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User Guide

Determination of ammonia in wastewater by ion selective electrode measurement

Orion Pro Star PI214, PI217
UG-005NH3WW-E Rev 01 1024
Revision Date: October 2024

Introduction

Ammonia nitrogen is present in surface and wastewaters as result of natural processes, including the bacterial decomposition of organic nitrogen compounds (such as proteins) and the hydrolysis of urea[1,2]. In the wastewater plant, influent ammonia consumes oxygen and alkalinity during aerobic biological treatment. Effluent ammonia poses a potential threat to waters that support fish and wildlife. Under certain conditions, dissolved ammonia ($NH3_{aq}$) is a toxic pollutant to aquatic organisms. Monitoring ammonia concentrations throughout the wastewater treatment plant allows for better process control and may be necessary for regulatory permit requirements.

Ammonia in wastewater has been measured directly using a Thermo Scientific™ Orion™ 9512HPBNWP high-performance ammonia electrode and Thermo Scientific™ Orion™ pH/ion bench meter. The only sample preparation required is the addition of ion strength adjuster (ISA) to the sample before testing. The addition of ISA raises the pH to greater than 11 and converts the ammonia to the gaseous form, which diffuses across the membrane. The diffused ammonia changes the internal solution pH and is sensed by the pH electrode. The diffusion across the membrane and the ISA formulation (which removes ammonia-complexing ions) eliminates many common interfering ions and allows ammonia to be measured directly without the need for distillation.

Key words

Ammonia in wastewater, ammonia electrode, 9512HPBNWP, 9512BNWP, Standard Methods 4500-NH3 D, Ammonia Nitrogen, ASTM D1426, ISO 6778, IS 3025-34, proficiency testing, simple nutrients.

Equipment

- Thermo Scientific™ Orion™ pH/ion bench meter
- Thermo Scientific™ Orion™ ammonia electrode (9512HPBNWP or 9512BNWP)
- Thermo Scientific™ Orion™ automatic temperature compensation (ATC) probe
- Thermo Scientific™ Orion™ automatic stirrer probe or a magnetic stirrer with stir bars
- Graduated cylinders, 50 and 100 mL
- Beakers, 100 mL
- Pipets or pipettor, 1 and 10 mL
- Volumetrics flasks, 1000 mL (1L)
- Laboratory timer
- Laboratory wash bottle filled with reagent grade water
- Laboratory wipes (optional)

Required solutions

- Thermo Scientific™ Orion™ ammonia standard, 1000 ppm (mg/L)
- Thermo Scientific™ Orion™ ammonia standard, 100 ppm (mg/L)

- Thermo Scientific™ Orion™ ammonia ISA with blue dye
- Thermo Scientific™ Orion™ ammonia electrode filling solution
- Thermo Scientific™ Orion™ ammonia electrode storage solution
- Thermo Scientific™ Orion™ pre-assembled outer body and membrane cap
  - Or Thermo Scientific™ Orion™ loose membranes
- Reagent grade water (RGW), free of ammonia

Please see the last page of this document for ordering details.

Electrode setup

See the ammonia electrode user guide and the ammonia electrode quick start guide for the first time preparation procedure and general use instructions. See Table 3 Best practices for ammonia electrode care for a summary of daily, monthly, and as-needed care for the electrode.

Meter setup

1. Connect the ammonia electrode (9512HPBNWP or 9512BNWP) to the BNC connector on the meter.
2. Connect the ATC probe to the ATC connector on the meter.
3. Connect the automatic stirrer probe to the meter.
   a. If not using the automatic stirrer probe, use a magnetic stirrer and a stir bar to stir each standard and sample. Stirring is required for mixing the ISA into the sample and liberating ammonia from the sample. Stirring also provides the fastest response and best method performance.
4. Power up the meter, open Main Menu / Settings / Instrument Settings, set the Read Type to Continuous, and then tap the Back icon to return to Settings.
5. Select pH/Ion Settings. Set Measure Mode to “Ion”, ISE Type to “Ammonia (NH3)”, Ion Units to “mg/L”, and Ion Resolution to “3 digits”. Select Ion Standard Sets / Custom. Enter Standard 1 = “1”, Standard 2 = “10”, and Standard 3 = “100”. Set Ion Calibration Points to “3”, Stirrer to “On”, and Slope Type to “segmented”. Select Create Method and type in the desired method name, then tap Enter / Save / Load.
   a. Note about mg/L and ppm units. For Orion ammonia standard solutions, mg/L and ppm are equivalent. As mg/L units are generally preferred for reporting, we recommend choosing mg/L as the units during setup.

