CENTER FOR RESERVOIR AND AQUATIC SYSTEMS RESEARCH (CRASR)

STANDARD OPERATING PROCEDURE #5.0 – Determination of NH3-N in Water

Analytical Range: 10 to 1000 μg N/L

Revision 1
10 January 2013

1. Method Description

This method is based on the Berthelot reaction. Ammonia reacts with alkaline phenol, then with sodium hypochlorite to form indophenol blue. Sodium nitroprusside (nitroferricyanide) is added to enhance sensitivity. The absorbance of the reaction product is measured at 630 nm, and is directly proportional to the original ammonia concentration in the sample.

2. Equipment and Supplies

2.1. Balance -- analytical, capable of accurately weighing to the nearest 0.0001 g.

2.2. Glassware -- Class A volumetric flasks and pipettes or plastic containers as required. Samples may be stored in plastic or glass.

2.3. Flow injection analysis equipment designed to deliver and react sample and reagents in the required order and ratios.

2.3.1. Autosampler

2.3.2. Multichannel proportioning pump

2.3.3. Reaction unit or manifold

2.3.4. Colorimetric detector

2.3.5. Data system

2.3.6. Acid washed glassware: All glassware used in the determination of phosphate should be washed with 10% muriatic acid and triple rinsed with distilled water. Preferable, this glassware should be used only for the determination of phosphate and after use it should be rinsed with distilled water and kept covered until needed again.

2.4. Special Apparatus

2.4.1. Heating unit Lachat Part No. A85X00 (X=1 for 110V, X=2 for 220V)

2.4.2. Glass calibration vials must be used. Lachat Part No. 21304 for XYZ samplers.

2.4.3. PVC PUMP TUBES MUST BE USED FOR THIS METHOD

3. Sample Collection and Preservation – See CRASR SOP # 2 for details.

4. Standards

a. Ammonia-N Stock solution (1000 mg N/L) – Add 500 ml deionized water to a 1000 ml volumetric flask. Carefully weigh out 3.8188 g of ammonium chloride (NH_4Cl; FW=53.49) and add it to the flask. Dissolve material, dilute to 1000 ml, and mix well. Solution may be stored indefinitely in refrigerator if also using a laboratory control standard (LCS) as a calibration verification in analyses. If not using LCS as a calibration verification, the ammonia-N stock solution should be prepared fresh monthly.

b. DNP Mixed Standard (1.0 mg N / L) – The Mixed Standard is used for the simultaneous determination of PO_4-P, NH_3-N and NO_2-N+NO_3-N in unpreserved water samples. To prepare, add 500 ml of water to a 1000 ml volumetric flask. Carefully pipette 1 ml of 1000 mg/L Phosphate-P Stock Solution, 1 ml of Ammonia-N Stock Solution and 1 ml of Nitrate-N Stock Solution into the flask. Dilute to 1000 ml and mix well by inverting flask three times. The DNP Mixed standard may be stored in the refrigerator for up to 48 hours.

c. Mixed working standards – Mixed working standards are used as calibration and continuing calibration verification (CCVs) standards in the simultaneous determination of PO_4-P, NH_3-N and NO_2-N+NO_3-N. Prepare mixed working standards at concentrations (high range) of 5, 10, 25, 50, 100, 250, 500, and 1000 ppb (µg/L) PO_4-P, or (low range) 5, 10, 25, 50, 75, 100, 150, 200 and 250 ppb or (µg/L) PO_4-P. The following table outlines preparation, use pipet:

HIGH RANGE

Target Concentration (µg/L)	Total volume (ml)	DNP Mixed Standard Concentration (µg/L)	Volume of DI Water (ml)	Volume of Mixed Standard (ml)
5	50	1000	49.75	0.25
10	50	1000	49.50	0.50
25	50	1000	48.75	1.25
50	50	1000	47.50	2.50
100	50	1000	45.00	5.00
250	50	1000	37.50	12.50
500	50	1000	25.00	25.00
1000	50	1000	0.00	50.00

LOW RANGE

Target Concentration (µg/L)	Total volume (ml)	DNP Mixed Standard Concentration (µg/L)	Volume of DI Water (ml)	Volume of Mixed Standard (ml)
5	50	1000	49.75	0.25
10	50	1000	49.50	0.50
25	50	1000	48.75	1.25
50	50	1000	47.50	2.50
75	50	1000	46.25	3.75
100	50	1000	45.00	5.00
150	50	1000	42.50	7.50
200	50	1000	40.00	10.00
250	50	1000	37.50	12.50

Mixed working standards may be stored in the refrigerator for up to 48 hours.

d. Laboratory Control Standard – A laboratory control standard of 150 ppb should be prepared from a purchased certified aqueous ammonia-nitrogen standard. This standard should be certified by the external source from which it was purchased.

5. Reagents

Degassing with helium:

To prevent bubble formation, degas all solutions except the standards with helium. Use He at 140kPa (20 lb/in^2) through a helium degassing tube (Lachat Part No. 50100.) Bubble He through the solution for one minute.

