

Ammonium in Soils

Photometric determination using the indophenol blue method after extraction with calcium chloride solution

Introduction

Ammonium (NH_4^+) is one of the most important sources of nitrogen for plant nutrition and it is formed with the mineralization of decomposed organic matter.¹ Research also has established that ammonium is the naturally preferred source of nitrogen for plant growth.² With the increasing global demand for organic food products, farmers are resorting to the use of compost and manure. While ready-made ammonium fertilizers have defined concentrations of the cation and related compounds in them, it is important to analyze composts and manure for their accurate ammonium concentration. As the early phases of decomposition account for the loss of nitrogen as ammonia, and there is a natural loss of ammonium as the compost matures.³ Excess ammonium has also been reported to be toxic in higher plants.⁴

Experimental

This application note details the photometric determination of ammonium in soils, using the indophenol blue method, after its extraction with calcium chloride solution.

Method

Ammonium nitrogen ($\text{NH}_4\text{-N}$) occurs partly in the form of ammonium ions and partly as ammonia. A pH-dependent equilibrium exists between the two forms.

In strongly alkaline solution ammonium nitrogen is present almost entirely as ammonia, which reacts with a chlorinating agent to form monochloramine. This in turn reacts with a substituted phenol to form a blue indophenol derivative that is determined photometrically. Due to the intrinsic yellow coloration of the reagent blank, the measurement solution is yellow-green to green in color.

The method is analogous to EPA 350.1, APHA 4500-NH₃ F, ISO 7150-1, and DIN 38406-5.

Measuring Range

Spectroquant® Ammonium Cell Test (1.14739)	Test kit: 0.013–2.432 mg/L NH_4
	Method: 0.026–4.864 mg/kg NH_4
Spectroquant® Ammonium Cell Test (1.14558)	Test kit: 0.26–10.30 mg/L NH_4
	Method: 0.52–20.6 mg/kg NH_4
Spectroquant® Ammonium Cell Test (1.14544)	Test kit: 0.6–20.6 mg/L NH_4
	Method: 1.2–41.2 mg/kg NH_4
Spectroquant® Ammonium Cell Test (1.14559)	Test kit: 5.2–103.0 mg/L NH_4
	Method: 10.4–206 mg/kg NH_4
Spectroquant® Ammonium Test (1.14752)	Test kit: 0.013–3.86 mg/L NH_4
	Method: 0.026–7.72 mg/kg NH_4
Spectroquant® Ammonium Test (1.00683)	Test kit: 2.6–193 mg/L NH_4
	Method: 5.2–386 mg/kg NH_4

Applicable Sample

Soil samples

Reagents, Instruments and Materials

Test/Reagent Kit(s)

For the measurement one of the following Spectroquant® test kits is necessary:

- Spectroquant® Ammonium Cell Test (1.14739)
- Spectroquant® Ammonium Cell Test (1.14558)
- Spectroquant® Ammonium Cell Test* (1.14544)
- Spectroquant® Ammonium Cell Test (1.14559)
- Spectroquant® Ammonium Test (1.14752)
- Spectroquant® Ammonium Test (1.00683)

* not compatible with Move 100

Instrument(s) & Devices

For the measurement one of the following Spectroquant® photometers is necessary:

- Spectroquant® VIS Spectrophotometer Prove 100 plus (**1.73026**)
- Spectroquant® UV/VIS Spectrophotometer Prove 300 plus (**1.73027**)
- Spectroquant® UV/VIS Spectrophotometer Prove 600 plus (**1.73028**)
- Spectroquant® Colorimeter Move 100 (**1.73632**)

Note: Also, legacy Spectroquant® instruments are suitable.

Software for Data transfer

- Optional Spectroquant® Prove Connect to LIMS software package (**Y.11086**) to transfer your data into an existing LIMS system.

Instrument Accessories

- Rectangular cells 10 mm (**1.14946**)
- Rectangular cells 20 mm (**1.14947**)
- Rectangular cells 50 mm (**1.14944**)

Other Reagents and Accessories

- Calcium chloride dihydrate for analysis (**1.02382**)
- Water for analysis (**1.16754**)
- Charcoal activated for soil tests
- Analytical balance
- Standard laboratory glassware (e.g., glass beakers) and pipettes
- Spatula
- Stirring plate or shaker
- Drying kiln
- Folded filter

Analytical Procedure

Calcium chloride solution 0.025 mol/L

Dissolve 3.68 g calcium chloride dihydrate for analysis with 1 L of water for analysis.

