## Nitrate Analysis

A major inorganic nutrient that drives primary production in aquatic habitats is Nitrate ($NO_3^-$); therefore nitrate analyses help explain ecosystem dynamics. High nitrate concentrations increase productivity because algae require nitrate for growth. However, high concentrations may also lead to animal or fish kills, thus reducing system productivity.

Nitrate analyses are used in:

Biological Oceanography to:
- Determine physiological response of phytoplankton
- Estimate primary production

Water resource management to:
- Estimate nutrient budgets
- Identify nutrient sources and sinks
- Establish nutrient protocols

Fisheries to:
- Predict seasonal productivity
- Evaluate resources

Aquacultures to:
- Maintain algal crop
- Maximize production

Nitrate, the most abundant fixed nitrogen source in the ocean, varies in concentration depending on habitat. Eutrophication leads to high nitrate concentrations, which are observed in lakes, rivers, estuaries, and coastal habitats. Offshore surface water contains low nitrate concentrations because of algal uptake; therefore accumulation of nitrate occurs below the photic zone. The average oceanic nitrate concentration is 30 micromolar.

Increased nitrate concentrations can occur from:
- Rainfall
- Erosion
- Human pollution
- Upwelling
- Decomposition
- Animal excretions
- Natural breakdown of minerals

## Nitrate ($NO_3^-$)

Nitrate ($NO_3^-$) results from a process known as oxidation or nitrification, which is the stepwise addition of oxygen atoms to a nitrogen atom:

$NH_4^+ \leftrightarrow NH_3 \leftrightarrow NO_2^- \leftrightarrow NO_3^-$

Nitrate represents nitrogen in its most oxidized form. This stable ion is:
A powerful oxidizer used by bacteria as a substitute for oxygen in anoxic environments
Taken up by phytoplankton, algae, or plants and reduced to help build nucleotides
Because high nitrate concentrations are toxic, the Environmental Protection Agency (EPA) determined that the Maximum Contamination Limit (MCL) for nitrate in water is 10 parts per million (ppm) (http://www.epa.gov/OGWDW/contaminants/dw_contamfs/nitrates.html).

## Nitrite ($NO_2^-$)

Natural water has a low nitrite concentration because bacteria quickly convert Nitrite ($NO_2^-$) to other more stable nitrogen ions. Therefore, nitrate measurements typically represent nitrate+nitrite concentrations.

Nitrite is:
- An unstable nitrogen ion
- An intermediate ion in nitrification/denitrification process
- A nitrogen source for algae or phytoplankton
- More toxic than nitrate to fish, animals, and humans

The EPA's stated MCL for nitrite in water is 1 ppm (http://www.epa.gov/OGWDW/contaminants/dw_contamfs/nitrates.html).

## Methods

Environmental Protection Agency (EPA's) or the American Society for Testing and Materials (ASTM's) approved and published methods are used to determine nitrate concentrations in water.
(http://www.epa.gov/nerlcwww/m353_4.pdf)
(http://www.astm.org/cgi-bin/SoftCart.exe/DATABASE.CART/REDLINE_PAGES/D3867.htm?L+mystore+znmb0275)

## Cadmium Column Reduction Method

An EPA and ASTM approved and preferred method for estimating nitrate in water is the Strickland and Parsons (1968) Cadmium Column Reduction Method. Turner Designs has developed a Nitrate/Nitrite Module (P/N: 7200-074) for the Trilogy, which can be used to estimate nitrate in water following the general two step process outlined in the Cadmium Column Reduction Method:

1. Quantitative reduction from nitrate to nitrite - Samples are run through a prepared cadmium column because cadmium quantitatively reduces nitrate to nitrite. The reduced samples are further processed to create a purple colored solution (See Step 2).

2. Colorimetric determination of nitrite using the Griess Reaction (1879) - Adding a diazotizing agent, in acidic media, to the reduced samples forms a temporary diazonium salt. Then, immediately adding a coupling agent forms a stable azo compound resulting in a purple solution. The absorbance of the solution is directly proportional to the concentration of nitrite in the sample.

The Cadmium Column Reduction Method, when used with the Turner Designs Trilogy Nitrate/Nitrite Module (P/N: 7200-074), offers:

- Linearity to 1000 micromolar Nitrate
- A detection limit of 0.5 micromolar Nitrate
- Greater than 90% accuracy of Automated Cadmium Column Analyzers

## Test Kit Method

More recent methods use copper cadmium granules and powders. These methods are easier, quicker, and comparable to results produced from traditional cadmium column reduction methods. When used with Turner Designs Trilogy Nitrate/Nitrite Module (P/N: 7200-074), Test Kit Methods offer:

- Linearity to 1000 micromolar Nitrate
- A detection limit of 3 micromolar Nitrate
- Greater than 90% accuracy of Automated Cadmium Column Analyzers

Accuracy of estimates will reflect the accuracy and precision of the laboratory techniques used for preparing and measuring samples.

## Other Approved Methods

Approved methods for nitrate analyses, without using cadmium reduction, are available. However, they may require using expensive and analytical instruments and can be quite tedious. A few methods are listed below:

- UV estimation using spectrophotometers and an array of wavelengths – UV wavelengths at 220nm and 275nm are used to analyze prepared water samples and estimate nitrate concentrations. This method has been proposed for samples containing low organic content because of interference's from dissolved organic matter.

- Nitrate electrodes – an electrode used *in situ* to determine ionic nitrate concentrations in water systems. Ion electrodes are expensive and may have ionic interference. pH is also an issue when using ion electrodes. If pH is not held constant erratic readings may persist.

## References:

J. D. H. Strickland and T. R. Parsons, 1972. A practical handbook of seawater analysis. Second Edition, Bulletin 167. Fisheries Research Board of Canada, Ottawa

Timothy R. Parsons, Maita Y. and Lalli C. M., 1984. A manual of chemical and biological methods for seawater analysis. First Edition. Pergamon Press Ltd., Great Britain

Standard Methods for the Examination of Water and Wastewater, 18th edition 1992. American Public Health Association, American Water Works Association, Water Environment Federation. Method 4500-E. ISBN 0-87553-207.

Robert D. Cox, 1980. Determination of nitrate and nitrite at the parts per billion level by chemiluminescence. Analytical Chemistry, Vol. 52, No. 2: 332-335

K. Grasshoff, M. Ehrhardt, K. Kremling. Methods of seawater analysis. 2nd Edition.

Yves Collos, 1998. Nitrate uptake, nitrite release and uptake, and new production estimates. Marine Ecology Progress Series, Vol. 171: 293-301