

Nitrogen (Total) in Soils

Photometric determination subsequent to extraction with calcium chloride solution

Introduction

Plants require nitrogen as a nutrient for their healthy growth and development, and nitrogen is imperative for protein synthesis. Three major sources of nitrogen for plants are ammonia (NH_3), nitrates (NO_3^-), and nitrites (NO_2^-). Extensively, ammonia and nitrates present in fertilizer and manure-amended soil function as major nitrogen suppliers for plants. One parameter of soil health for growing crops is the concentration of total nitrogen and hence it is important to test total nitrogen in soils. The Kjeldahl method, or Kjeldahl digestion is a widely used analytical method to determine organic and ammonium nitrogen.¹ In this application note, we detail an alternative, quantitative photometric determination of total nitrogen test of soils (not just organic and ammonium nitrogen) with either dimethyl phenol (DMP) or a benzoic acid derivative, depending on the used test kit, after extraction with calcium chloride solution and digestion by Koroleff's method.

Experimental

This application note details the photometric determination of total Nitrogen in soils after extraction and sample digestion.

Method

Organic and inorganic nitrogen compounds are transformed into nitrate according to Koroleff's method by treatment with an oxidizing agent in a thermoreactor.

For test 1.14537: In concentrated sulfuric acid, this nitrate reacts with a benzoic acid derivative to form a red nitro compound that is determined photometrically.

The digestion is analogous to DIN EN ISO 11905-1.

For tests 1.14763 and 1.00613: In a solution acidified with sulfuric and phosphoric acid, this nitrate reacts with 2,6-dimethylphenol (DMP) to form 4-nitro-2,6-dimethylphenol that is determined photometrically.

The method (digestion and determination) corresponds to ISO 23697-1.

The digestion is analogous to EN ISO 11905-1.

The determination of nitrate is analogous to DIN 38405-9.

Measuring Range

Spectroquant® Nitrogen (total) Cell Test (1.14537)	Test kit: 0.5–15.0 mg/L N Method: 1.0–30.0 mg/kg N
Spectroquant® Nitrogen (total) Cell Test (1.00613)	Test kit: 0.5–15.0 mg/L N Method: 1.0–30.0 mg/kg N
Spectroquant® Nitrogen (total) Cell Test (1.14763)	Test kit: 10–150 mg/L N Method: 20–300 mg/kg N

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® Nitrogen (total) Cell Test (**1.14537**)
- Spectroquant® Nitrogen (total) Cell Test (**1.00613**)
- Spectroquant® Nitrogen (total) Cell Test (**1.14763**)

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.

For the digestion, one of the following devices is necessary:

- Spectroquant® Thermoreactor TR 320 (**1.71200**)
- Spectroquant® Thermoreactor TR 420 (**1.71201**)
- Spectroquant® Thermoreactor TR 620 (**1.71202**)

Software for Data transfer

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

Other Reagents and Accessories

- Calcium chloride dihydrate for analysis (**1.02382**)
- Water suitable for UV/Vis spectroscopy, EMSURE® (**1.16754**)
- Analytical balance
- Standard laboratory glassware (e.g., glass beakers) and pipettes
- Shaker or stirring plate
- Spatula
- Drying kiln
- Charcoal activated for soil test
- Folded filter

Analytical Procedure

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

Calcium chloride solution 0.025 mol/L

Dissolve 3.68 g calcium chloride dihydrate 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.

Using Cat. No. 1.14537: Procedure and Measurement

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

Procedure

- Pipette 10 mL Pretreated sample into an empty cell.
- Add 1 level blue microspoon (in the cap of the N-1K bottle) Reagent N-1K and mix.
- Add 6 drops Reagent N-2K, close cell tightly and mix. Hold the bottle vertically while adding the reagent.
- Heat the cell at 120 °C in the preheated thermoreactor for 1 hour.
- Allow the closed cell to cool to room temperature in a test-tube rack. Do not cool with cold water!
- Shake the cell briefly after 10 min. (Turbidity or precipitation frequently occurs in the digestion solution.)
- Place 1 level microspoon (in the cap of the N-3K bottle) Reagent N-3K into a reaction cell, immediately close the cell tightly, and shake vigorously for 1 min.
- Very slowly and carefully allow 1.5 mL digested, cooled sample (use clear supernatant or filtrate in the event of turbidity or precipitation) to run from the pipette down the inside of the tilted reaction cell onto the reagent (Wear eye protection! The cell becomes hot!). Immediately close the cell tightly and mix briefly. The cell must be held only by the screw cap!
- Leave the hot cell to stand for 10 min (reaction time). Do not cool with cold water!
- 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 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. 1.00613: Procedure and Measurement

