Stable isotope ratio measurements on biological samples by cryo-LA-ICP-TOF-MS

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Abstract

The use of stable isotopes as tracers of trace metal uptake in toxicological investigations has proven useful, especially for trace elements with micro-nutrient status (e.g. zinc and copper). In an effort to develop a method to measure and map the distribution of zinc and copper isotope tracers in exposed tissue samples, cryo-LA-ICP-TOF-MS was employed.

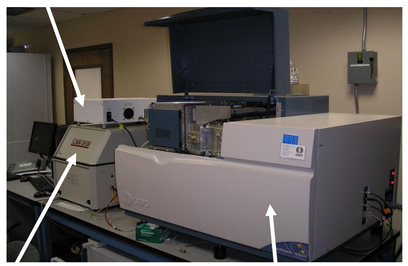
Five tissue standard reference materials (SRM 2976, SRM 1566b, RM 8414, TORT-2, DOLT-4) were pressed into pellets and analyzed with a GeoMed Analytical cryo-cell-fitted LA-ICP-TOF-MS system from GBC Scientific. Optimal cryo-cell and laser ablation conditions were determined for the collection of both zinc and copper isotope ratios. Conditions were optimized to decrease uncertainty and limit mass bias. Zinc and copper isotope ratios were determined for each reference material. It should be noted that any natural fractionation associated with the true ratio in the samples analyzed should be small, if present at all, based on the potential for natural fractionation in these two isotope systems. Therefore, it is assumed that any deviation from the natural isotopic ratio is an analytical artifact.

For $^{68}Zn/^{66}Zn$, all of the SRMs are within 2.5 % of the natural abundance ratio of 0.672, exhibiting very little laser or instrumental induced mass bias. Ratios for $^{65}Cu/^{63}Cu$ in the same SRMs are more variable with three of the five SRMs within 12 % of the natural abundance ratio of 0.446. The SRMs outside this range contain less than 5 mg kg^{-1} Cu and the resulting low signal/noise ratio is most likely responsible for the apparent mass bias. For comparison, the SRMs were digested in 7N nitric acid and hydrogen peroxide for analysis by traditional quadrupole ICP-MS. Using the ICP-MS in standard solution mode resulted in $^{68}Zn/^{66}Zn$ and $^{65}Cu/^{63}Cu$ ratios for the SRMs that are all within 7 % and 11 % of the natural abundance ratios, respectively. These results compare favorably with the cryo-LA-ICP-TOF-MS results and suggest that the ICP-TOF-MS may actually be a more stable platform for this type of isotope ratio measurement.

Analytical Equipment

GeoMed Analytical Cryocell 10

The GBC Optimass 9500 ICP-TOF-MS measures concentrations of inorganic elements as well as isotopic ratios. A CETAC LSX-213 laser ablation system allows for the measurement of solid samples, and a GeoMed Analytical Cryocell 10 coupled to the laser ablation system allows for the direct sampling of solid biological samples.



CETAC LSX-213 GBC Optimass 9500 ICP-TOF-MS

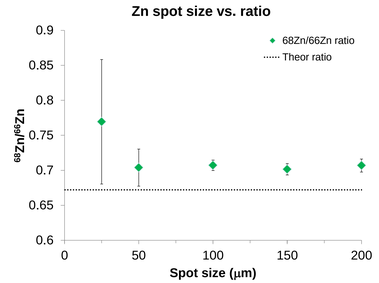
Thermo (VG) PQ ExCell ICP-MS

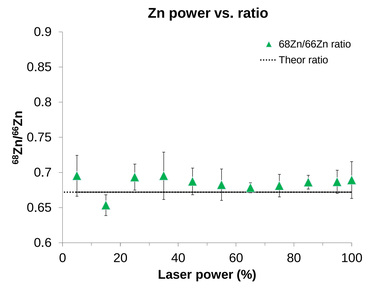
The Thermo (VG) PQ ExCell ICP-MS system measures concentrations of inorganic elements as well as isotopic ratios. A CETAC ASX-520HS auto-sampler allows for unattended operation.

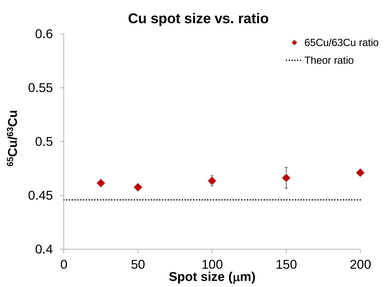
Method Development

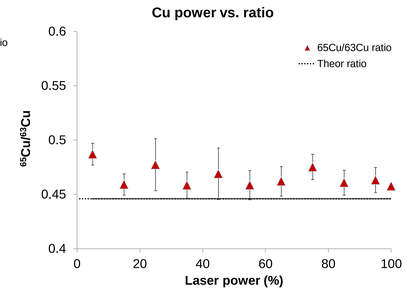
For the cryo-LA-ICP-TOF-MS, standard reference material TORT-2 (lobster hepatopancreas, NRC Canada) was used to develop the method for copper $^{65}Cu/^{63}Cu$ analysis. Standard reference material RM8414 (bovine muscle powder, NRC Canada) was used to develop the method for zinc $^{68}Zn/^{66}Zn$ analysis. 1.5 g of each reference material was placed in an aluminum cup inside a Spex 31 mm pellet die set, and pressed with 25 tons of pressure, held for 3 minutes, and released over 1 minute.