Temperature is an important factor when testing with an ISE. Best results are achieved when the calibration standards and samples are at the same temperature. Generally, within 1° or 2° C is recommended. The ATC readings are a simple way to monitor and document that standards and sample temperatures are at the same stable temperature during the calibration and testing.

User prepared solutions

- **User Prepared Standards:** Prepare the standards at the desired concentration by dilution of a 1000 mg/L (ppm) or 100 mg/L (ppm) ammonia standard with RGW. Use clean volumetric flasks, graduated cylinders, and pipets to prevent errors from cross contamination.
  - To prepare all standards from 1000 mg/L (ppm) standard
    1. Prepare a 100 mg/L standard. Using a clean graduated cylinder, measure 100 mL of 1000 mg/L standard and transfer into a clean 1000 mL volumetric flask. Dilute to the mark with RGW.
    2. Prepare a 10 mg/L standard. Pipet 10 mL of 1000 mg/L standard into a clean 1000 mL volumetric flask. Dilute to the mark with RGW.
    3. Prepare a 1.0 mg/L standard. Pipet 1.0 mL of 1000 mg/L standard into a clean 1000 mL volumetric flask. Dilute to the mark with RGW.

  - To prepare 10 and 1 mg/L standards from 100 mg/L (ppm) standard
    1. Prepare a 10 mg/L standard. Using a graduated cylinder, measure 100 mL of 100 mg/L standard and transfer into a clean 1000 mL volumetric flask. Dilute to the mark with RGW.
    2. Prepare a 1.0 mg/L standard. Pipet 10 mL of 100 mg/L standard into a clean 1000 mL volumetric flask. Dilute to the mark with RGW.

- **Short Term Storage Solution:** Pour 50 mL of the 1 mg/L standard into a 100 mL beaker. Add 1 mL of ammonia ISA with blue dye. (If using Orion alkaline reagent, add 0.5 mL) Label as “short term storage”. Prepare daily. Using this solution during the day can speed up the testing, reduce drift, and improve precision and accuracy.

Calibration

1. Prior to calibration, condition the ammonia electrode by placing in the short-term storage solution for about 15 minutes.
2. Prepare the standards for testing as follows: for each standard, pour 50.0 mL of the standard into a clean dry 100 mL beaker.
   a. Do not add ISA at this time.

3. Allow the standards to come to room temperature (RT), if they are not already at RT.
4. Check that your saved ammonia method name is displayed on the Measurement screen.
    a. If not, load the ammonia method. From the Main Menu, select Methods, and tap the Play icon to load the method of choice.
5. Select Calibrate to begin a three point calibration using the 1.0, 10, and 100 ppm (mg/L) standards.
    a. Remove the ammonia electrode from the short-term storage solution.
    b. Rinse the electrode, ATC, and stirrer with a generous amount of RGW before and after each standard reading. Gently shake to remove excess water drops. Do not touch or blot the ammonia membrane.
    c. Lower the electrode, ATC, and stirrer into the 1.0 mg/L calibration standard so that about 1 inch of the electrodes is immersed.
        i. Ensure there are no bubbles clinging to the membrane. If so, tap the electrode until the bubbles are released.
        ii. Note the temperature of the standards. If not at room temperature (RT), bring the standards to RT (+/- 1° or 2° C ideally) before proceeding with the calibration. ISE results are affected by changes in temperature.
    d. Add 1 mL of the ammonia ISA with blue dye.
    e. Press Start (or Continue) when measuring the second and third calibration points). If the automatic stirrer is connected, it will start automatically. If using a magnetic stirrer, start the stirrer.
    f. Start a 1 minute timer. Allow at least 1 minute for the standard to reach a stable reading. (“Reading stable” is displayed.)
        i. To ensure good results, it is important to wait at least 1 minute* for a truly stable calibration point. Do not rush the calibration.
        ii. A 9512HPBNWP ammonia electrode in good working order will typically take from 1 – 2 minutes to stabilize fully during a calibration from 1 to 100 mg/L and when coming from short term storage solution.
        iii. A 9512BNWP ammonia electrode may take longer (for example, 30 to 60 seconds longer) to stabilize fully[3]. Set the timer for 90 to 120 seconds, if needed to achieve full stability.
    g. After at least 1 minute and when the standard reading becomes stable, the meter will automatically display the value of 1.00 for the first standard or will display the Custom prompt for the drop down menu of the custom standard set.
        i. If the 1.00 value is displayed, press Next to accept.
        ii. If the Custom prompt is displayed, tap to open the drop down menu and select the standard value that matches your standard - in this case, the 1.0 value. Press Next to accept the value.
    h. Repeat steps b. through g. for the 10 and 100 (mg/L) standard.
        i. In step g., the value of 10 will automatically be displayed for the second standard and a value of 100 will automatically be displayed for the third standard.