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

Procedure

- Pipette 10 mL Pretreated sample into an empty cell.
- Add 1 level blue microspoon (in the cap of the N-1K bottle) Reagent N-1K and mix.
- Add 6 drops Reagent N-2K, close cell tightly and mix. Hold the bottle vertically while adding the reagent.
- Heat the cell at 120 °C in the preheated thermoreactor for 1 hour.
- Allow the closed cell to cool to room temperature in a test-tube rack. Do not cool with cold water!
- Shake the cell briefly after 10 min. (Turbidity or precipitation frequently occurs in the digestion solution.)
- Pipette 1.0 mL digested, cooled sample (use clear supernatant or filtrate in the event of turbidity or precipitation) into a reaction cell. Do not mix content!
- Add 1.0 mL Reagent N-3K with a pipette, close the cell tightly and mix (Wear eye protection! The cell becomes hot! The cell must be held only by the screw cap!)
- Leave the hot cell to stand for 10 min (reaction time). Do not cool with cold water!
- 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 color of the measurement solution remains stable for 30 min after the end of the reaction time stated above. (After 60 mins the measurement value would have increased by 5%.)

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. 1.14763: Procedure and Measurement

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

Procedure

- Pipette 1 mL Pretreated sample into an empty cell.
- Add 9 mL water (recommended is **1.16754**, or use distilled water).
- Add 1 level blue microspoon (in the cap of the N-1K bottle) Reagent N-1K and mix.
- Add 6 drops Reagent N-2K, close cell tightly and mix. Hold the bottle vertically while adding the reagent.
- Heat the cell at 120 °C in the preheated thermoreactor for 1 hour.
- Allow the closed cell to cool to room temperature in a test-tube rack. Do not cool with cold water!
- Shake the cell briefly after 10 min. (Turbidity or precipitation frequently occurs in the digestion solution.)
- Pipette 1.0 mL digested, cooled sample (use clear supernatant or filtrate in the event of turbidity or precipitation) into a reaction cell. Do not mix content!
- Add 1.0 mL Reagent N-3K with a pipette, close the cell tightly and mix (Wear eye protection! The cell becomes hot! The cell must be held only by the screw cap!)
- Leave the hot cell to stand for 10 min (reaction time). Do not cool with cold water!
- 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 color of the measurement solution remains stable for 30 min after the end of the reaction time stated

above. (After 60 mins the measurement value would have increased by 5%.)

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.

Analytical Quality Assurance

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

To check the photometric measurement system (test reagents, measurement device, handling) and the mode of working, the nitrogen (total) standard solutions (see section 5 of the respective packaging insert) or Spectroquant® CombiCheck 50 (**1.14695**) or CombiCheck 70 (**1.14689**) respectively can be used. Besides a standard solution with 5.0 mg/L N or 50 mg/L N, respectively, 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.

Calculation

Total Nitrogen content in mg/kg N = analysis value in mg/L N x 2

References

1. Nelson, D. W.; Sommers, L. E. Total Nitrogen Analysis of Soil and Plant Tissues. *J. AOAC* **1980**, 63 (4), 770–778. DOI: [10.1093/jaoac/63.4.770](https://doi.org/10.1093/jaoac/63.4.770).

Featured Products

Description	Cat. No.
Test Kits	
Spectroquant® Nitrogen (total) Cell Test	1.14537
Spectroquant® Nitrogen (total) Cell Test	1.00613
Spectroquant® Nitrogen (total) Cell Test	1.14763
Instruments & Accessories	
Spectroquant® VIS Spectrophotometer Prove 100 plus	1.73026
Spectroquant® Thermoreactor TR 320	1.71200
Reagents	
Calcium chloride dihydrate for analysis	1.02382
Water suitable for UV/Vis spectroscopy, EMSURE®	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
Spectroquant® Thermoreactor TR 420	1.71201
Spectroquant® Thermoreactor TR 620	1.71202
Reference Materials	
Spectroquant® CombiCheck 50	1.14695
Spectroquant® CombiCheck 70	1.14689
Nitrogen (total) standard solution, 2.50 mg/L N	1.25043
Nitrogen (total) standard solution, 12.0 mg/L N	1.25044
Nitrogen (total) standard solution, 100 mg/L N	1.25045
Reagents & Accessories	
MQuant® Chloride Test, measuring range 500–3000 mg/L Cl ⁻	1.10079
Spectroquant® COD Cell Test, measuring range 25–1500 mg/L	1.14541
Spectroquant® COD Cell Test, measuring range 300–3500 mg/L	1.14691
Spectroquant® COD Cell Test measuring range 5000–90 000 mg/L	1.01797
Empty cells 16 mm with screw caps (25 pcs)	1.14724

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