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Soil quality — Determination of nitrate nitrogen, ammonium nitrogen and total soluble nitrogen in air-dry soils using calcium chloride solution as extractant

Qualité du sol — Détermination de l'azote nitrique, de l'azote ammoniacal et de l'azote soluble total dans les sols séchés à l'air en utilisant le chlorure de calcium comme solution d'extraction

Reference number
ISO 14255:1998(E)

Foreword

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International Standard ISO 14255 was prepared by Technical Committee ISO/TC 190, *Soil quality*, Subcommittee SC 3, *Chemical methods and soil characteristics*.

Annexes A and B of this International Standard are for information only.

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Soil quality — Determination of nitrate nitrogen, ammonium nitrogen and total soluble nitrogen in air-dry soils using calcium chloride solution as extractant

1 Scope

This International Standard describes a method for the determination of soluble nitrogen fractions (nitrate, nitrite, ammonium and organic nitrogen) in a 0,01 mol/l calcium chloride extract of soil samples.

It is applicable to air-dry soil samples, pretreated in accordance with ISO 11464.

2 Normative references

The following standards contain provisions which, through reference in this text, constitute provisions of this International Standard. At the time of publication, the editions indicated were valid. All standards are subject to revisions, and parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 3696:1987, *Water for analytical laboratory use — Specification and test methods*.

ISO 11464:1994, *Soil quality — Pretreatment of samples for physico-chemical analyses*.

ISO 11465:1993, *Determination of dry matter and water content on a mass basis — Gravimetric method*.

3 Principle

By means of a simple extraction with a 0,01 mol/l calcium chloride ($CaCl_2$) solution of air-dry soil samples, different soluble nitrogen fractions are extracted (see references [1] and [2]). The concentrations of the inorganic nitrogen compounds nitrate (+ nitrite) and ammonia are measured directly in the extract using automated spectrometric methods.

For the determination of total soluble nitrogen, part of the extract is first digested whereafter the produced ammonia is converted to nitrate. This, together with the originally present nitrate (+ nitrite) and ammonia, is then measured using an automated spectrometric method. The content of soluble organic nitrogen is calculated by subtracting the content of nitrate (+ nitrite) and ammonium nitrogen from the content of soluble total nitrogen.

NOTES

1 The content of soluble organic nitrogen may give an indication of the easily mineralizable fraction of the organic matter.

2 The content of extractable ammonium and organic nitrogen of the soil is changed very often by drying, compared to the fresh soil sample. The way of drying affects the results (see reference [3]).

3 Since manual determinations for the different nitrogen fractions cause problems, in this International Standard measurements are specified using automated continuous flow techniques. Other ways of detection are permissible provided the results are in agreement with the results achieved by the specified automated procedures.

4 Laboratory samples

The fraction of the air-dry soil samples, pretreated in accordance with ISO 11464, which has passed a sieve of 2 mm aperture shall be used. Part of this sample shall be used to determine the water content in accordance with ISO 11465.

5 Extraction

5.1 Principle

Air-dry soil is extracted with a 0,01 mol/l calcium chloride ($CaCl_2$) solution in a 1:10 ratio (*m*/*V*) at 20 °C ± 1 °C. After reaching equilibrium (2 h), the solution is centrifuged for the determination of the different nitrogen fractions.

NOTES

1 The extraction is performed at a constant temperature of 20 °C because the amounts of extractable ammonium nitrogen and soluble organic nitrogen are affected by the temperature of the extracting solution (see reference [4]). For special climatic conditions, another constant temperature may be chosen. The used extraction temperature should be mentioned in the test report.

2 Centrifuging is preferred because most filter papers either absorb substances or may be contaminated by ammonia. When the extracts are to be filtered, the filter papers should be stored in a desiccator over concentrated sulfuric acid or a drying medium containing sulfuric acid, one week at least before use, and the first 20 ml of the filtrate should be discarded.

5.2 Reagents

Use only reagents of recognized analytical grade.

5.2.1 Water, with a specific conductivity not higher than 0,2 mS/m at 25 °C (water according to grade 2 of ISO 3696).

5.2.2 Calcium chloride solution, $c(CaCl_2) = 0{,}01$ mol/l, for the extraction.

Dissolve 1,47 g of calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$) in water (5.2.1). Dilute with water to make 1 litre.

