Measuring Total Ammonia

1. SCOPE AND APPLICATION

1.1. This method is based on SM 4500-NH3 F, the Denver® Model 225 meter and ammonia ISE electrode instructions. It is used to determine total ammonia in aqueous samples.

Note: Analysis for total ammonia in the toxicity lab is only as an aid in determining potential causes of any noted toxicity. Values are not used in official results.

2. SUMMARY OF METHOD

2.1. Meter check and probe preparation are performed (as needed due to meter malfunction or when a new probe is connected). Meter calibration through determination of slope is performed. Calibration checks using two different brands of standard are performed. Samples are prepared (salinity diluted to < 10 ppth if needed) and then pH adjusted by the addition of ionic strength adjusting solution (~ 10N sodium hydroxide). Ammonia measurements are made and recorded in appropriate logbooks.

2.2. Interferences

2.2.1. Volatile amines are a positive interference. Some metallic ions such as mercury and silver can complex ammonia, causing falsely low results in direct measurement (Reference 10.2 and 10.3).

2.3. Definitions

2.3.1. ISA – Ionic Strength adjusting solution

2.3.2. MV - millivolts

3. EQUIPMENT AND SUPPLIES

3.1. Denver Instruments Co. Model 225 pH/ISE meter

3.2. Ammonia specific ISE electrode (ex. Orion® 95-12)

3.3. Fisher® Dilu-vial, plastic, 30-mL vials

3.4. Volumetric flasks, Class A, 100 mL (4)

3.5. Specimen cups, 200-mL

3.6. Volumetric pipets, 1.0 mL and 10 mL, with repipettors

3.7. Large-bore plastic transfer pipette for use with pH adjusting solution

3.8. Magnetic stirrer

3.9. Magnetic stir bars, 6 mm

3.10. Wash bottle, 500 mL filled with DI water

3.11. pH test strips (range pH 10-14)

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3.12. Para-Film®, four ~ 2.5 cm x 2.5 cm squares

3.13. Beaker (400 mL minimum volume)

3.14. BNC shorting strap

3.15. Labeling tape

3.16. Black permanent marker

4. REAGENTS AND STANDARDS

4.1. Deionized (DI) water

4.2. Ammonia Standards, 0.1M, two different brands (we use Fisher, Ricca, Orion, etc.)

4.3. Ammonia pH adjusting Ionic Strength Adjustor (ISA) solution

5. CALIBRATION AND MEASUREMENT

5.1. **Meter check (**performed only if needed- for performance issue(s) not attributed to electrode/standards)

5.1.1. Disconnect the electrode from the meter.

5.1.2. Connect the BNC grounding cap to the **Channel B** input located on the back of the meter. Press the [Channel} button. Use the arrow keys to highlight **Channel B**. Press the [Enter} button.

5.1.3. Press the [Mode] button. Use the arrow keys to highlight **MV** (not **Rel MV**). Press the [Enter] key.

5.1.4. The meter should display 0±0.1 mv. If not, report issue to laboratory supervisor before continuing.

5.1.5. Remove the BNC grounding cap from the **Channel B** input. Connect the ISE electrode to the **Channel B** input on the back of the meter.

5.2. **Electrode Preparation** (previously assembled per instruction booklet)

5.2.1. Tighten the top cap on the electrode.

5.2.2. Leave the probe tip in the storage solution (0.05M ammonia standard without pH ISA).

5.3. **Standard Preparation**

5.3.1. Label the 100-mL volumetric flasks (1.4 mg/L Brand A; 14 mg/L Brand A; 1.4 mg/L Brand B; 14 mg/L Brand B).

5.3.2. Half fill each of the four labeled 100 mL flasks with DI water

5.3.3. Place 1 mL of Brand A ammonia standard and place 1 mL of Brand B ammonia standard into the appropriately labeled 14 mg/L flasks.

5.3.4. Fill the 14 mg/L flasks to the 100 mL line with DI water. Cover with Para-Film®, invert several times to mix.

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5.3.5. Half fill the two labeled 1.4 mg/L 100-mL flasks with DI water.

5.3.6. Use separate 10 mL pipettes and transfer 10 mL from each 14 mg/L standard to the appropriately labeled 1.4 mg/L flask (same brand to same brand).

