Standard Operating Procedure

Ammonia and Nitrate/Nitrite Lachat operation
Soil Environmental Chemistry Program, The Ohio State University
Version 3

1.0 Scope

Lachat may be used to determine the following ions in solution: Nitrite (NO_2^-), Nitrate (NO_3^-), Ammonia (NH_3).

1.1 The application ranges:

- 0.01-2.0 mg N/L : 0.20-20 mg N/L as NO_2^- and NO_3^- Sample loop 40.5 cm: Microloop
- 0.01-2.0mg N/L as NO_2^-.
- 0.10- 20 mg N/L as NH_4

1.2 Throughput:

- 30 injections/hour

2.0 Definitions

2.1 Check standard: Standard that is independently made, that is used for Quality control.

2.2 Matrix: also known as the carrier, it matches the substance the samples are in.

3.0 Equipment and Supplies

3.1 Balance

3.2 500 ml beaker

3.3 Volumetric flask (v.f.)

3.4 Hamilton Autodiluter

3.5 Various graduated cylinders

3.6 Stir bars

3.7 Ethylenediamne tetraacetic acid disodium salt dihydrate (EDTA)

3.8 ACS grade Sodium hydroxide (NaOH)

3.9 Sodium phosphate dibasic Heptahydrate (SPDH)

3.10 Sodium salicylate

3.11 Sodium nitroprusside

3.12 Sodium hypochlorite

3.13 ACS grade Ammonium Hydroxide

3.14 85% Phosphoric acid

3.15 Sulfanilamide

3.16 N-1-naphthylethylenediamine dihydrochloride (NED)

3.17 ACS grade Potassium Nitrate

3.18 ACS grade Potassium Nitrite

3.19 Hydrochloride acid (HCl)

3.20 ACS grade Ammonium Chloride, previous dried for two hours at 110ºC

4.0 Reagents and Standards
***Note strong bases and acids will be use, caution is advised.**

4.1 Reagent 1: EDTA Solution
- 4.1a Dissolve 66g EDTA in about 500 mL of DI water
- 4.1b Adjust pH to 7.0 with 4M NaOH
- 4.1c Dilute to 1000 mL volume in a 1000 mL v.f.
- 4.1d Store in HDPE screw top bottle, good for 2 months.

4.2 Reagent 2: Buffer
- 4.2a Dissolve 28.0g NaOH completely in 800 mL DI water using a 1000 mL v.f.. Then add 50.0 g SPDH and bring to volume
- 4.2b Store in HDPE screw top bottle, good for 5 days

4.3 Reagent 3: Salicylate- Nitroprusside Color reagent
- 4.3a Dissolve 150g Sodium Salicylate and 1.0g Sodium Nitroprusside in 800 mL DI water in a 1000 mL v.f. Bring to volume
- 4.3b Store in light-proof screw top bottle, good for 1 month

4.4 Reagent 4:Hypochlorite Reagent
- 4.4a Dilute 250ml 5.25% sodium hypochlorite with DI water in a 500 mL v.f.

4.4b Store in HDPE screw top bottle, good for 1 month

4.5 Reagent 5: Ammonium Chloride buffer
4.5a Add 700 mL DI to 1000 mL v.f. and mix in 105 mL HCl, then 95 mL ammonium hydroxide, and 1.0g EDTA. Adding ammonium hydroxide before HCl will create fumes. Bring to volume
4.5b Store in HDPE screw top bottle, good for 2 months

4.6 Reagent 6: Sulfanilamide Color reagent
4.7a Mix100ml of phosphoric acid, 40.0g sulfanilamide and 1.0g NED in 800 mL DI in a 1000 mL v.f. Bring to volume
4.7b Store in dark screw top bottle, discard when pink

4.7 Reagent 7: Copper Sulfate
4.6a Mix 0.80g of Copper Sulfate in half-filled 500mL v.f. Bring to volume.
4.6b Good for one week.
4.6c Add 5 drops/1000mL to reagent 5.

