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Measuring ammonium (NH_4^+) concentrations in water samples

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Jacob Waldbauer¹, Amy Zimmerman²

¹University of Chicago; ²Pacific Northwest National Lab



Jacob Waldbauer

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We use this protocol and it's working

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Abstract

Fluorometric assay for precise measurements of ammonium from 0.3 to 50 $\mu\text{mol}\cdot\text{L}^{-1}$ concentrations. Reaction reaches maximum fluorescence after ~2 hours and remains stable for several more hours. Single, non-hazardous working reagent is stable for months. NOTE: This protocol is written for measurement in 96-well plates—adjust reaction volumes if using fluorometer for measurements.

Reference: Holmes, R.M., A. Aminot, R. Ke?rouel, B.A. Hooker, B.J. Peterson. (1999) A simple and precise method for measuring ammonium in marine and freshwater ecosystems. *Can. J. Fish. Aquat. Sci.* 56: 1801–1808.

Materials

Fluorometer (or plate reader)
200 and 1000 μL filter tips
200 and 1000 μL pipettes
microcentrifuge tubes
Tube racks
15 mL conical tubes (for standards)
Vortexer
96-well microplate with lid, black (preferable)
Working reagent
Ammonium standards

- 1 1. Prepare 200 μ M stock solution: Dilute 1:500 from 0.1 M solution $\Rightarrow$ 20 μ L + 9.980 mL nanopure water.
- 2 Dilute the stock solution to the following concentrations (μ M) in nanopure water: 0, 0.5, 1.25, 2.5, 5, 7.5, 10. NOTE: 1 mL of each standard is necessary for triplicate reactions.
- 3 Prepare Reagents and Solutions.
 - 3.1 1. Sodium sulfite solution: Add 1 g of sodium sulfite (Sigma S-4672) to 125 mL of nanopure water. The resulting solution is stable for ~1 month when stored at room temperature in a glass bottle.
 - 3.2 2. Borate buffer solution: Add 80 g of sodium tetraborate (Sigma S-9640) to 2 L of nanopure water. Stir or shake thoroughly to dissolve.
 - 3.3 3. OPA solution: Add 4 g of OPA (Sigma P-1378) to 100 mL of ethanol (use a high-grade ethanol because impurities in ethanol can autofluoresce). OPA is light sensitive, so it should be protected from light while dissolving in ethanol and stored in the dark.
 - 3.4 4. Working Reagent: In a large (>2 L) brown polyethylene bottle, mix 2 L of borate buffer solution, 10 mL of sodium sulfite solution, and 100 mL of OPA solution. Ideally, allow the WR to "age" for 1 day or more prior to use because its blank will decrease over time. The resulting WR is stable for at least 3 months when stored in the dark at room temperature, or longer when stored at 4°C. The final WR should contain the chemicals at the following final concentrations: borate buffer (40 g·L⁻¹, 21 mM), sodium sulfite (40 mg·L⁻¹, 0.063 mM), and OPA in ethanol (50 mL·L⁻¹).
- 4 A. Assay set-up.NOTE: This procedure can be modified for 24-well plates by increasing reaction volumes 4X and preparing reactions in 15 mL conical tubes. 24-well plates hold up to 2.5 mL per well.
 - 4.1 1. Label one microcentrifuge tube for each sample, blank, and all standards (7).
 - 4.2 2. Working under dimmed light, aliquot 1 mL of Working Reagent (or nanopure water, as appropriate) to each reaction (or blank) tube.
 - 4.3 3. Add 250 μ L of sample, standard, or nanopure water to each corresponding tube.
 - 4.4 4. Mix (invert or vortex) and incubate in the dark at room temperature for 2-3 hours.

- 4.5 5. Transfer 250 μ L of each reaction to triplicate wells of a 96-well microplate and measure fluorescence on plate reader.
- 5 B. Reading plates.
 - 5.1 1. Turn on Tecan Infinite 200 PRO plate reader 20-30 minutes prior to use.
 - 5.2 2. Once warmed up, open the iControl software on MLCLab-PC.
 - 5.3 3. Open file "OPAammonia_96well" (or "OPAammonia_24well" if appropriate).
 - 5.4 4. Load the plate—check whether the "plate with cover" box is checked (can read with lid if using clear plates but make sure to remove lid if using black plates).
 - 5.5 5. Read plate at 350 ± 9 nm excitation and 422 ± 20 nm emission (fluorescence top mode, manual Z-position set to 24995 μ m, 25 flashes, manual gain of 100, 20 μ s integration time). Program automatically opens an Excel file that documents parameters and data.
- 6 C. Analyzing data.
 - 6.1 1. Subtract the fluorescence values of the samples mixed with nanopure water (or borate buffer, preferably) instead of the Working Reagent (sample blanks) from the corresponding reacted sample fluorescence's (= corrected sample fluorescence).
 - 6.2 2. Subtract the average fluorescence of the nanopure water tubes (i.e., 0 μ M ammonium) mixed with borate buffer instead of Working Reagent (standard blanks) from the fluorescence values of all the standards.
 - 6.3 3. Plot corrected fluorescence (y) vs. concentration (x) for all standards to establish a standard curve with linear regression.
 - 6.4 4. Use the equation of the standard curve to calculate sample concentration from fluorescence.