5.3.7. Fill the 1.4 mg/L flasks to the 100 mL line with DI water. Cover with Para-Film®, and invert several times to mix.

5.4. Determine Electrode Slope

5.4.1. Press the [Standardize] button. Select option #3 (clear standards) and press [Enter].

5.4.2. Select option #1 (yes) and press [Enter].

5.4.3. Press [Standardize]. Select option #1 (enter a Standard) and press [Enter]. The "Select Ion" screen will display.

5.4.4. Select option #8 NH3 and press [Enter].

5.4.5. Select option #6 (mg/L) and press [Enter].

5.4.6. Label 3 each of the 30-mL vials (0.0 mg/L, 1.4 mg/L Brand A; 14 mg/L Brand A; 1.4 mg/L Brand B, 14 mg/L Brand B).

5.4.7. Place a stir bar in each of the labeled vials.

5.4.8. Place 10 mL of DI water into the 0.0 vials; pour 10 ml of each standard (1.4 mg/L and 14 mg/L) into the appropriately labeled vials.

5.4.9. Remove the electrode from the storage solution. Ensure that the electrode cap is tight. Thoroughly rinse the electrode in DI water. (Caution: do not allow the electrode to remain out of a solution for more than 1 minute)

5.4.10. Place a 0.0 vial on the magnetic stirrer. Place the electrode tip into solution (allow room for the stir bar to spin). Turn the stirrer on to a moderate speed (mix the solution but not a vortex).

5.4.11. Use the transfer pipette to add 3 drops of ISA solution to the vial.

5.4.12. Use the pH test strip to ensure that the pH of the solution is ≥ pH 11. Add additional ISA if necessary.

5.4.13. Follow instructions on the meter display. When the (Enter Standard) screen displays, use the keypad to type in 0.0. Press the [Enter] button. When the meter output stabilizes and the display value is accepted, an S will flash on the screen.

Note: It takes several minutes for the output to stabilize. If S does not display, manually accept the value by pressing [Enter] when the value changes back and forth (several times) between two numbers or stops on one value for ≥ 4 seconds.

5.4.14. Remove the electrode from the 0.0 vial. Rinse the electrode with DI water.

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5.4.15. Repeat 5.17 and 5.23 – 5.28 (typing in the appropriate value (1.4 and 14) first with one vial of Brand A 1.4 mg/L, then with one vial of Brand A 14 mg/L standard.

5.4.16. After Brand A 14 mg/L has been accepted (flashing S or manual entry), press the [Cal Data] button. The slope (-54 – -60) will appear and should be ((-54) – (-60)). Record the slope in the *Ammonia Bench Sheets* logbook.

Note: If there are any issues/problems with the meter slope, repeat the process one time and then notify the lab supervisor if the issue remains.

5.5. **Checking the meter with Ammonia Standards**

5.5.1. Press the [Meter] button.

5.5.2. Place the rinsed electrode in one of the two unused 0.0 vials (containing DI water). Add ISA (5.25) and check the pH (5.26).

5.5.3. When the reading stabilizes, remove the electrode from the vial; remove the vial from the stirrer. Thoroughly rinse the electrode.

5.5.4. Record the displayed value in the *Ammonia Bench Sheets* logbook.

5.5.5. Repeat 5.32 – 5.34 for the remaining 0.0 vial, the two 1.4 mg/L vials, and the two 14 mg/L vials.

Note: Values should be less than 0.017 mg/L for the 0.0 vials and ±10% (1.26 – 1.54 for 1.4 standard and 12.6 -15.4 for the 14 mg/L standard). (Checking the meter with the 14.0 mg/L liter standards is not necessary unless ammonia values are expected to be above 1.4 mg/L). If not, repeat the process and notify supervisor if second attempt fails.

5.6. **Maintenance of Electrode**

Note: Maintenance of the electrode is limited to replacing the sensing membrane and the internal fill solution. The membrane is replaced when: the electrode slope doesn't fall within the range of (-54) – (-60) after three attempts to determine the slope, the membrane appears dirty, or the membrane appears to be damaged (holes, tears, etc.). The internal fill solution is always replaced when the membrane is replaced.

