

Nitrite in Soils

Photometric determination using the Griess Method After Extraction with a Calcium Chloride Solution

Introduction

Nitrite is an anion with formula NO_2^- and a rich source of nitrogen found in soil, for plants. In the intermediate step of nitrification, ammonia (NH_3) is aerobically oxidized first to nitrites.¹ Nitrites are found in soil amended with composts matured to be used to improve crop yield. It has been studied that nitrites get accumulated in composts after the exudence of ammonia, when only ammonia-oxidizing bacteria get proliferated, with the natural eradication of nitrite oxidizing bacteria (NOB).² Nitrite accumulation leads to nitrous oxide (N_2O), a greenhouse gas³ emitted from soil. It is important to analyze compost-amended soils for nitrite, to mitigate nitrous oxide emission and the consequential ill-impact on the environment.

Experimental

This application note details the photometric determination of nitrite in soils using the Griess method, after extraction with a calcium chloride solution.

Method

In acidic solution nitrite ions react with sulfanilic acid to form a diazonium salt, which in turn reacts with *N*-(1-naphthyl) ethylenediamine dihydrochloride to form a red-violet azo dye. This dye is determined photometrically.

The method is analogous to EPA 354.1, APHA 4500- NO_2^- -B, DIN EN 26777, and ISO 6777.

Measuring Range

Spectroquant® Nitrite Cell Test (1.14547)	Test kit: 0.03–2.30 mg/L NO_2^- Method: 0.06–4.60 mg/kg NO_2^-
Spectroquant® Nitrite Test (1.14776)	Test kit: 0.007–3.28 mg/L NO_2^- Method: 0.014–6.56 mg/kg NO_2^-

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® Nitrite Cell Test (1.14547)
- Spectroquant® Nitrite Test (1.14776)

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

Reagent Preparation

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 a 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.
- A drying of the sample before the determination is not advisable because of the fast change in the nitrogen forms.

Note: 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. 1.14547: Procedure and Measurement

For more information on the measurement, see the packaging insert for the test.

Procedure

- Pipette 5.0 mL Pretreated sample into a reaction cell, close the cell tightly, and shake vigorously until the reagent is completely dissolved
- Leave to stand for 10 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.

- The pH of the measurement solution must be within the range 2.0–2.5.
- The color of the measurement solution remains stable for at least 60 min after the end of the reaction time stated above.

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. 1.14776: Procedure and Measurement

For more information on the measurement, see the packaging insert for the test.

Procedure

- Pipette 5.0 mL Pretreated sample into a test tube
- Add 1 level blue microspoon (in the cap of the NO₂⁻ 1 bottle) Reagent NO₂⁻1 and shake vigorously for 1 minute until the reagent is almost completely dissolved. The pH must be within the range 2.0–2.5. Check with MQuant® pH-indicator strips. Adjust the pH, if necessary, with sodium hydroxide solution or sulfuric acid.
- Leave to stand for 10 min (reaction time), then fill the sample into a cell, and measure in the photometer.

Notes: For measurement in the 50 mm cell both the sample volume as well as the quantity of reagent NO₂⁻1 must be doubled. Alternatively, the semi-microcell (1.73502) can be used. It 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.
- A minimum bottom sediment may be formed occasionally due to undissolved reagent. This has no influence on the functionality of the test kit. While transferring the measuring solution into the measuring cell, make sure that it remains in the test tube, in order to avoid influences on the result.
- Measurement of turbid solutions yields false-high readings.
- The pH of the measurement solution must be within the range 2.0–2.5.
- The color of the measurement solution remains stable for at least 60 min after the end of the reaction time stated above.

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® nitrite standard solution (see section 5 of the respective test kit instruction) can be used.

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

Nitrite content in mg/kg NO₂ = analysis value in mg/L NO₂ x 2

References

1. Beeckman, F.; Motte, H.; Beeckman, T. Nitrification in Agricultural Soils: Impact, Actors and Mitigation. *Curr. Opin. Biotechnol.* **2018**, *50*, 166–173. DOI: [10.1016/j.copbio.2018.01.014](https://doi.org/10.1016/j.copbio.2018.01.014).
2. Fukumoto, Y.; Inubushi, K. Effect of Nitrite Accumulation on Nitrous Oxide Emission and Total Nitrogen Loss during Swine Manure Composting. *Soil Sci. Plant Nutr.* **2009**, *55* (3), 428–434. DOI: [10.1111/j.1747-0765.2009.00376.x](https://doi.org/10.1111/j.1747-0765.2009.00376.x).
3. Wilson, T.; Warren, J.; Arnal, B. *Nitrous Oxide Emissions from Soil*; Oklahoma State University Extension, 2017. <https://extension.okstate.edu/fact-sheets/nitrous-oxide-emissions-from-soil> (accessed 2024-08-08).

Featured Products

Description	Cat. No.
Test Kits	
Spectroquant® Nitrite Cell Test	1.14547
Spectroquant® Nitrite Test	1.14776
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	
Nitrite standard solution, 0.200 mg/L NO ₂ ⁻ N	1.25041
Reagents Test Strips & Accessories	
MQuant® Nitrite Test, measuring range 0.5–10 mg/L NO ₂ ⁻ (0.15–3.0 mg/L NO ₂ ⁻ N)	1.10057
MQuant® Universal indicator strips pH 0–14	1.09535
MQuant® pH-indicator strips pH 0–6.0	1.09531
Sulfuric acid 0.5 mol/L Titripur®	1.09072
Sodium hydroxide solution 1 mol/L Titripur®	1.09137
Semi-microcells 50 mm (2 pcs)	1.73502

For more information visit us at SigmaAldrich.com/photometry



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