Sample Preparation

- In a glass bottle mix 50 g of naturally moist sample, free from coarse stones, with 100 mL of the calcium chloride solution 0.025 mol/L.
- Add a spatula-tip full of charcoal activated for soil tests and shake the closed bottle in a shaking machine for 1 hour (alternative stir in a beaker).
- Let the suspension settle and filter it through a folded filter.
- For determination of the water content dry a similar sample to constant weight in the drying kiln at 105 °C.

Note: A drying of the sample before determination is not advisable because of the fast change in the nitrogen forms. The analysis should take place very quickly. Storing

the sample in plastic bags at room temperature changes the analysis values after a short time already.

Using Cat. No. 1.14739: Procedure and Measurement

For more information on the measurement see the packaging insert of the test

Procedure

- Pipette 5.0 mL Pretreated sample into a reaction cell, close the cell, and mix.
- Add 1 dose Reagent $\text{NH}_4\text{-1K}$, close the cell tightly, and shake vigorously until the reagent is completely dissolved.
- Leave to stand for 15 min (reaction time), then measure the sample in the photometer.

Note: White crystals may be present in the reaction cells. However, these dissolve when the sample is added and do not influence the measurement result.

Measurement

- For photometric measurement the cells must be clean. Wipe, if necessary, with a clean dry cloth.
- Measurement of turbid solutions yields false-high readings.
- Ammonium-free samples turn yellow on addition of reagent $\text{NH}_4\text{-1K}$.
- The pH of the measurement solution must be within the range 11.5–11.8.
- The color of the measurement solution remains stable for at least 60 min after the end of the reaction time stated above.
- In the event of ammonium concentrations exceeding 100 mg/L, other reaction products are formed, and false-low readings are yielded. In such cases it is advisable to conduct a plausibility check of the measurement results by diluting the sample (1:10, 1:100).

Hints for Measurement

- It is recommended to zero the method each new working day. To do this, open the method, either by manually selecting the method or by inserting a barcoded cell. Tap the <Settings> button and select the <ZERO ADJUSTMENT> menu item. After prompting, insert the 16 mm zero cell through the corresponding opening. The zero adjustment is performed automatically. Confirm the performance of the zero-adjustment procedure by clicking on <OK>.
- After the zero has been performed, insert the barcoded Spectroquant® round cell through the corresponding opening, ensuring that the white position mark on the cell is aligned with the positioning mark on the spectrophotometer. The measurement starts automatically.
- Read off the result in mg/L from the display.

Hint: The above written measurement description is only valid for the Spectroquant® Prove (plus) series

photometer. If a different instrument is used, please consult the corresponding instrument manual for more details on how to perform the measurement.

Using Cat. No. 1.14558: Procedure and Measurement

For more information on the measurement see the packaging insert of the test

Procedure

- Pipette 1.0 mL Pretreated sample into a reaction cell, close the cell, and mix.
- Add 1 dose Reagent $\text{NH}_4\text{-1K}$, close the cell tightly, and shake vigorously until the reagent is completely dissolved.
- Leave to stand for 15 min (reaction time), then measure the sample in the photometer.

Measurement

- For photometric measurement the cells must be clean. Wipe, if necessary, with a clean dry cloth.
- Measurement of turbid solutions yields false-high readings.
- Ammonium-free samples turn yellow on addition of reagent $\text{NH}_4\text{-1K}$.
- The pH of the measurement solution must be within the range 11.5–11.8.
- The color of the measurement solution remains stable for at least 60 min after the end of the reaction time stated above.
- In the event of ammonium concentrations exceeding 500 mg/L, other reaction products are formed, and false-low readings are yielded. In such cases it is advisable to conduct a plausibility check of the measurement results by diluting the sample (1:10, 1:100).

Hints for Measurement

- It is recommended to zero the method each new working day. To do this, open the method, either by manually selecting the method or by inserting a barcoded cell. Tap the <Settings> button and select the <ZERO ADJUSTMENT> menu item. After prompting, insert the 16 mm zero cell through the corresponding opening. The zero adjustment is performed automatically. Confirm the performance of the zero-adjustment procedure by clicking on <OK>.
- After the zero has been performed, insert the barcoded Spectroquant® round cell through the corresponding opening, ensuring that the white position mark on the cell is aligned with the positioning mark on the spectrophotometer. The measurement starts automatically.
- Read off the result in mg/L from the display.

