How reproducible are methods to determine nanomolar concentrations of nitrate and nitrite?

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As a limiting element for biological productivity, nitrogen (N) occupies a central role in ocean biogeochemistry, exerting a significant influence on cycles of many other elements, in particular carbon and phosphorus (Gruber, 2008). Among the forms of Dissolved Inorganic Nitrogen (DIN) nitrate (NO_3^-) is the principal form of fixed DIN assimilated by organisms (Patey *et al*., 2008). The concentrations of nitrates and phosphates in many natural waters especially over much of the world's surface oceans are below the detection limits of conventional colorimetric analysis (Yao *et al*., 1998, Patey *et al*., 2008). This is so because in surface waters, biological uptake depletes these nutrients. Therefore highly sensitive nutrient analyses are needed to better understand the DIN and also phosphate relationships in the euphotic zone; and provide much needed insights into the mechanism of new production and the significance of new N input via N_2-fixation (Yao *et al.,* 1998; Moore *et al*., 2009). To address this challenge, techniques have been developed and methods are now available for the shipboard analysis of nanomolar (nM) nitrate and phosphate concentrations with a high sample throughput (Patey *et al*., 2008). The most frequently applied method of nitrate analysis employs colorimetric detection with Griess reagents whereby Cadmium (Cd) column is used to reduce nitrate to nitrite. Nitrite is then determined spectrophotometrically (at 540nm) following formation of a highly colored dye through diazotisation with sulphanilamide and coupling with N-(1-naphthyl)-ethylenediamine dihydrochloride (NEDD). This analytical method determines the sum of the nitrate and nitrite concentrations; Nitrite is then analysed without use of the Cd column and nitrate is calculated as the difference of the outcome with and without reduction of nitrate. This method as described in (Patey *et al*., 2008) has 2 limitations. Cadmium toxicity (Schnetger and Lehners, 2014); and a comparably higher limit of detection (LOD) which is approximately 0.1µM for nitrate. Therefore variations in nanomolar nitrate concentrations will pass unobserved in oligotrophic ocean regions where these nutrients control primary production (Patey *et al*., 2008).

Most surface samples collected in the Bay of Biscay and the Iberian Margin during the Belgica 2014/14 expedition in May 2014 were very low in nitrate concentrations after analysis on a continuous flow QuAAtro auto-analyser (Seal Analytical, UK). This therefore required a more sensitive method to lower detection limits in order to correctly quantify the low, nM range, nitrate concentrations.

First to enhance sensitivity of the spectrophotometry analytical system during analysis of nitrates and nitrites, the most feasible approach is to increase optical pathlength of the measurement cells (Zhang, 2006). In spectrophotometry, the utilization of absorbance signals for quantitative analysis relies upon the Lambert-Beer law, according to which the magnitude of absorbance signals is proportional to the optical pathlength, the molar absorptivity, and the concentration of the substance under investigation (Zhang, 2006). Secondly ensure complete reduction of nitrate to nitrite by use of acidic Vanadium(III) instead of a column with toxic Cd metal.
Sensitivity, accuracy and reproducibility of the methods were investigated. The first results will be presented for the Belgica 2014/14 cruise.

References

Gruber N. 2008. The marine nitrogen cycle: overview and challenges. Nitrogen in the marine environment. p.1-50.

Moore C.M., M.M. Mills, E.P. Achterberg, R.J. Geider, J. LaRoche, M.I. Lucas, E.L. McDonagh, X. Pan, A.J. Poulton, M.J.A. Rijkenberg, D.J. Suggett, S.J. Ussher, and E.M.S. Woodward. 2009. Large-scale distribution of Atlantic nitrogen fixation controlled by iron availability. Nature Geoscience 2:867-871.

Patey M.D., M.J. Rijkenberg, P.J. Statham, M.C. Stinchcombe, E.P. Achterberg, and M. Mowlem. 2008. Determination of nitrate and phosphate in seawater at nanomolar concentrations. TrAC Trends in Analytical Chemistry 27(2):169-182.

Schnetger B. and C. Lehners. 2014. Determination of nitrate plus nitrite in small volume marine water samples using vanadium (III) chloride as a reduction agent. Marine Chemistry 160:91-98.

Yao W., R.H. Byrne, and R.D. Waterbury. 1998. Determination of nanomolar concentrations of nitrite and nitrate in natural waters using long path length absorbance spectroscopy. Environmental science & technology 32(17):2646-2649.
Zhang J.Z. 2006. Enhanced sensitivity in flow injection analysis using a long pathlength liquid waveguide capillary flow cell for spectrophotometric detection. Analytical Sciences 22(1):57-60.