2+}, which is then readily eluted from the resin.

In spite of the 6 M HCl rinses that are applied before the elution of Cu (Table 1), residual traces of ascorbic acid (and/or its

Table 3 Major molecular interferences that may be problematic for biological samples and the Ni, Cu isotopes of interest

Element	Mass	Interferences
Ni	60	$^{23}Na^{37}Cl^+$, $^{24}Mg^{36}Ar^+$, $^{44}Ca^{16}O^+$
	62	$^{23}Na_2{}^{16}O^+$, $^{24}Mg^{38}Ar^+$, $^{26}Mg^{36}Ar^+$, $^{31}P_2{}^+$, $^{46}Ti^{16}O^+$
Cu	63	$^{23}Na^{40}Ar^+$, $^{25}Mg^{38}Ar^+$, $^{26}Mg^{37}Cl^+$, $^{31}P^{16}O_2{}^+$, $^{47}Ti^{16}O^+$
	65	$^{25}Mg^{40}Ar^+$, $^{32}S^{33}S^+$, $^{33}S^{16}O_2{}^+$, $^{49}Ti^{16}O^+$, $^{130}Ba^{2+}$

degradation products) were found to be present in the Cu fraction. To prevent problematic matrix effects during the isotopic analysis, it is therefore imperative that the organic matter is either removed or destroyed prior to the isotope measurements. A number of trials showed that near-quantitative removal of ascorbic acid by oxidation is most reliably achieved by drying down with $HClO_4$, whilst application of concentrated HNO_3 or H_2SO_4 was found to be inadequate.

3.2. Copper yields and blanks

Sample recovery must be 100% for the ion exchange separation procedure, as Cu isotopes are readily fractionated on ion exchange resins.[14,20] To determine the yield of the procedure, the Cu content of Cu fractions was precisely measured with the aid of Cu/Ni ratios. To this end, mixed Cu–Ni standard solutions with 250 ppb Ni and 0, 30, 50 and 100 ppb Cu were prepared gravimetrically to give variable Cu/Ni ratios (*x* axis, Fig. 1) and the $^{65}Cu/^{60}Ni$ ratios (*y* axis, Fig. 1) of these solutions were measured by MC-ICP-MS. The calibration line defined (typically with an r^2 value of better than 0.9996) was then used to determine the Cu/Ni ratio of sample solutions that were doped with 250 ppb of Ni, based on the $^{65}Cu/^{60}Ni$ ratio measured. This procedure was applied to both pure metal reference solutions and FBS samples with known Cu concentrations that were processed though the column separation technique and all analyses confirmed yields of $100 \pm 2\%$.

The quantitative Cu yields were further corroborated by analyses of the combined "matrix elution" and "Cu oxidation" fractions (Table 1) that were collected for a sample of FBS. The Cu content of this solution was analyzed by quadrupole-ICP-MS at the Natural History Museum, London by monitoring of ^{65}Cu. This measurement revealed that the solutions contained no Cu for a detection limit of 0.03 ppb, which includes any uncertainties from the formation of $^{25}Mg^{40}Ar^+$ (Table 3). This result demonstrates that the "early" elute fractions hold no more than 1–2% of the total Cu budget of the respective sample.

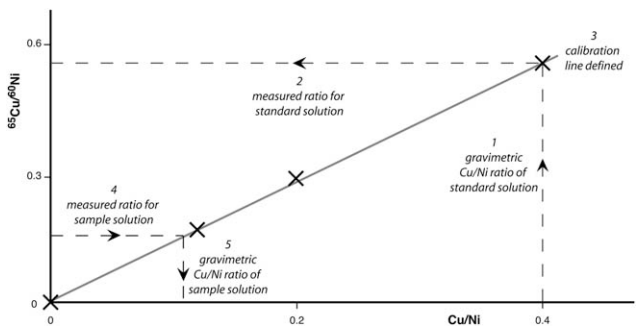

Fig. 1 Calibration line that was used to determine the Cu yields of the ion exchange separation procedure. Several mixed gravimetric Cu–Ni standard solutions with variable Cu/Ni ratios (1) were analyzed by MC-ICP-MS for the determination of $^{65}Cu/^{60}Ni$ (2) and these data define the slope of the calibration line (3). Sample solutions were then analyzed to determine $^{65}Cu/^{60}Ni$ (4), and these results can be translated into (gravimetric) Cu/Ni ratios using the calibration line (5). The Cu content of a sample solution can then be calculated, as the concentration of the admixed Ni is known.