4.8 Ammonia Standard: 500.0 mg N/L as NH_3
4.11a Stock Solution: Dissolve 1.9095g of ammonium chloride in 800 mL matrix in a 1000 mL v.f. Bring to volume
4.11a Repeat for check standard

4.9 Nitrate Standard : 200 mg N/L as NO_3^-
4.12a Stock solution: Dissolve 1.444g Potassium Nitrate in 800 mL matrix in a 1000 mL v.f. Bring to volume
4.12b Repeat for check standard

4.10 Nitrite Standard : 200 mg N/L as NO_3^- and NO_2
4.13a Stock solution: Dissolve 1.214 Potassium Nitrite in 800 mL matrix in a 1000 mL v.f. Bring to volume
4.13b Repeat for check standard

4.11 Blank Standard: Matrix

5.0 Sample Collection, Preservation and Storage

5.1 Note if samples can't be run within 48 hours, samples should be adjusted to pH <2 with sulfuric acid and refrigerated to 4ºC. Samples can be held in the refrigerator up to 28 days.

5.2 Samples should be filtered with a .45um Nylon filter that have been checked for contaminants and if possible new syringes to make sure all particles are free from the sample and to avoid contamination.

5.3 Sample Runlists

5.3.1 Sample runlists (ie. Lachat 16-1.xls) should be created and stored to Wdrive>SEC Lab>Lachat> >year (i.e 2016)>Runlist. A runlist template is in Wdrive>SEC Lab>Lachat.

5.3.2 Each time the method is started or stopped and restarted, the runlist must be updated immediately with the timestamp corresponding to the sample.

6.0 Lachat Setup

6.1 Turn on all instrument components and then start the OMNION software.

6.2 Clamp down tubing on the pump, Turn on pump by hitting manual start/stop, make sure the speed is 35.

6.3 Pump DI water through all the reagent lines and check for leaks and smooth flow. Switch to reagents one by one and wait for the system to equilibrate until a stable baseline is achieved for each one.

6.4 If running for Nitrate/Nitrite then turn on the Cadmium Column and wait for the system to equilibrate.

6.5 Templates are in Documents> OmnionData> OSU>Templates>NOx or NO2

Commented [WU1]: We need separate templates for NO2 and NO2+NO3. The analyte should be clearly indicated in the method.

6.5.1 Open template

6.5.2 Modify the template if necessary and save the file in Document>Omnion Data>OSU> Data>(Current year)>(runlist #)

6.6 Templates are created by imputing the following data system parameters:

In the timing Tab:

Run:

Cycle period: 90 s

Min. probe in wash period: 40 s

Probe in sample period: 30 s

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Ammonia:

Peak Base Width: 30 s

Inject to peak start: 46 s

Channel 2:

Load Period: 18 s

Inject period: 42 s

Time to valve: 27 s

Under the Ammonia Analyte Tab:

Chemistry: Direct

Calibration Rep: Average

Calibration fit type: 2nd order polynomial

Weighting method: none

Force through zero: no

Concentration Unit: mg/L

Name: Ammonia

Clear calibration: should be checked, unless otherwise

Nitrate/ Nitrite :

Peak Base Width: 35 s

Inject to peak start: 13 s

Channel 3:

Load Period: 17.9 s

Inject period: 27.1 s

Time to valve: 27 s

Under the Nitrate/ Nitrite Analyte Tab:

Chemistry: Direct/ Bipolar

Calibration Rep: Average

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Calibration fit type: 2nd order polynomial

Weighting method: 1/X

Force through zero: no

Concentration Unit: mg/L

Name: Nitrate/Nitrite

Clear calibration: should be checked, unless otherwise

6.7 From the stock 200 mg N/L Nitrate or Nitrite standard, prepare 50mL solutions, using the matrix as diluent, of 2.0, 1.0, 0.20, 0.10, 0.05,and 0.01 mg N/L. This can be done manually or using the auto diluter on the Lachat.

6.7a Using the Autodiluter

6.7a1 Put standard in its position
6.7a2 In Omnion select where the standard is going to be taken from.
6.7a3 If it need diluted click the ADF check mark and impute the auto dilution factor in the ADF box
6.7a4 Then under sample in run properties, impute the known concentration of the standard you are achieving by dilution.

