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HOLTGRIEVE ECOSYSTEM ECOLOGY LAB
CONCENTRATION OF NITRATE IN PUGET SOUND WATER SAMPLES

INTRODUCTION

This protocol outlines basic calibration methods for a YSI EX02 multiparameter water quality sonde, specifically dissolved oxygen, conductivity, and pH calibration. Additional calibration of other sensors (e.g., Chlorophyll RFU, fDOM, Turbidity, etc.) is not detailed in this protocol.

SAFETY

None.

MATERIALS

- 3-mL empty fritted SPE tubes (Sigma Aldrich, 54221-U)
- Polyethylene frits for 3-mL tubes (Sigma Aldrich, 57180-U)
- Adapters (Sigma Aldrich, 57020-U)
- Resin, AG1-X8, 200-400 mesh, chloride form (BioRad, 140-1451)
- Poly prey chromatography columns (BioRad, 731-1550)
- 250-mL econo-column funnel (BioRad, 731-0003)
- 15-mL clear Nalgene HDPE bottles

BEFORE YOU BEGIN: DOES YOUR SAMPLE NEED TO BE CONCENTRATED?

Calculate nanomoles nitrate in sample from concentrations provided by Manchester Laboratory and/or Kathy Krogsland's lab. Determine whether the amount of nitrate in the volume of sample will enable you to analyze a volume equal to or less than 10mL of sample containing 100-200 nanomoles of nitrate. A 200 nmol injection is the ideal target, and you cannot inject more than 10 mL of sample when using the denitrifier method. If this target is not reachable given low nitrate concentrations, then the sample must be concentrated.

ION EXCHANGE RESIN IN COLUMN

1. In a small, clean beaker, mix roughly 50% of resin and 50% of 1 M NaCl (V/V). (1 M NaCl: 14.61 g to 250 mL of DI water; Resin: BioRad, AG 1-X8, 200-400 mesh, chloride form, density 0.75/mL). Make new resin on the day of the concentration to avoid resin degradation.

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2. Shake the beaker vigorously to homogenize the resin solution.
3. Prepare the resin column (SUPELCO, 3-mL empty fritted SPE tubes) by pipetting 0.6 mL (600 uL) of the above resin solution and transferring to the tube. Verify that you have effectively ~0.3 mL of resin. Use the syringe and tubing to pull liquid from the resin column. Put the frit on the top of the resin bed in the tube. Use tweezers to place the frit snugly against the top of the resin layer.
4. Attach the rest of filtration setup on manifold.
5. Rinse the resin with 3 x 3 mL of NaCl (1 M) and following with 3 x 3 mL of MiliQ water (using syringe). Add 3 mL of NaCl (1 M). Then use syringe to draw out 3 mL and dump into waste container or down sink. Repeat last two steps twice to have a total of 3 x 3 mL NaCl rinses. Repeat steps for DI water 3 x 3 mL.
 - a. This step introduces Cl ions to resin.

TRAP AND COLLECT NITRATE

1. Pour thawed water sample (125 mL) into column and allow gravity to pull it through the resin. Nitrate in the sample gets trapped in the resin.
2. Once passed, rinse column with 10-15 mL of MiliQ water to rinse tube and column. Drain into sink.
3. Place a small clean container (15 mL dry, pre-cleaned bottle; i.e., acid-washed) below the column. Label the bottles with the sample name, including collection site, date (month, yr), sample type (freshwater, snow, rain), and project name.
 - a. Note: for saltwater samples, the process for concentrating samples is much more complicated. You cannot use this method given that a NaCl resin is used as the exchange ion during the concentration process. Refer to method by B. Chang for more information.
4. Elute the column with 5 x 2 mL 1 M NaCl (using the syringe to push the solution through), collect the solution in the bottle; put caps on and seal the bottles. Freeze bottled samples until ready to analyze in IsoLab. The NaCl eluent releases the nitrate from the resin (nitrate ions switch with Cl ions) and is released into the sample bottle liquid.
5. Rinse the resin with 3 mL of DI water (using syringe). The sample columns are ready to be used for another sample.
6. IF the resin turns grey or black, or if you suspect any contamination, dispose of column and make new ones.

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Depending on the purity of the sample, the same resin can be used multiple times.

WHAT TO CONCENTRATE IN ADDITION TO YOUR SAMPLES

1. A typical run on Brave Irene includes 40 vials: 16 standards (IAEA, USGS34, USGS32, and USGS35) + 22 samples. Anytime you plan to concentrate samples, you should also include concentrated:
 - a. Blanks (i.e., MiliQ water source you use during concentration steps)
 - b. IAEA standards (use 20 uM IAEA stock solution from IsoLab)
2. Blanks (2-3)
 - a. Run 125 mL MiliQ water through resin steps
 - b. Be consistent with your water source as different water MiliQ sources have been found to contribute varying amounts of nitrate to the measurement. SAFS MiliQ water is what we have been using, but it has a higher nitrate contribution than the IsoLab source. Keep this in mind are you proceed with this method.
3. IAEA (2-3)
 - a. Use 20 uM IAEA stock solution to run 200 uM through resin method
 - b. Can inject directly through resin or add to MiliQ water to assimilate 125 mL sample