A prerequisite for any technique of trace metal isotope analysis is that it features blank levels, which are low or insignificant in comparison to the initial analyte content of the sample. A total of five blank samples were processed and analyzed during the course of this study and yielded an average total procedural blank of 0.2 ± 0.1 ng for Cu, which is equivalent to less than 0.3% of the indigenous Cu of the samples analyzed during this study.

3.3. Spectral interferences

Two isotopes each of Cu and Ni are monitored in this study for the acquisition of mass bias corrected $^{65}Cu/^{63}Cu$ isotope ratios. Whilst these four isotopes are not isobaric to any other stable isotopes, their measurement can be impeded by a number of other spectral interferences that may form from elements that can be present at relatively high concentrations in biological materials (Table 3). A number of measurements were therefore conducted on the Cu fractions isolated from the FBS samples to ascertain that elements which can form problematic spectral interferences are either essentially absent (*i.e.*, present at background concentration levels) or present at negligible concentrations.

To this end, mass scans were carried out to check for the presence of Na, Mg, S, P, Ti and Ba in the sample solutions. All relevant, potentially inference-forming isotopes (Table 3) were found to be present at only negligible levels, with the exception of ^{23}Na. Based on a typical $^{23}Na^{40}Ar^+$ formation rate of less than 0.03% (as determined from analyses of pure Na solutions), the levels of Na present in the FBS samples were too low, however, to alter the measured $\delta^{65}Cu$ values by more than 0.01‰, which is insignificant. In addition, we also carried out mass scans at a higher mass resolution in the vicinity of the ^{60}Ni, ^{62}Ni, ^{63}Cu and ^{65}Cu peaks obtained for the Cu separates of FBS samples. These scans utilized a mass resolution ($M/\Delta M$) of about 4000, which is sufficient to at least partially resolve all interferences listed in Table 3, and they revealed no significant presence of peaks from interfering species.

3.4. Accuracy and reproducibility obtained for standard solutions

An efficient technique for the mass bias correction of the measured $^{65}Cu/^{63}Cu$ isotope ratios is important to facilitate the acquisition of precise and accurate $\delta^{65}Cu$ values for samples. In the early stages of this study, both the use of admixed Zn and Ni were tested for instrumental mass bias correction of Cu as both methods have been used with success in previous Cu isotope studies.[6,21–25,30–34] Our analyses, conducted over a period of more than 3 months, confirmed that, in principle, both elements are suitable for precise Cu isotope data acquisition. The use of Ni was preferred in this study, however, as its application (i) is more robust because Ni is less susceptible to contamination than Zn and (ii) was also found to consistently provide slightly more precise mass bias corrected Cu isotope data for standard solutions. This conclusion is in accordance with the results of a number of other recent Cu isotope investigations.[6,31–34]

In addition, we also performed multiple analyses over a period of three months to investigate whether the mass bias correction of the measured $^{65}Cu/^{63}Cu$ ratios is best carried out using

Table 4 Cu isotope data obtained for pure standard solutions and biological samples[c]

Sample	Standard	*n*	δ^{65}Cu (‰)	2*s* (‰)
50 ppb Romil Cu (AEC)	50 ppb Romil Cu (AEC)	22	0.00	±0.04
50 ppb Romil Cu	50 ppb Romil Cu (AEC)	5[a]	0.20	±0.04
50 ppb NIST 976 Cu (AEC)	50 ppb Romil Cu (AEC)	4[b]	0.21	±0.07*
50 ppb NIST 976 Cu	50 ppb Romil Cu	5[a]	0.20	±0.08
Foetal Bovine Serum (FBS)	50 ppb Romil Cu (AEC)	3	1.30	±0.15*
FBS Matrix–50 ppb Romil Cu	50 ppb Romil Cu (AEC)	3	0.00	±0.08*

[a] The results are based on *n* repeated analyses of a single Cu–Ni solution. [b] Results are based on data obtained for *n* samples that were separately processed through the ion exchange chromatography. [c] Results are based on data obtained for *n* samples from a single digest but that were processed separately through the column chemistry. (All analyses were carried out with mixed Cu–Ni solutions that contained 250 ppb Ni. AEC = denotes solutions that were processed through the anion exchange chemistry summarized in Table 1. *n* = number of individual analyses. * the quoted precision denotes the deviation of the individual results from the given mean value.)