5.6.1. Replace the membrane and internal fill solution in accordance with the instructions found in the electrode manual. Record replacement in the maintenance record book and the ammonia logbook.

6. MEASUREMENT

6.1. Place DI water (used as blank) and samples for ammonia analyses into each of two vials. Dilute any sample having a salinity ≥ 10 ppth (parts per thousand) to a salinity of < 10 ppth.

Note: If a sample has to be diluted, the MDL (0.017 mg/L) changes by the dilution factor. Ex. if a sample is diluted by 50% (0.5 dilution) the new MDL will be (0.017/0.5) = 0.034.

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6.2. Place a magnetic stir bar in each of the vials.

6.3. Place a 'Blank' vial on the magnetic stirrer. Place the rinsed probe in the vial (keep tip above but not touching the stir bar). Turn the stirrer on moderate speed.

6.4. Use the transfer pipette to add three drops of ISA pH adjusting solution to the vial (on the magnetic stirrer). Use the pH test strips to verify that the pH is ≥ 11. Add additional ISA as needed.

6.5. Allow the meter to stabilize (reading should be < 0.017 for the blank). Record the result as replicate 1 in the *Ammonia Bench Sheet* logbook.

6.6. Remove the electrode from the vial. Rinse the electrode with DI water.

6.7. Repeat 5.38 – 5.41 for the second blank replicate (record as second replicate) then for the first and second replicates for the samples.

6.8. When finished with the last measurement, turn off the stirrer, rinse the electrode, and return it to the storage bottle (with storage solution) making certain the tip is immersed. Loosen but do not completely remove the cap from the electrode.

6.9. Rinse all vials, stir bars, and flasks with DI water. Clean up the area. Put away all material.

6.10. **Maintenance of Electrode** - Maintenance of the electrode is limited to replacing the sensing membrane and the internal fill solution. The membrane is replaced when: the electrode slope doesn't fall within the range of (-54) – (-60) after three attempts to determine the slope, the membrane appears dirty, or the membrane appears to be damaged (holes, tears, etc.). The internal fill solution is always replaced when the membrane is replaced.

6.10.1. Replace the membrane and internal fill solution in accordance with the instructions found in the electrode manual.

7. DATA ARCHIVAL

7.1. Use a black, waterproof pen for all bench sheets and logbooks.

7.2. Record calibration and measurements in the *Ammonia Bench Sheets* logbook.

7.3. To correct raw data entries, place a single line through the incorrect entry, write the corrected entry near the error, date and initial the correction. Do not overwrite entries.

7.3.1. The corrected entry is written as near its appropriate space as possible or else in the comment box on the appropriate bench sheet.

7.3.2. If the correction requires an explanation, write a comment and reference the error. Initial and date the explanation.

7.3.3. Use sequential alphabetical annotation to identify errors and corresponding comments (use A for first error and use A for corresponding explanatory comment; use B for second error, etc.).

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8. QUALITY CONTROL

8.1. Refer to QA manual for corrective action and handling out-of-control data.

9. SAFETY/HAZARDOUS WASTE MANAGEMENT

9.1. Proper personal protection, including lab hood or ventilation system, lab coat, gloves and safety glasses, is required when working with chemicals and unprocessed samples.

9.2. Refer to the Laboratory Quality Manual for sample disposal

9.3. Refer to the Laboratory Health and Safety Manual and Hazardous Waste Management

10. REFERENCES

10.1. Instruction manual for Ammonia Electrode

10.2. Operation manual for the Denver Instrument Company Model 225 pH/Ion Meter

10.3. Standard Methods for the Examination of Water and Wastewater, 20th Edition

10.4. Laboratory Quality Manual http://www.dep.state.fl.us/labs/library/lab_sops.htm

10.5. Laboratory Health and Safety Manual and Hazardous Waste Management https://www.fldepnet.org/content/dear/bureau-laboratories-safety-documents

APPENDIX OF CHANGES

12/12/2007 Added Scope and Application; Summary of Method; and Appendix of Changes. Added “with probe (YSI model 5750)” in Step 1 of *Materials*. Replaced “read”

08/09/2013 Reformatted SOP.

02/09/2015 Formatted for SOP compliance.

07/11/2016 Updated links.

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