Reagent 1. Sodium Phenolate

CAUTION: Wear gloves. Phenol causes severe burns and is rapidly absorbed into the body through the skin.

By Volume: In a **1 L volumetric flask** dissolve **88 mL** of **88% liquified phenol** or **83 g crystalline phenol** (C_6H_5OH) in approximately **600 mL DI water**. While stirring, slowly add **32 g sodium hydroxide** (NaOH). Cool, dilute to the mark, and invert to mix. Do not degas this reagent. Prepare fresh every 3 to 5 days. Discard when reagent turns dark brown.

Hach sells this premade reagent (Part no. 52005). This reagent has a shelf life of at least two months if refrigerated.

Reagent 2. Sodium Hypochlorite By Volume: In a **500 mL volumetric flask** dilute **250 mL 5.25% sodium hypochlorite** (NaOCl), to the mark with **DI water**. Invert to mix. Prepare fresh daily

Reagent 3. Buffer

By Volume: In a **1 L volumetric flask**, dissolve **50.0 g disodium ethylenediamine tetraacetate dihydrate** ($Na_2EDTA \cdot 2H_20$) and **9.0 g sodium hydroxide** (NaOH) in

approximately **900 mL DI water.** Dilute to the mark and mix with a magnetic stirrer until dissolved. Prepare fresh monthly

Reagent 4. Sodium Nitroprusside

By Volume: To a **1 L volumetric flask** dissolve **3.50 g sodium nitroprusside** (Sodium Nitroferricyanide [$Na_2Fe(CN)_5NO.2H_2O$]). Dilute to the mark with **DI water** and invert to mix. Prepare fresh every 1 to 2 weeks

Reagent 5. Carrier and Diluent (DI water)

6. Procedure

6.1. Prepare reagent and standards as described in sections 4 and 5 of this document.

6.2. Set up ammonia manifold as shown in section 9 of this document (manifold diagram is on the last page of the SOP).

6.3. Make sure power is on to all portions of the instrument (autosampler, manifold pump, and main instrument) then open the Omnion software. On the main screen click on the "Configuration" pull down menu then click on "Autosamplers". When the pop up menu opens, click the button that says "Initialize Autosampler". This should cause the autosampler to re-center itself over the rinse tube then move permanently down into the rinse tube.

6.3. After you have initialized the autosampler, open the NH3-N template by going to the "Run" pull down menu and clicking on "Open". Navigate to the folder Omnion/templates and click on the file named NH3-N.omn. When the file opens, the software will ask if you would like to change the setpoints of the relevant heaters. Click yes. Click again on the "Run" pull down menu, then click "Save As". Navigate to the folder Omnion/Data/Inorganic Nutrients. Save the template as the batch ID (yyyymmddPRJmANL; code on Chain of Custody Form) number associated with the sample set you are running. After the template has been saved, click the "Preview" button on the toolbar. This will allow you to view the baseline signal from the flowcell.

6.3. Secure the pump tubes to the pump by clicking down the tubing shafts. Turn on the pump by pressing the manual flow button on the top left of the pump (blue button). Make sure that the probe rinse pump line is submerged in DI water and that the probe is down in the rinse tube on the autosampler. Put all reagent lines into DI water. Pump DI water through all reagent lines and check for leaks and smooth flow. After any air in the system has passed through the flow cell, the baseline in the preview screen should be completely flat. Switch to reagents and allow the system to equilibrate until a stable baseline is achieved. This will probably take at least 15 minutes for all chemicals to come into equilibrium in the mixture.

6.4. Place standards in the sampler according to the slot locations in the method template. Fill in samples with the appropriate duplicates and spikes. All information on duplicate and spike samples has already been set up in the template for the analytical method, the only thing that needs to change are the sample IDs.

6.5. Once all sample data has been entered into the sample sheet, save the run again as described earlier. Once saved, check the baseline to insure that reagents have come through and that the baseline is stable. If stable, press the "Run" button on the tool bar.

6.6. The software will check that the LCS, and that all CCVs, duplicates, and spikes meet appropriate QA/QC criteria. If the LCS fails to meet QA/QC criteria, the run will automatically terminate. However, if the LCS passes and one of the CCVs, duplicates, or spikes fails to meet QA/QC criteria, the run will continue. It is imperative that the analyst check the QA/QC results for all CCVs, duplicates, and spikes and rerun any and all sample sets that do not adhere to QA/QC requirements outlined in CRASR SOP #8.

6.7. After run is completed, place all reagent lines in 10% H2SO4 for at least 20 minutes. After this time transfer all lines to DI water for an additional 30 minutes.

7. Quality Control/Quality Assurance – See CRASR SOP # 8 (Quality Assurance and Quality Control) for details on QA/QC criteria.

8. References

Lachat Method # 10-107-06-1-B. Determination of ammonia (phenolate) by flow injection analysis colorimetry.