^{62}Ni/^{60}Ni or ^{61}Ni/^{62}Ni. Our measurements showed that both Ni isotope ratios can provide precise Cu isotope data. The use of ^{62}Ni/^{60}Ni was preferred during the course of this study, however, because the Cu results were typically marginally more precise and as ^{60}Ni and ^{62}Ni were found to be consistently free of spectral interferences in the Cu fractions of the FBS samples.

Repeated analyses of solutions prepared from "unprocessed" zero-delta Romil Cu isotope standard yielded a δ^{65}Cu value of +0.2‰, when measured relative to the same Romil Cu that that had "processed" through the anion exchange chemistry summarized in Table 1 (Table 4). This offset probably reflects minor matrix effects from either traces of $HClO_4$ or residual ascorbic acid and/or resin material (and their degradation products).[35] In order to account for this offset, the sample analyses are best conducted relative to a zero-delta reference solution, which has been processed through the anion exchange separation that is applied for samples. This solution is then used as the bracketing zero-delta standard during sample analyses. The application of this approach is validated by a number of results. Firstly, the offset of $\delta^{65}Cu = 0.2‰$ between processed and unprocessed Romil Cu was found to be constant over a period of several months. Secondly, identical differences of $\delta^{65}Cu = 0.2‰$ were obtained for analyses of (i) unprocessed SRM (standard reference material) NIST 976 Cu, which served as a secondary standard, relative to unprocessed Romil Cu and (ii) processed NIST 976 Cu relative to processed Romil Cu (Table 4).

The reproducibility of the methods was investigated using standard solutions in various ways. Multiple analyses of the Romil Cu standard that were evaluated using standard-sample bracketing provide a precision of ±0.04‰ (2*s*), regardless of whether the "sample" is unprocessed or processed through the ion exchange separation (Table 4). Furthermore, multiple analyses of NIST 976 Cu relative to Romil Cu were found to display a precision of approximately ±0.07‰, again regardless of whether the NIST 976 Cu sample was taken through the column chemistry or not. In summary, these results demonstrate that our new techniques can provide δ^{65}Cu data for pure standard solutions that have a precision of about ±0.05‰.

3.5. Accuracy and reproducibility obtained for samples

Further experiments were carried out to validate that the method can deliver accurate and reproducible Cu isotope data not only for Cu reference solutions, but also for matrix-rich biological samples.

In order to test the performance of the method for biological samples, three aliquots of the FBS reference material were processed with our technique and the Cu isotope compositions analyzed. The δ^{65}Cu data obtained for these samples display a reproducibility of ±0.15‰, based on the deviation of the individual measurements from the mean result (Table 4).

To test the accuracy of the method, the "matrix elution" fractions (Table 1) from the Cu column chemistry of the FBS samples were collected, doped with about 50 ng Romil Cu, dried down and then processed again through the chromatographic procedure. Importantly, these analyses yielded a result of $\delta^{65}Cu = 0.00 \pm 0.08‰$, which is identical to the expected value of $\delta^{65}Cu = 0$ (Table 4) and this demonstrates that the method can provide accurate results for biological samples. Furthermore, the precision obtained for these analyses is similar to the reproducibility determined for multiple analyses of the FBS samples, and only slightly worse than the precision that was determined for multiple analyses of standard solutions (Table 4). This indicates that the new methodology can provide Cu isotope data with a precision of about ±0.10‰ for biological samples and therefore the method is suitable for the resolution of small natural Cu stable isotope effects, as previous work has shown that biological samples have δ^{65}Cu values that vary by about 3‰, with published results that range from −2.13‰ to +0.62‰ relative to NIST 976 Cu isotope reference materials.[14,24]

4. Conclusion

A novel ion exchange method for the separation of Cu from biological materials prior to isotopic analysis has been developed. This procedure makes use of the differing affinity of Cu^+ and Cu^{2+} to anion exchange resin. In this technique, Cu^+ is first retained on the anion exchange resin whilst the matrix is comprehensively eluted. The univalent Cu is then oxidized to Cu^{2+} on the resin and a clean Cu fraction is collected at a defined elution volume. A number of validation experiments were carried out. These demonstrate that the method provides quantitative Cu yields and effectively isolates Cu from elements that may produce problematic spectral interferences. In addition, it is shown that the method is suitable for Cu stable isotope analyses of biological materials with a precision of about ±0.10‰.

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