

# Submicromolar Measurement of $\delta^{15}\text{N}$ Bulk Isotope Ratios in Chlorophyll-a Using Elemental Analysis-Isotope Ratio Mass Spectrometry (EA-IRMS)

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Previous efforts to measure  $\delta^{15}\text{N}$  ratios by EA-IRMS have used cryofocusing techniques or numerical correction of the N blank to yield accurate results with 0.1‰ precision for samples with 500 to 5000 nMol  $\text{N}_2$  (Avak and Fry, 1999). By reducing flow, sending more sample to the IRMS, minimizing the  $\text{N}_2$  blank, and without numerical manipulation, we demonstrate 0.05‰ accuracy with 0.2‰ precision for samples containing as little as 150 nMol  $\text{N}_2$ .

A Carlo Erba/Fisons 1108 EA interfaced via a Conflo II to a Finnigan-MAT DeltaPlus was used to measure  $\delta^{15}\text{N}$  ratios in IAEA N1 (ammonium sulfate,  $\delta^{15}\text{N} = 0.4\text{‰} \pm 0.2$ ) reference material and chlorophyll pigment (Sigma Chemical, St. Louis). Modifications were made including the replacement of the injector used for routine elemental analysis with an atmosphere excluding injector. The  $\text{O}_2$  valve of the EA 1108, with its neoprene diaphragm, was replaced with a Valco rotary valve. This was done as atmospheric  $\text{N}_2$  was noted to be diffusing through the diaphragm into the analytical side of the valve. Only the highest purity  $\text{O}_2$  and He were used for isotopic analyses. The use of these gases significantly reduced the  $\text{N}_2$  background while allowing atmospheric leaks (monitored as Ar+) to be easily detected. The EA furnace was replaced with a customized design (Anderson Glass, Inc., Fitzwilliam N.H.) (Figure 1). Reduction of the cross sectional area of the furnace allowed for a 60% reduction in total volumetric flow. The original 12 mm X 11 cm  $\text{CO}_2$ /water trap was replaced with a 6 mm X 35 cm tube and joined to the furnace and chromatographic column with Cajon Ultratorr fittings. This long, narrow configuration reduced mixing and turbulent flow while more efficiently trapping  $\text{CO}_2$  and water. The teflon 8 mm X 1 m chromatographic column was replaced with a 3 mm X 30 cm stainless steel column which eliminated atmospheric diffusion, and was packed with smaller mesh activated charcoal (Darco G-60, -100 mesh, Aldrich). The thermal conductivity detector (TCD), not in use, was removed from the flow path. The internal diameter of the transfer capillary from the EA to the IRMS was increased from 100  $\mu\text{m}$  to 150  $\mu\text{m}$  to allow the greatest flow acceptable to the vacuum requirements of the ion source. The overall result of these changes included an increased proportion of sample admitted to the IRMS, a threefold increase in sensitivity, and a reduction of the  $\text{N}_2$  background.

A reference tank of ultra high purity  $\text{N}_2$  was standardized against N1 reference material. Chlorophyll-a samples containing 20-600 nMol N were analyzed interspersed with reference pulses. N1 reference material, also interspersed throughout the chlorophyll-a analyses, yielded an overall  $\delta^{15}\text{N}$  of 0.51‰  $\pm$  0.11. Raw chlorophyll-a sample data at the 600 nMol N level yielded a  $\delta^{15}\text{N}$  of -1.60‰  $\pm$  0.04. Off-line data analysis of the entire data set using a commercial curve-fitting algorithm showed the isotopic value of the chlorophyll-a to be -1.65‰. Graphical interpretation using the method of Fry (Fry et al, 1992) resulted in an isotopic value of -1.62‰. All data treatments yielded consistent results to the 150 nMol N level. Below that level, precision diminished and accuracy was significantly degraded, presumably by contributions from the  $\text{N}_2$  blank.

## References

Avak H., and Fry B., 1999, EA-IRMS: Precise and Accurate Measurement of  $\delta^{15}\text{N}$  on  $<10\mu\text{g N}$ , *Finnigan MAT Application Flash Report No. G 29*.

