GORDON AND BETTY MOORE FOUNDATION

Team Karl Highlight

Direct determination of dissolved organic nitrogen, phase 1: Validation of titanium (III) trichloride as a reducing agent for nitrate and nitrite in seawater

Submitted by: Rhea Foreman, Mariona Segura-Noguera, Susan Curless and David M. Karl

Currently, no method exists for the direct determination of dissolved organic nitrogen (DON) in natural waters: DON is reported as the difference between total nitrogen (TN) and inorganic nitrogen (nitrate plus nitrite, N+N). This approach works relatively well in the ocean's uppermost water column, since TN is a significantly larger number than N+N, meaning error estimates on DON remain tolerable. In deeper water (~300m) at Station ALOHA, N+N accounts for ~50% of TN and, below 1000m, values for TN and N+N are nearly identical, resulting in intolerable errors and sometimes even negative values for DON! A direct method to quantify DON is needed in order to establish a basic mass balance of nitrogen in the lower 80% of the open ocean.

The protocol we are developing is two-fold. The first step is to eliminate N+N from the seawater sample. The second step is to convert the thousands of unique DON compounds into a single, measurable species of nitrogen. We have completed step 1 by using titanium (III) trichloride ($TiCl_3$) to reduce nitrate and nitrite to NO gas, which is then purged from solution. A similar reducing chemistry has been used for the low-level, chemiluminescent detection of N+N itself, using ferrous ammonium sulfate and ammonium molybdate (Garside, 1982, *Marine Chemistry* 11; 159-167); however, if the goal is to measure the nitrogen content *remaining* in the seawater, it is made impossible if the reducing agents create an overwhelming contamination of nitrogen (i.e. from the ammonium).

To validate step 1, we have done an extensive study to show that the $TiCl_3$ reagent quantitatively reduces N+N and that the treatment does not result in loss of DON. Figure 1 shows a comparison of N+N determined by the chemiluminescent method using both the Fe(II)+Mo(VI) reagent and the Ti(III) reagent. Recovered values of N+N in natural seawater and prepared solutions are statistically identical between the two reagents. Concentrations of N+N using the Ti(III) chemiluminescent method are also statistically identical to N+N concentrations obtained by traditional colorimetric analyses, indicating that only nitrate and nitrite are lost as NO gas and that DON concentrations are preserved – and with negligible contamination from the $TiCl_3$.

In developing the Ti(III) chemiluminescent method, we found significant advantages over the traditionally-used Fe(II)+Mo(VI) chemistry, including a 30% reduction in the sulfuric acid catalyst needed for fast reaction rates, and have actually adopted this method for our standard low-level N+N measurements. A manuscript detailing the Ti(III) method will be submitted to *Marine Chemistry* in April, 2016. Also, data produced with this method are currently available for samples collected during the HOE-BOE I and HOE-Legacy 2 cruises in the North Pacific Subtropical Gyre.

Initial work on Phase 2 of the direct determination of DON has also begun, using ultraviolet (UV) light and hydrogen peroxide as an advanced oxidation process to convert all DON compounds to nitrate. Nitrate can subsequently be measured using the traditional colorimetric method, or if

concentrations are low, using our new, low-level chemiluminescent method. The protocol's chemistry and operational parameters will be optimized using a novel UV assembly in which light from a microwave-powered UV bulb is diffused into a highly-reflective, enclosed chamber; preliminary tests show this system to be much more efficient (in terms of light absorption and exposure times) than any other previously tested system.

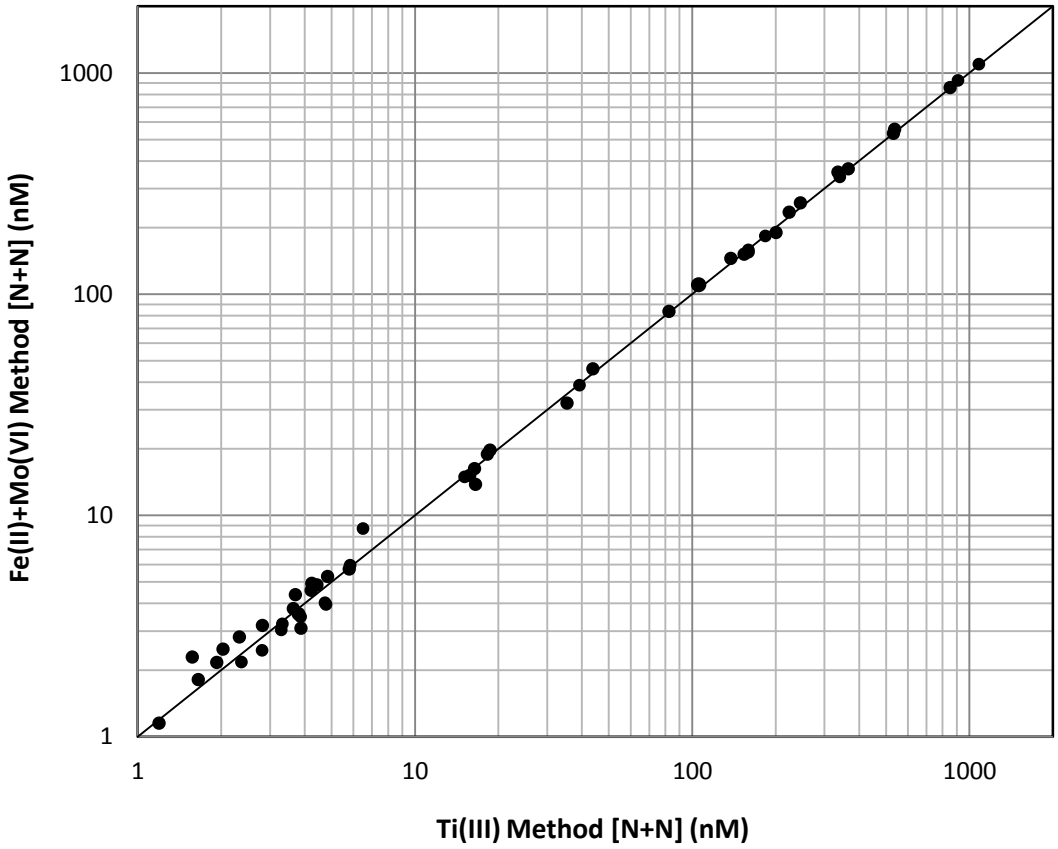

Figure 1. Comparison of reducing agents in the chemiluminescent detection of nitrate and nitrite. Data points include Wako CSK nitrate standards (n=9), Wako CSK and OSIL nitrite standards (n=3), and seawater samples (n=41) from station ALOHA, north of Oahu, Hawaii, USA.