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Measuring nitrate/nitrite (NO_x) concentrations in water samples

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We use this protocol and it's working

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Abstract

Colorimetric (i.e., UV-visible spectroscopy) assay for measurements of nitrate/nitrite down to $0.5 \mu\text{mol} \cdot \text{L}^{-1}$ concentration. Vanadium(III) reduces nitrate to nitrite prior to measurement with traditional Griess reaction. Nitrate and nitrite concentrations can be measured for the same sample. NOTE: This protocol is written for measurement in 96-well plates.

Materials

Spectrophotometer (or plate reader)

200 and 1000 μL filter tips

200 and 1000 μL pipettes

microcentrifuge tubes

Tube racks

15 mL conical tubes (if necessary)

Vortexer

96-well microplate with lid, clear

saturated VCl_3

2% SULF

0.1% NEDD

- 1 Making standards (sodium nitrate and sodium nitrite)
 - 1.1 Prepare 200 μ M stock solution: Dilute 1:500 from 0.1 M stock solution $\Rightarrow$ 20 μ L + 9.980 mL nanopure water.
 - 1.2 Dilute the stock solution to the following concentrations in nanopure water: 0, 10, 20, 30, 40, 60, 100, 150, 200 μ M.
- 2 Making Reagents and Solutions.
 - 2.1 Saturated vanadium(III) chloride (VCl_3) solution: Dissolve 100 mg in 12.5 mL 1 M HCl. Remove excess precipitates with nylon-66 syringe filter. DO NOT HEAT. Store solution at 4°C protected from light. Use within 2 weeks. NOTE: Appears viable after 2 months. Development of a lighter blue color indicates oxidation— authors suggest discarding solution, but light blue color observed in our lab shortly after preparation, and worked fine.
 - 2.2 2% (w/v) Sulfanilamide (SULF) solution: Add 1 g to 50 mL HCl. Heat to dissolve (in water bath set to ~55°C). Filter to remove trace particulates. Store solution at 4°C protected from light. Stable for several months. Discard if colored.
 - 2.3 0.1% (w/v) N-(1-Naphthyl)ethylenediamine dihydrochloride (NEDD) solution: Add 50 mg to 50 mL nanopure water. Heat to dissolve (in water bath set to ~55°C). Filter to remove trace particulates. Store solution at 4°C protected from light. Stable for several months. Discard if colored.
- 3 Assay set-up.
 - 3.1 Calculate total volume of SULF/NEDD mixture needed to run triplicate wells for each standard and sample: (# standards + # samples + 1 extra) $\times$ 3 $\times$ 80 μ L = total vol (μ L). a. If quantifying both total NO_x and NO_2^- , double the # samples. b. Account for both sample (no added SULF/NEDD mixture) and reagent blanks (nanopure plus reagents, i.e., 0. μ M NO_x).
 - 3.2 Prepare the total volume of SULF/NEDD mixture needed by mixing equal parts of the reagents (i.e., if need 3 mL total volume, add 1.5 mL SULF to 1.5 mL NEDD in 15 mL tube). Vortex to mix.
 - 3.3 Aliquot 80 μ L of each sample/standard to the appropriate wells of a clear 96-well plate.

- 3.4 Add 80 μL of VCl_3 to each well for total NO_x concentrations and 80 μL of nanopure water to each well for nitrite concentrations. Mix briefly by pipetting (use multichannel pipet).
- 3.5 Quickly add 80 μL of SULF/NEDD mixture to each well. Mix briefly by pipetting.
- 3.6 Incubate at room temperature for 30 minutes (45 minutes maximum).
- 3.7 Measure absorbance on plate reader.
- 4 Reading plates.
 - 4.1 Turn on Tecan Infinite 200 PRO plate reader 20-30 minutes prior to use.
 - 4.2 Once warmed up, open the iControl software on MLCLab-PC.
 - 4.3 Open file "Miranda_NOx_96well" (or "Miranda_NOx_24well" if appropriate).
 - 4.4 Load the plate—check whether the "plate with cover" box is checked (since using clear plates for this, can be read with lid on).
 - 4.5 Read absorbance at 520 ± 9 nm (25 flashes). Program automatically opens an Excel file that documents read parameters and data.
- 5 Analyzing data.
 - 5.1 Subtract the absorbance values of the samples mixed with diluting solution/medium instead of the SULF/NEDD mixture (sample blanks) from the corresponding reacted sample absorbances (= corrected sample absorbance).
 - 5.2 Subtract the average absorbance of the nanopure water tubes (i.e., 0 μM NO_x) mixed with diluting solution/medium instead of the SULF/NEDD mixture (standard blanks) from



the absorbances of all the standards.

- 5.3 Plot corrected absorbance (y) vs. concentration (x) for all standards to establish a standard curve with linear regression.
- 5.4 Use the equation of the standard curve to calculate sample concentration from fluorescence.