Fry B., Brand W., Mersch F.J., Tholke K., and Garritt R., 1992, Automated analysis system for coupled  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  measurements, *Analytical Chemistry*, 64, 288-291.

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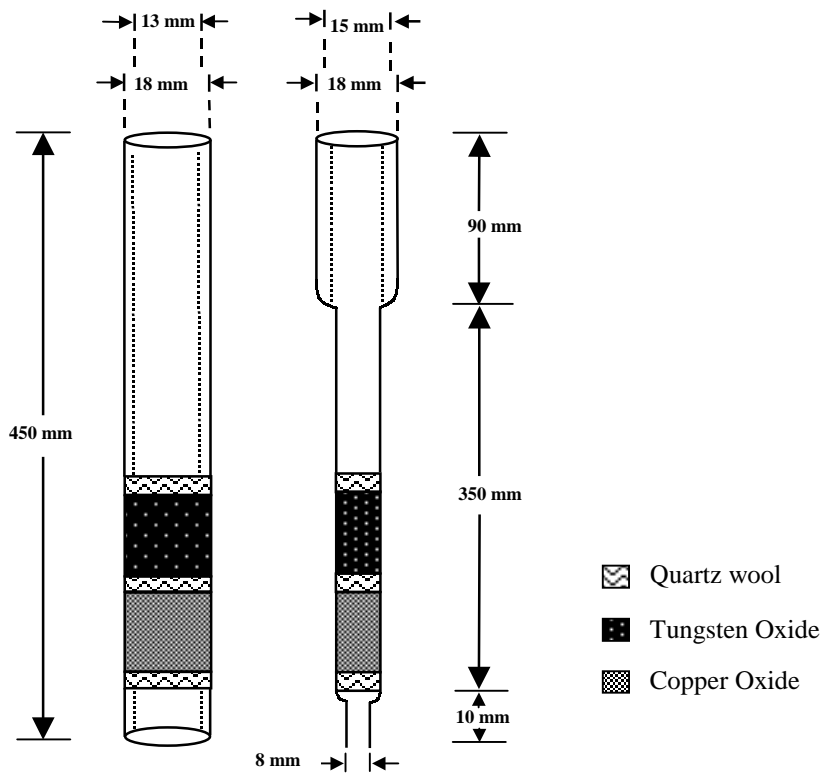


Figure 1. Dimensions and composition of the original EA combustion furnace (left) and the modified furnace (right). The cross sectional area was reduced from  $130\text{mm}^2$  to  $50\text{mm}^2$ , respectively.

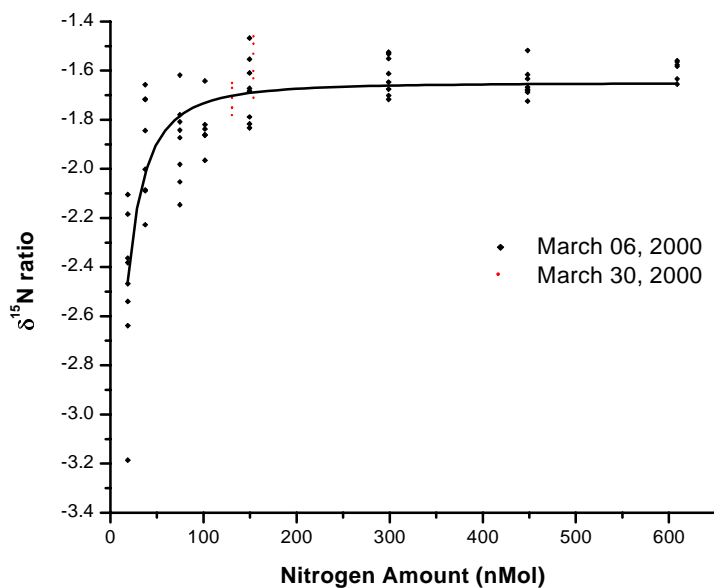


Figure 2.  $\delta^{15}\text{N}$  ratio vs. nitrogen amount (nMol) from chlorophyll-a.