6.8 From the 200mg N/L Nitrate or Nitrite check standard, prepare 50 mL solution, using the matrix as diluent of 1.0 mg N/L.

6.9 From the stock 100 mg N/L Ammonia standard, prepare 50mL solutions, using the matrix as diluent, of 20, 10, 2.0, 1.0, 0.20,and 0.10 mg N/L. This can be done manually or using the auto diluter on the Lachat.

6.10 From the 100mg N/L Ammonia check standard, prepare 50 mL solution, using the matrix as diluent of 10 mg N/L.

6.11 Double check that the test tube racks are the test tube racks being used. That is under the racks tab.

6.12 Using the standards run a calibration curve.

6.12a Calibration curve should have a straight line and r= 1±.0010

6.13 Run the Check standard and blank after calibration is completed

6.14 Before running samples create a Composite (Comp), comp spk, and comp x5 solution.

6.14.1 **Comp**- make a solution with a mixture that is a composite of all the samples going to be run. Make about 15 ml of solution.
6.14.2 **Comp spk**- Take 5 ml of comp solution and add 0.125 mL of 10 mg N/L of Nitrate and Ammonia solution.
6.14.3 **Comp x5-** dilute Comp solution x5
6.14.4 Calculate the % recovery for each. Should be approx. ±10%

6.15 Run samples as unknowns and check standard and blank after every 10 samples.

7.0 Quality Control

7.1 Run the Check standards, 1.0 mg N/L as prepared in 6.7 and blank on the Lachat.

Commented [WU2]: Do we need a low limit check standard, low calibration standard maybe?

7.1.1 Results: should fall with in ±10%

7.2 Check standards as prepared in 6.7 and blank should be run after ever 10 samples.
7.2.1 If check standards fail then recalibrate instrument and re-run the previous 10 samples and check standards and blank
7.2.2 If it continues to fail then remake standards and recalibrate and test using check standards
7.2.3 If check standards fail then remake check standards and re-run check standard
7.3 When equilibrating in 6.1, after each reagent is added, it should go back down to the baseline after equilibration if it does not, check lines and remake reagent if necessary.

8.0 Data Summary

8.1 Data automatically goes to excel sheet in the Document>Omnion Data>OSU> Data>(Current year).
8.1.1 This can be found and/or modified in the Configuration tab>Options>Data export> Data Items.
8.2 After a run is completed go into the Document>Omnion Data>OSU> Data>(Current year) folder and change the names of the excel sheet(s) and method(s) to match the Runlist id.

8.3 See Lachat Summary SOP for Data Summary.

9.0 Lachat Power off procedure

9.1 Turn off Cadmium Column

9.2 Rinse all lines with DI water for 10 minutes

9.3 Allow air to pump through lines for five minutes

9.4 Restart Auto-sampler so that the sampling wand ends out of rinse.

9.5 Turn off all devices and computer

10.0 Waste

10.1 All Waste lines from the Lachat should be positioned into a carboy, not down the sink

11.0 Cadmium Column Efficiency Testing

11.1 Cadmium Column can go inefficient over time, it is good to test it once a month or more depending on usage

11.2 To test the Column:

11.2.1 Start up the instrument as in 6.0, but making sure Column is open and using the NO_3 Standard as your calibration.

11.2.2 Run a check standard of your NO_3 and NO_2 of the same concentration.

11.2.3 Using the following formula: $\frac{NO3}{NO2} * 100\% = E$

11.2.4 E should be greater than 90%.

12.0 References

12.1 Lachat Applications Group. Determination of Nitrate in 2 M KCl Soil Extracts by Flow Injection Analysis Calorimetry. QuickChem Method 12-107-04-1-F. 2015

12.2 O'Dell, J.W. Determination of Nitrate-Nitrite by Automated Colorimetry. Environmental Monitoring Systems Laboratory. United States Environmental Protection Agency. Method 353.2. 1993

12.3 United States Geological Survey. Methods for Determination of Inorganic Substances in Water and Fluvial Sediments. Brook 5 Chapter A1. United States Department of the Interior. Method I-2601-78