i. After calibrating with the three standards, press Done to finish the calibration. The calibration is automatically logged into the meter.
j. Review the calibration summary. There should be three standards at 1.0, 10, and 100 mg/L, and the slopes should ideally be between -54 and -60 mV/decade for a well-functioning electrode.

6. If the slopes are good, return to Measure mode. Rinse as before and place the electrode in short-term storage solution for 5 minutes before proceeding.
    a. If the slopes are not acceptable, see the Electrode Performance Check section below.
7. Recommended calibration verification: Pour a fresh 1.0 or 10 ppm (mg/L) standard to verify the calibration. Add ISA and lower the clean electrode, ATC, and stirrer into the standard. Check and release bubbles. Set a timer, start the stirrer, and take the reading after 2 minutes. If desired, take another reading at 3 minutes to ensure a stable reading has been achieved.
    a. In general, expect results to be within +/- 10% of the expected value or within limits as determined by your quality department.
    b. If the calibration verification is not acceptable, take corrective actions.
        i. Review the meter calibration log to verify the calibration proceeded as expected. If not, check the standards, replace if needed, and recalibrate.
        ii. Check for drift by collecting mV readings in the low standard at 1 minute intervals until the results stabilize to +/- 0.5 mV/minute. Readings should ideally stabilize within 3 minutes (or within 4 minutes for 9512BNWP). If not, follow the Electrode Care steps in Table 3.
        iii. Consult the troubleshooting section of the Ammonia ISE user guide.
    c. Ensure a good calibration verification reading is achieved before proceeding with sample testing.
8. Proceed to sample preparation and analysis after a good calibration and good calibration verification.

Electrode performance check

The ammonia electrode determines the performance of the test method. Follow the electrode care and maintenance recommendations in Table 3 to help ensure the fastest and most reliable ammonia readings.

If the calibration slopes are not between -54 to -60 mV, check the slope according to the Checking Electrode Operation (slope check) procedure described in the electrode user manual.

1. Use the Orion 1000 ppm standard (or the Orion 0.1 M standard) to make the additions. During the procedure, check for drift by collecting mV readings at 1 minute intervals (after each addition) until the results stabilize. Ideally, results will stabilize within 2 - 3 minutes for the 9512HPBNWP (or within 4 minutes for the 9512BNWP).

2. A good slope during this check indicates that the electrode is working properly, so a poor slope during the calibration is a signal to check your calibration standards and calibration practices for issues.
3. If the electrode slope is low (< -54 mV) or the electrode drifts during this check (e.g., takes more than 3 or 4 minutes to stabilize to +/- 0.5 mV/minute), perform maintenance as described in Table 3 Best practices for ammonia electrode care. For more information, consult the troubleshooting section of the ammonia electrode manual.

Sample Preparation

1. Allow the samples to come to room temperature (RT).
2. Using a clean 50 mL graduated cylinder, measure 50 mL of the water sample and add to a clean 100 mL beaker.
3. The sample is ready for analysis.