Hint: The above written measurement description is only valid for the Spectroquant® Prove (plus) series photometer. If a different instrument is used, please consult the corresponding instrument manual for more details on how to perform the measurement.

Using Cat. No. 1.14544: Procedure and Measurement

For more information on the measurement see the packaging insert of the test

Procedure

- Pipette 0.50 mL Pretreated sample into a reaction cell, close the cell, and mix.
- Add 1 dose Reagent $\text{NH}_4\text{-1K}$, close the cell tightly, and shake vigorously until the reagent is completely dissolved.
- Leave to stand for 15 min (reaction time), then measure the sample in the photometer.

Measurement

- For photometric measurement the cells must be clean. Wipe, if necessary, with a clean dry cloth.
- Measurement of turbid solutions yields false-high readings.
- Ammonium-free samples turn yellow on addition of reagent $\text{NH}_4\text{-1K}$.
- The pH of the measurement solution must be within the range 11.5–11.8.
- The color of the measurement solution remains stable for at least 60 min after the end of the reaction time stated above.
- In the event of ammonium concentrations exceeding 1000 mg/L, other reaction products are formed, and false-low readings are yielded. In such cases it is advisable to conduct a plausibility check of the measurement results by diluting the sample (1:10, 1:100).

Hints for Measurement

- It is recommended to zero the method each new working day. To do this, open the method, either by manually selecting the method or by inserting a barcoded cell. Tap the <Settings> button and select the <ZERO ADJUSTMENT> menu item. After prompting, insert the 16 mm zero cell through the corresponding opening. The zero adjustment is performed automatically. Confirm the performance of the zero-adjustment procedure by clicking on <OK>.
- After the zero has been performed, insert the barcoded Spectroquant® round cell through the corresponding opening, ensuring that the white position mark on the cell is aligned with the positioning mark on the spectrophotometer. The measurement starts automatically.
- Read off the result in mg/L from the display.

Hint: The above written measurement description is only valid for the Spectroquant® Prove (plus) series photometer. If a different instrument is used, please consult the corresponding instrument manual for more details on how to perform the measurement.

Using Cat. No. 1.14559: Procedure and Measurement

For more information on the measurement see the packaging insert of the test

Procedure

- Pipette 0.10 mL Pretreated sample into a reaction cell, close the cell, and mix.
- Add 1 dose Reagent NH₄-1K, close the cell tightly, and shake vigorously until the reagent is completely dissolved.
- Leave to stand for 15 min (reaction time), then measure the sample in the photometer.

Measurement

- For photometric measurement the cells must be clean. Wipe, if necessary, with a clean dry cloth.
- Measurement of turbid solutions yields false-high readings.
- Ammonium-free samples turn yellow on addition of reagent NH₄-1K.
- The pH of the measurement solution must be within the range 11.5–11.8.
- The color of the measurement solution remains stable for at least 60 min after the end of the reaction time stated above.
- In the event of ammonium concentrations exceeding 5000 mg/L, other reaction products are formed, and false-low readings are yielded. In such cases it is advisable to conduct a plausibility check of the measurement results by diluting the sample (1:10, 1:100).

Hints for Measurement

- It is recommended to zero the method each new working day. To do this, open the method, either by manually selecting the method or by inserting a barcoded cell. Tap the <Settings> button and select the <ZERO ADJUSTMENT> menu item. After prompting, insert the 16 mm zero cell through the corresponding opening. The zero adjustment is performed automatically. Confirm the performance of the zero-adjustment procedure by clicking on <OK>.
- After the zero has been performed, insert the barcoded Spectroquant® round cell through the corresponding opening, ensuring that the white position mark on the cell is aligned with the positioning mark on the spectrophotometer. The measurement starts automatically.
- Read off the result in mg/L from the display.

Hint: The above written measurement description is only valid for the Spectroquant® Prove (plus) series photometer. If a different instrument is used, please consult the corresponding instrument manual for more details on how to perform the measurement.

Using Cat. No. 1.14752: Procedure and Measurement

For more information on the measurement see the packaging insert of the test

Procedure

- Add 0.60 mL Reagent NH₄-1 with pipette and mix.
- Add 1 level blue microspoon (in the cap of the bottle NH₄-2) Reagent NH₄-2 and shake vigorously until the reagent is completely dissolved. Leave to stand for 5 min (reaction time A).
- Add 4 drops Reagent NH₄-3 and mix. Hold the bottle vertically while adding the reagent!
- Leave to stand for 5 min (reaction time B), then fill the sample into the cell, and measure in the photometer.

