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Measuring nitrate/nitrite (NOx) 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 µmol•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 µM stock solution: Dilute 1:500 from 0.1 M stock solution ⇒ 20 µL + 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 µM.

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) x 3 × 80 µL = total vol (µL). 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. µM NOx).

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 µL 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 NOx 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 NOx) 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.