Sample Analysis

1. Organize samples so that cleaner samples with lower levels of ammonia are analyzed first, followed by stronger samples with higher levels of ammonia.
2. Rinse electrode, ATC probe and stirrer with generous amounts of RGW and gently shake to remove excess water drops. Do not touch or blot the ammonia membrane.
3. Place all probes in sample and start the stirrer.
    a. Ensure there are no bubbles clinging to the membrane. If so, tap the electrode until the bubbles are released.
    b. Note the sample temperature. If it is not at RT (+/- 1 or 2°C ideally), allow to reach RT before proceeding.
4. Add 1 mL of ammonia ISA with blue dye.
5. Set the timer for 2 minutes and start timing.
    a. If using the 9512BNWP, allow extra response time, if needed to achieve full stability.
6. After 2 minutes, record the reading or save the reading by pressing Log/Export. Consider allowing another minute (3 minutes total or 4 minutes for the 9512BNWP) before recording the reading if the reading is still changing or is not as expected. To achieve a truly stable measurement, allow time for the electrode to respond fully.
7. After the measurement, rinse the electrode, ATC, and stirrer with generous amounts of RGW, and gently shake off excess water drops. Do not touch or blot the ammonia membrane.
8. Repeat steps 2 through 7 until all the samples have been measured.

9. For storage between samples or throughout the day, place the electrode into the prepared short-term storage solution.

After Sample Analysis

1. When testing is done for the day, rinse the electrode with a generous amount of RGW and place the electrode into the ammonia overnight storage solution.
2. Discard used ammonia standards after each use. Do not save for reuse.

Quality control (QC)

Recommended QC procedures may include: calibration verification (described above); analysis of a prepared or purchased QC sample; sample duplicates, and/or matrix spikes. Consult your Standard Operating Procedures (SOPs) or your quality department for requirements.

Ammonia ISE testing performance demonstration

For this user guide, a proficiency testing (PT) quality control certified reference material (QC CRM) standard was analyzed to demonstrate the Orion ammonia ISE method for testing ammonia in water. The QC CRM standard is formulated for simple nutrients testing and was purchased from an ISO 17034 certified reference material provider [4].

> Certified laboratories will typically participate in a proficiency testing study at least once a year. Test results within the study acceptance limits are needed for these laboratories to maintain their certification.

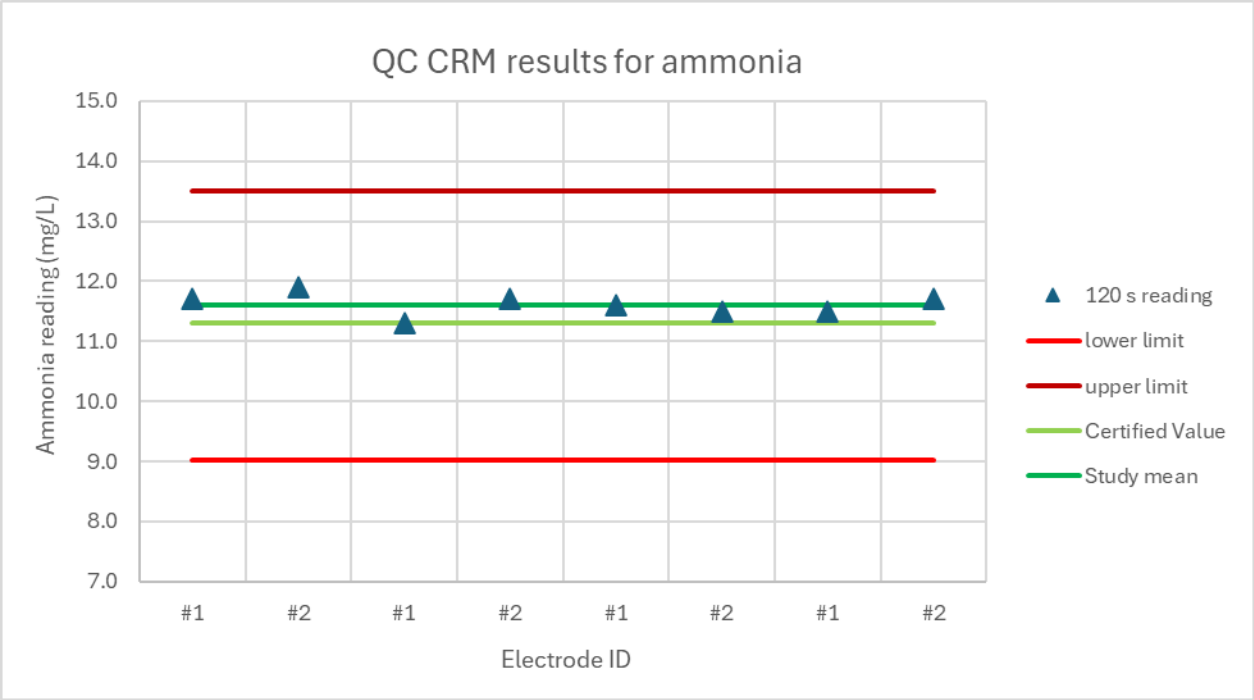
- Four replicates of the QC CRM were tested on two 9512HPBNWP ammonia ISEs for a total of eight measurements (n = 8).
- Reproducibility is good and the measured ammonia results are close to the certified concentration and the study mean value.
- All ammonia ISE results are well within the acceptance limits of the certificate of analysis.
- The analysis of ammonia in the QC CRM following this user guide indicates the suitability of the Orion ammonia ISE, meter, and solutions for this application.