Notes:

- For measurement in the 50 mm cell both the sample volume as well as the quantities of reagents NH₄-1, NH₄-2, and NH₄-3 must be doubled. Alternatively, the semi-microcell (**1.73502**) can be used.
- When using the 50-mm cell is recommended to measure against an own prepared blank sample (preparation as per measurement sample, but with distilled water instead of sample) to increase the accuracy. Configure the photometer for blank measurement.

Measurement

- Certain photometers may require a blank (preparation as per measurement sample, but with distilled water instead of sample).
- For photometric measurement the cells must be clean. Wipe, if necessary, with a clean dry cloth.
- Measurement of turbid solutions yields false-high readings.
- Ammonium-free samples turn yellow on addition of reagent NH₄-3.
- The pH of the measurement solution must be approx. 12.5.
- The color of the measurement solution remains stable for at least 60 min after the end of the reaction time B stated above.
- In the event of ammonium concentrations exceeding 100 mg/L, other reaction products are formed, and false-low readings are yielded. In such cases it is advisable to conduct a plausibility check of the measurement results by diluting the sample (1:10, 1:100).

Hints for Measurement

- It is recommended to zero the method for each new working day. To do this, open the method by inserting the barcode, tap the <Settings> button and select the <ZERO ADJUSTMENT> menu item. Fill the same cell which will be used for the sample measurement with distilled water. After prompting, insert the filled rectangular cell into the cell compartment. The zero adjustment is performed automatically. Confirm the performance of the zero-adjustment procedure by clicking on <OK>.
- After the zero adjustment, fill the measurement sample into the same or a matched rectangular cell and insert the cell into the cell compartment. The measurement starts automatically.
- Read off the result in mg/L from the display.

Hint: The above written measurement description is only valid for the Spectroquant® Prove (plus) series photometer. If a different instrument is used, please consult the corresponding instrument manual for more details on how to perform the measurement.

Using Cat. No. 1.00683: Procedure and Measurement

For more information on the measurement see the packaging insert of the test

Procedure measuring range 2.6–96.6 mg/L NH₄

- Pipette 5.0 mL Reagent NH₄-1 into a test tube.
- Add 0.20 mL Pretreated sample with pipette and mix.
- Add 1 level blue microspoon (in the cap of the NH₄-2 bottle) Reagent NH₄-2 and shake vigorously until the reagent is completely dissolved.
- Leave to stand for 15 min (reaction time), then fill the sample into a 10-mm cell, and measure in the photometer.

Procedure measuring range 6–193 mg/L NH₄

- Pipette 5.0 mL Reagent NH₄-1 into a test tube.
- Add 0.10 mL Pretreated sample with pipette and mix.
- Add 1 level blue microspoon (in the cap of the NH₄-2 bottle) Reagent NH₄-2 and shake vigorously until the reagent is completely dissolved.
- Leave to stand for 15 min (reaction time), then fill the sample into a 10-mm cell, and measure in the photometer.

Measurement

- Due to the strong temperature dependence of the color reaction, the temperature of the reagents should be between 20 and 30 °C.
- Certain photometers may require a blank (preparation as per measurement sample, but with distilled water instead of sample).
- For photometric measurement the cells must be clean. Wipe, if necessary, with a clean dry cloth.
- Measurement of turbid solutions yields false-high readings.
- Ammonium-free samples turn yellow on addition of reagent NH₄-2.
- The pH of the measurement solution must be within the range 11.5–11.8.
- The color of the measurement solution remains stable for at least 60 min after the end of the reaction time stated above.
- In the event of ammonium concentrations exceeding 2500 mg/l, other reaction products are formed, and false-low readings are yielded. In such cases it is advisable to conduct a plausibility check of the measurement results by diluting the sample (1:10, 1:100).

Hints for Measurement

- It is recommended to zero the method for each new working day. To do this, open the method by inserting the barcode, tap the <Settings> button and select the <ZERO ADJUSTMENT> menu item. Fill the same cell which will be used for the sample measurement with distilled water. After prompting, insert the filled rectangular cell into the cell compartment. The zero adjustment is performed automatically. Confirm the performance of the zero-adjustment procedure by clicking on <OK>.
- After the zero adjustment, fill the measurement sample into the same or a matched rectangular cell and insert the cell into the cell compartment. The measurement starts automatically.
- Read off the result in mg/L from the display.

