OPA Method for NH_4^+ Analysis

Holmes et al. 1999, Protocol A

Working Reagent (WR):

Sodium sulfite solution

Add 1g of sodium sulfite to 125mL of DI water.

Borate buffer solution

Add 80g of sodium tetraborate to 2L of DI water.
Stir or shake thoroughly to dissolve.

OPA solution

Add 4g of OPA to 100mL of ethanol.
Protect OPA from light while dissolving in ethanol, b/c it is light sensitive, and should be stored in the dark.

WR

Mix 2L of borate buffer solution, 10mL of sodium sulfite solution and 100mL of OPA solution in a >2L amber polyethylene bottle.

Allow the WR to age for 1+ days, b/c its blank will decrease over time. Is stable for at least 3mo when stored in dark at room temp.

Standard Additions Protocol I:

The background-corrected fluorescence of 3-5 samples with known amounts of NH_4^+ added and 1 sample with no NH_4^+ added are regressed against their nominal spike concentrations. The ME-corrected concentration of the sample is estimated by extrapolating the curve to the *x*-axis.

1. Plot the background-corrected fluorescence of the standard additions against their nominal concentrations.
2. Fit a linear regression.
3. Then extrapolate the line to the *x*-axis; the absolute value of the *x*-axis intercept ($|c_x| = b/m$) is the concentration of the sample (i.e., the standard addition with no NH_4^+ added)

c_x = NH_4^+ concentration
y = BF-corrected sample fluorescence

Field procedures

All sample bottles should be acid washed in a bath of 10% HCl and rinsed with Milli-Q water prior to collecting samples.

1. Rinse bottle once w/sample water and then fill to the 40mL mark. Repeat for each sub-sample of the sample water (for NH_4^+ analysis, for standard samples to be spiked, and for determination of background fluorescence).
2. Prepare the time zero sample first. Add 10mL of working reagent (WR), cap, shake, and pour sample into a 25mm cuvette. Measure raw fluorescence immediately (<30s). This is the background fluorescence (BF).
3. Prepare standards by spiking 3-5 samples with known concentrations of ammonium stock solution. (Concentrations should be pre-determined and range from 0.25-3x expected concentrations.) Add 10mL of WR, cap, shake, and store in dark at ambient temperature.
4. One sample will have no ammonium added. This will be the 0-NH_4^+ standard as well as the sample that concentration will be determined from.
5. These steps will be repeated for each depth of water sampled (each Niskin bottle on the CTD rosette). Always do the BF samples immediately.

Laboratory procedures

1. After incubating for at least 2-3hrs, pour samples and standards into 25mm test tubes and immediately read using the field fluorometer.
2. Record all sample readings in raw units.
3. Calculate sample ammonium concentration. The procedure for calculating a sample's concentration involves four steps:
   (i) subtracting BF ($F_{\text{sample time-zero}}$) from the raw readings
   (ii) correct for ME
   (iii) calculate sample concentration using the standard regression

To account for **BF** use the equation from Taylor et al. 2007:

$F_{\text{sample time-zero}}$ is the fluorescence of a sample with WR added and measured immediately (incubated <30s).

To account for **ME** use Protocol I of the method of multiple standard additions (ASTM 2006).

To obtain the sample fluorescence corrected for ME, use the equation from Taylor et al. 2007:

Materials:

Reagents:

Makes 2L of Working Reagent

Sodium sulfite – 1g (Sigma S4672)
Sodium tetraborate – 80g (Fisher S249500)
Orthophthaldialdehyde (OPA) – 4g (Sigma P1378)
Ethanol – 100mL, high grade (Sigma E7023)
Ammonium Standard Stock Solution (Ricca 691-4)

Supplies/Equipment:

Pyrex media bottles (100mL, Corning Inc. 1395-100)
Pipette (100-1000 μL, Eppendorf 22472101)
Pipette tips (100-1000μL , sterile)
Amber polyethylene (PET) bottle (2L, Lab Safety Supply 155099)
HCl (for acid washing, Fisher SA481)
25mm Pyrex Cuvettes (Kimble 45060 25150)
Small graduated cylinder (to measure 20mL WR)
60mL plastic syringe

References:

ASTM Standard D5810-96 (2006). Standard Guide for Spiking into Aqueous Samples. ASTM International, West Conshohocken, PA. 2006. DOI: 10.1520/D5810-96R06. www.astm.org.

Holmes et al. 1999. A simple and precise method for measuring ammonium in marine and freshwater ecosystems. Can. J. Fish. Aquat. Sci. 56: 1801-1808.

Taylor et al. 2007. Improving the fluorometric ammonium method: matrix effects, background fluorescence, and standard additions. J. N. Am. Benthol. Soc. 26(2): 167-177.