QC CRM testing results according to this user guide are shown in Table 1 and Figure 1 below.

Table 1. QC CRM results for ammonia in wastewater			
QC Parameter	**Ammonia results (mg/L)***	**Certified concentration (mg/L)**	**Study mean concentration (mg/L)**
Average (n = 8)	11.6 ✓	11.3	11.6
Standard deviation	0.2 ✓	**Acceptance limits**	9.02 - 13.5

*readings at 120 seconds

Figure 1.



Wastewater sample testing results

Two 9512HPBNWP electrodes were used to test three synthetic wastewaters formulated to replicate primary and secondary effluents[5]. The calibration slopes were good, and the calibration verification readings were close to the expected values. The wastewater pH readings showed good repeatability and close agreement between the readings on the two electrodes. Relative standard deviations (RSD) amongst replicate sample tests were all within 3.3 % or better. Results are shown in Table 2.

Table 2. Performance of Orion 9512HPBNWP Ammonia ISE in wastewaters				
Calibration				
Average calibration slope	-58.4 mV/dec ✓			
Calibration Verification				
	Ammonia standard (mg/L)	**Average measured (mg/L)**	**%Recovery**	**Temp (°C)**
Before testing	1.0	1.0	100 % ✓	20.4
During testing	1.0	0.99	99 % ✓	20.3
During testing	10	9.6	96 % ✓	20.3
After testing	1.0	1.0	100 % ✓	20.3
After testing	10	10.7	107 % ✓	20.4
Wastewater Testing				
Wastewaters	**Primary effluent (mg/L)**	**Secondary effluent 1 (mg/L)**	**Secondary effluent 2 (mg/L)**	
N = # of readings	10	10	12	
Average mg/L*	20.8	1.16	1.53	
Standard deviation	0.5	0.03	0.05	
RSD	2.4 % ✓	2.6 % ✓	3.3 % ✓	
Temperature, °C	20.6	19.7	18.9	

*readings at 120 seconds

Hints and tips for ammonia testing in wastewater

- Saving the meter settings as a method simplifies the workflow and allows you to accept the correct standard values during calibration without the need to edit the values.
- Refer to the electrode user guide for details on cleaning, storage, and maintenance recommendations to keep the electrode performing well. Main points for electrode care are summarized below in Table 3 Best practices for ammonia electrode care.
- Consult the yellow insert titled “Tips for Optimizing Ammonia Measurements” that comes with the ammonia electrode for more useful hints.
- Conditioning the electrode in a portion of the low level standard for several minutes before calibration will speed up the calibration and improve stability and accuracy. Short-term storage in this solution during the day will also equilibrate the electrode, speed up sample results, and improve precision and accuracy.
- Ammonia standards are meant to be poured fresh before each use. Add ISA just before measuring each standard. Once a standard is tested, discard the standard, since ammonia is gradually lost from the solution after the addition of the ISA. Do not save standards for reuse.