Hint: The above written measurement description is only valid for the Spectroquant® Prove (plus) series photometer. If a different instrument is used, please consult the corresponding instrument manual for more details on how to perform the measurement.

Analytical Quality Assurance

Analytical quality assurance (AQA) is recommended before each measurement series.

To check the photometric measurement system (test reagent, measurement device, handling) and the mode of working, Spectroquant® ammonium standard solutions or CombiChecks (see section 5 of the respective test kit instruction) can be used. Besides a standard solution, CombiChecks also contains an addition solution for determining sample-dependent interferences (matrix effects).

Sample-dependent interferences (matrix effects) can be determined by means of standard addition or dilution.

To view additional notes, visit

[SigmaAldrich.com/qa-test-kits](https://sigmaaldrich.com/qa-test-kits).

Calculation

Ammonium content in mg/kg NH₄ = analysis value in mg/L NH₄ x 2

References

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2. Ganesh, S.; Khan, F.; Ahmed, M. K.; Velavendan, P.; Pandey, N. K.; Kamachi Mudali, U. Spectrophotometric Determination of Trace Amounts of Phosphate in Water and Soil. *Water Sci. Technol.* **2012**, 66 (12), 2653–2658. DOI: 10.2166/wst.2012.468.
3. Wanke, D. J.; Heichel, J.; Zikeli, S.; Müller, T.; Hartmann, T. E. Comparison of Soil Phosphorus Extraction Methods Regarding Their Suitability for Organic Farming Systems. *J. Plant Nutr. Soil Sci.* **2023**, 186, 599–608. DOI: 10.1002/jpln.202300129.
4. Britto, D. T.; Kronzucker, H. J. NH₄ + Toxicity in Higher Plants: A Critical Review. *J. Plant Physiol.* **2002**, 159 (6), 567–584. DOI: 10.1078/0176-1617-0774.

Featured Products

Description	Cat. No.
Test Kits	
Spectroquant® Ammonium Cell Test	1.14739
Spectroquant® Ammonium Cell Test	1.14558
Spectroquant® Ammonium Cell Test	1.14544
Spectroquant® Ammonium Cell Test	1.14559
Spectroquant® Ammonium Test	1.14752
Spectroquant® Ammonium Test	1.00683
Instruments & Accessories	
Spectroquant® VIS Spectrophotometer Prove 100 plus	1.73026
Rectangular cells 10 mm	1.14946
Rectangular cells 20 mm	1.14947
Rectangular cells 50 mm	1.14944
Reagents	
Calcium chloride dihydrate for analysis	1.02382
Water for analysis	1.16754

Related Products

Description	Cat. No.
Instruments & Software	
Spectroquant® UV/VIS Spectrophotometer Prove 300 plus	1.73027
Spectroquant® UV/VIS Spectrophotometer Prove 600 plus	1.73028
Spectroquant® Colorimeter Move 100	1.73632
Spectroquant® Prove Connect to LIMS software package	Y.11086
Reference Materials	
Spectroquant® CombiCheck 50	1.14695
Spectroquant® CombiCheck 20	1.14675
Spectroquant® CombiCheck 70	1.14689
Spectroquant® CombiCheck 10	1.14676
Spectroquant® Ammonium standard solution, 6.00 mg/L NH ₄ -N	1.25025
Spectroquant® Ammonium standard solution, 12.0 mg/L NH ₄ -N	1.25026
Spectroquant® Ammonium standard solution, 50.0 mg/L NH ₄ -N	1.25027
Spectroquant® Ammonium standard solution, 0.400 mg/L NH ₄ -N	1.25022
Spectroquant® Ammonium standard solution, 1.00 mg/L NH ₄ -N	1.25023
Spectroquant® Ammonium standard solution, 2.00 mg/L NH ₄ -N	1.25024
Spectroquant® Ammonium standard solution, 0.250 mg/L NH ₄ -N	1.32227
Reagents, Test Strips, & Accessories	
Semi-microcells 50 mm (2 pcs)	1.73502
MQuant® Ammonium Test, measuring range 10–400 mg/L NH ₄	1.10024
Sodium hydroxide solution 1.000 L Combi-Titrisol®, 5 mol/L	1.09913
MQuant® Universal indicator strips pH 0–14	1.09535
Sodium hydroxide solution 1 mol/L Titripur®	1.09137
Sulfuric acid 0.5 mol/L Titripur®	1.09072

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