The cryo-cell and laser ablation conditions were optimized to generate the most reproducible and accurate zinc and copper isotope ratios. The natural abundance isotope ratios for $^{68}Zn/^{66}Zn$ and $^{65}Cu/^{63}Cu$ (0.672 and 0.446, respectively) were assumed to be the true ratios of the reference materials, and any deviation from these values is assumed to be an analytical artifact. Laser spot size, laser burst count, cryo-cell temperature, and laser power were each analyzed individually to determine the optimum for each elemental isotope ratio. The optimal conditions determined for zinc analysis were: 100 µm spot size, 3 spots, 65% laser energy, 200 burst count, -10 °C cryo-cell temperature, and 0.5 L min^{-1} helium carrier gas flow rate. The optimal conditions determined for copper analysis were: 50 µm spot size, 3 spots, 100% laser energy, 200 burst count, -10 °C cryo-cell temperature, and 0.5 L min^{-1} helium carrier gas flow rate.







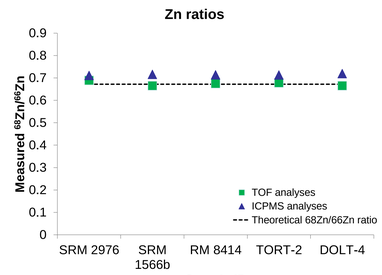


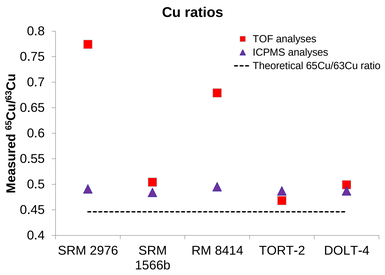
Five tissue standard reference materials, SRM 2976 (mussel tissue, NIST), SRM 1566b (oyster tissue, NIST), RM 8414, TORT-2, and DOLT-4 (dogfish liver, NRC Canada), were analyzed for both $^{68}Zn/^{66}Zn$ and $^{65}Cu/^{63}Cu$ ratios. The reference materials were prepared as described above. ^{13}C was assumed to be uniform in all samples and was used as the internal standard.

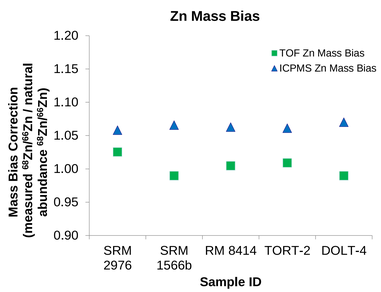
For traditional quadrupole ICP-MS analysis, 50 mg of each standard reference material was digested overnight in 7N nitric acid, and subsequently digested overnight in 30 % hydrogen peroxide. These digests were taken to dryness and then diluted with 50 ml of a 2% nitric acid solution spiked with 1 ppb Indium.

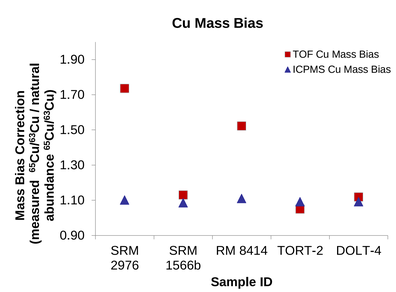
Results and Discussion

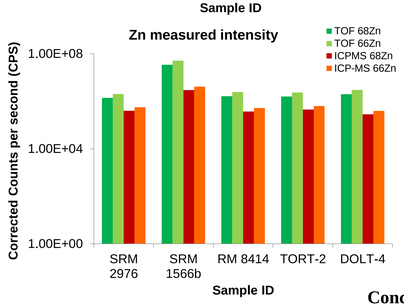
The cryo-LA-ICP-TOF-MS generated very accurate values for both $^{68}Zn/^{66}Zn$ and $^{65}Cu/^{63}Cu$, with all but two of the reference materials being within 2.5 % and 12 % of the natural abundance ratios, respectively. Very little laser or instrumental induced mass bias was evident. The two reference materials with $^{65}Cu/^{63}Cu$ ratios outside this range contain less than 5 mg kg^{-1} Cu and the resulting low signal/noise ratio is most likely responsible for poor accuracy. For comparison, analysis by traditional quadrupole ICP-MS resulted in $^{68}Zn/^{66}Zn$ and $^{65}Cu/^{63}Cu$ ratios for the reference materials that are all within 7 % and 11 % of the natural abundance ratios, respectively.

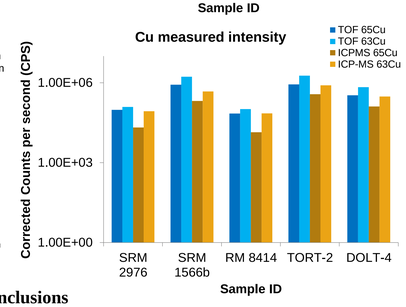












Conclusions

The zinc and copper isotope ratio values produced by the cryo-LA-ICP-TOF-MS are comparable or better than those produced by traditional quadrupole ICP-MS. Based on these results, the ease of preparation, and the ability to pinpoint and analyze specific regions of samples, the cryo-LA-ICP-TOF-MS system is superior for the analysis of zinc and copper ratios in biological materials.

Acknowledgements

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