- If needed, consult the Troubleshooting section of the ammonia electrode user manual for full details on symptoms and corrective actions.

Electrode Care

Refer to the electrode user manual for details on cleaning, storage, and maintenance recommendations to keep the electrode performing well. Main points for care are summarized below.

Table 3. Best practices for ammonia electrode care

Daily Care	Monthly Care	As Needed
•Remove the electrode from overnight storage solution and condition the electrode in short term storage solution (with ISA) for 15 minutes before calibration. •After testing is completed for the day, rinse the electrode thoroughly with RGW. •Soak 5 minutes in RGW before storage if electrode has become somewhat drifty or sluggish during testing or shows other signs of contamination on the membrane surface. •Store the clean electrode in Orion ammonia electrode storage solution 951213.	•Discard the used overnight storage solution. Replace with fresh ammonia electrode storage solution. •If the electrode fails the performance check due to low slope or drift (that is not resolved by soaking), change the membrane and fill solution.* •For long term storage (more than 1 or 2 weeks), dissasemble, drain electrode and rinse with RGW. Dry and reassemble the electrode without fill solution or membrane.	•Change membrane and replace fill solution. Disassemble, drain and rinse the electrode with RGW. Install a new membrane (or new preassembled body & membrane). Add fresh electrode filling solution and reassemble the electrode. Soak in electrode storage solution before using.* •If crystals form inside the electrode or the fill solution becomes discolored, drain and rinse the electrode. Replace the membrane and the filling solution.*

*See the electrode user manual for full details on all electrode care procedures, including disassembly and membrane replacement.

Ordering information

Product	Description	Cat. No.
Bench meter kit options	Orion Pro Star PI214 pH/ion bench meter ammonia kit w/ 9512HPBNWP ammonia electrode and ATC	PSTAR2146
	Orion Pro Star PI217 dual channel pH/ion bench meter pH and ammonia kit w/ electrodes (including 9512BNWP and ATC)	PSTAR2177
Solutions	Orion 1000 ppm ammonia as nitrogen standard	951007
	Orion 100 ppm ammonia as nitrogen standard	951207
	Orion ammonia ISA with blue dye	951211
	Alternate: Orion ammonia low level ISA with blue dye	951210
	Alternate: Orion alkaline reagent	951011
	Orion ammonia electrode filling solution	951209
	Orion ammonia electrode storage solution	951213
Other consumables	Orion pre-assembled outer body and membrane cap assembly for HP electrodes, 3 pack	951215
	Orion loose membranes	951214
Reagent Grade Water	Barnstead Smart2Pure 12 UV Water Purification System	50129890*

*Please contact your local Thermo Scientific™ representative for support to order a suitable water purification system for your application.

References

1) Standard Methods for the Examination of Water and Wastewater: 4500-NH3 Nitrogen (Ammonia), American Public Health Association (APHA), American Water Works Association (AWWA), Water Environment Federation (WEF), 24th edition, 2023.

2) Water Quality Control Handbook, , WEF Press, Alexandria, VA. 2nd edition, 2007. E. Roberts Alley, P.E. Chapter 3, p 3.12.

3) The Orion 9512BNWP is the original Orion design ammonia electrode and functions on the same principle as the 9512HPBNWP ammonia electrode. If desired, it can be used in place of the 9512HPBNWP at these ammonia concentrations (1 to 100 mg/L), but may have a somewhat longer response time. If so, allow extra time (for example, 30 to 60 seconds longer may be sufficient) for the electrode to respond and achieve full stability.

4) NSI Lab Solutions, QCI-138 Simple Nutrients QC CRM, lot N1123. Certified date: November 2, 2023.

5) Kargol AK, Burrell SR, Chakraborty I, Gough HL (2023) Synthetic wastewater prepared from readily available materials: Characteristics and economics. PLOS Water 2(9): e0000178. https://doi.org/10.1371/journal.pwat.0000178.

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