U.S. Environmental Protection Agency, **Methods for Chemical Analysis of Water and Wastes**, EPA-600/4-79-020, Revised March 1983, Method 350.1

U.S. Environmental Protection Agency, 40 CFR, Part 36 Table 1B, footnote 6, 1994.

9. Instrument Information

DATA SYSTEM PARAMETERS FOR QUIKCHEM 8000

The timing values listed below are approximate and ***will need to be optimized*** using graphical events programming.

Sample throughput:	60 samples/h, 60 s/sample
Pump Speed:	35
Cycle Period:	60

Analyte Data:

Concentration Units:	mg N/L
Peak Base Width:	29 s
% Width Tolerance:	100
Threshold:	10000
Inject to Peak Start:	30 s
Chemistry:	Direct

Calibration Data:

Level	1	2	3	4	5	6	7
Concentration mg N/L	5.00	2.50	1.25	0.50	0.10	0.05	0.00

Calibration Rep Handling: Average
Calibration Fit Type: 1st Order Polynomial
Weighting Method: None
Force through zero: No

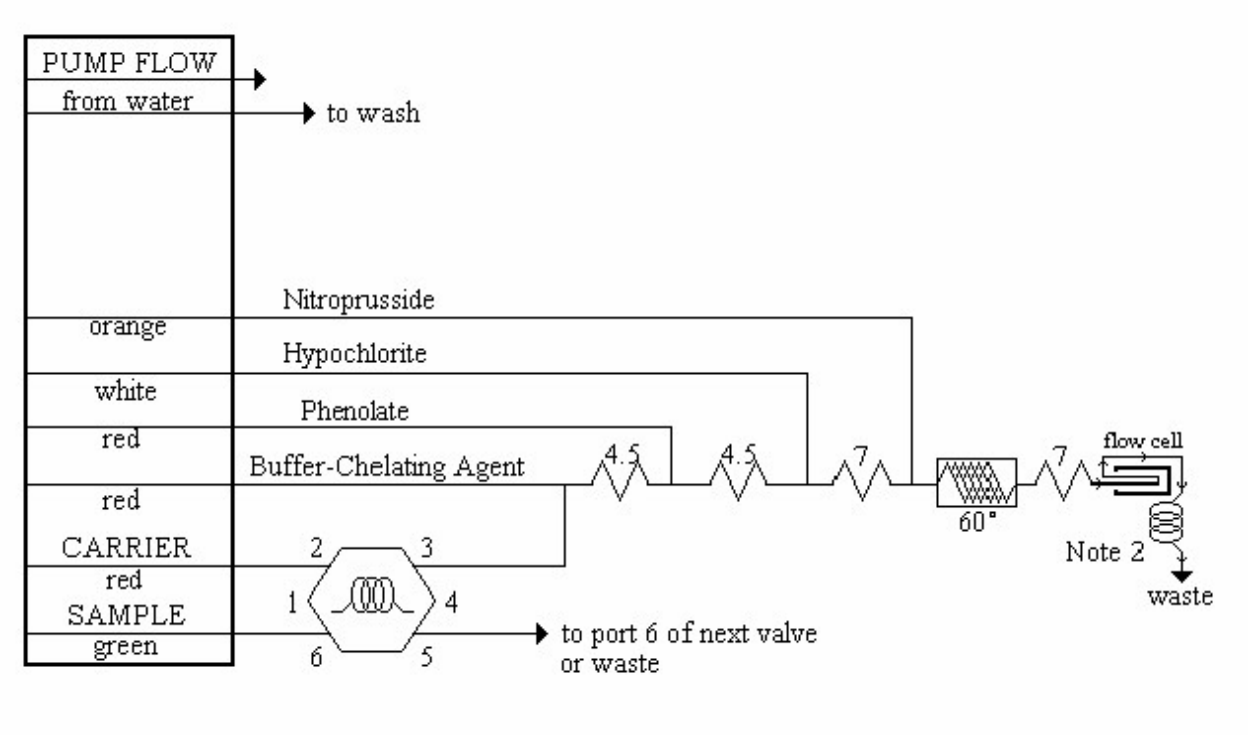
Sampler Timing:

Min. Probe in Wash Period: 5.0 s
Probe in Sample Period: 24 s

Valve Timing:

Load Time: 0 s
Load Period: 15 s Inject Period: 45 s

AMMONIA MANIFOLD DIAGRAM



Carrier: Reagent 5

Manifold Tubing: 0.8 mm (0.032 in) i.d. This is **5.2 µL/cm**.

AE Sample Loop: 75 cm **QC8000 Sample Loop:** 75 cm **Interference Filter:** 630 nm

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Apparatus: An injection valve, a 10 mm path length flow cell , and a colorimetric detector module is required. The shows 650 cm of tubing wrapped around the heater block at the specified temperature.

4.5: 70 cm of tubing on a 4.5 cm coil support

7: 135 cm of tubing on a 7 cm coil support

Note 1: PVC PUMP TUBES MUST BE USED FOR THIS METHOD
Note 2: 200 cm x 0.022” i.d. backpressure loop.