NOTE — $CaCl_2 \cdot 2H_2O$ may absorb water on standing. The reagent should be standardized.

5.3 Apparatus and glassware

5.3.1 Analytical balance, with an accuracy of 10 mg.

5.3.2 Polyethylene bottles, of nominal volume 250 ml, with screwcaps.

5.3.3 Shaking machine or reciprocating shaker, 150 r/min to 250 r/min.

5.3.4 Centrifuge, capable of holding the tubes used.

5.3.5 Polyethylene centrifuge tubes, of nominal volume 100 ml or other sufficient volume.

5.4 Procedure

Weigh 10,00 g of the laboratory sample into a polythene bottle (5.3.2).

Add 100,0 ml of calcium chloride solution (5.2.2) at a temperature of 20 °C. Shake mechanically (5.3.3) for 2 h at 20 °C.

Decant the necessary quantity of the extract suspension into centrifuge tubes (5.3.5) and centrifuge (5.3.4) for 10 min at about 3 000 *g*. Decant the supernatant solution in measuring cups and measure the contents of nitrate (+ nitrite), nitrite (if necessary), ammonium and total soluble nitrogen, as described in clauses 6, 7, 8 and 9 respectively.

Perform a blank test by adding only the calcium chloride solution (5.2.2) to the polythene bottle (5.3.2).

The soluble nitrogen fractions should be measured immediately, but not later than one day, after the extraction. If this is impossible, the extracts should be stored in a refrigerator at temperatures not exceeding 4 °C, for at maximum one week.

6 Determination of nitrate nitrogen

6.1 Principle

In a segmented flow analysis (SFA) system, the sample is first subjected to dialysis. Nitrate and nitrite ions from the samples pass in the membrane and are taken in an understream of ammonium chloride. The nitrate is then reduced to nitrite by means of cadmium. Next, α-naphthylethylenediamine dihydrochloride and sulfanilamide are added, so that a red-coloured diazo compound is formed in the acid medium. Its absorbance is measured at a wavelength of 543 nm.

NOTES

1 The dialysis serves to separate nitrate ions from interfering substances such as solid particles, colloids and coloured organic compounds.

2 The colour reagent is known as the Griess-Ilosvay reagent.

Normally, the content of nitrite in soils is negligibly small. In these cases, the sum of nitrate and nitrite corresponds to the content of nitrate. In special cases, when nitrification in the soils seems to be incomplete, the content of nitrite has to be determined separately.

6.2 Reagents

6.2.1 Wetting agent, polyoxyethylene lauryl ether (30 %).

NOTE — The wetting agent solution should not be stored for longer than 6 months.

6.2.2 Buffer solution

Dissolve, in a 1 litre volumetric flask, 25 g of ammonium chloride (NH_4Cl) in a little water. Add 12,5 ml of ammonium hydroxide solution [$w(NH_4OH) = 3$ %] and 1 ml of wetting agent (6.2.1). Make up to the mark with water and mix.

The pH of this solution should be between pH 6 and pH 8.

6.2.3 Cd/Cu reducing agent

Swirl approximately 5 g of cadmium powder (particle size 0,3 mm to 0,8 mm) for 1 min with about 30 ml of hydrochloric acid [$c(HCl) = 1$ mol/l]. Wash with water until acid free. Then add about 50 ml of a copper (II) sulfate solution [$\rho(CuSO_4 = 20$ g/l] and swirl for 3 min. Wash at least 10 times with water to remove any flocculated copper. Store the Cd/Cu reducing agent in a dark place.

6.2.4 Cd/Cu reduction tube

Fill the U-shaped column (6.3.2) with buffer solution (6.2.2), taking care not to introduce air bubbles. Introduce the activated cadmium powder (6.2.3) with the aid of a funnel on both sides of the column. Apply vibration now and then to pack the powder. Fill the column up to 5 mm from the top of the column and seal the ends with a small plug of glass wool.

The column is now ready for use and can be placed in the SFA system.

NOTE — To lengthen the lifetime of the Cd/Cu reducing agent, a copper wire should be installed in the beginning of the Cd/Cu reduction tube, and renewed if necessary.

6.2.5 Colour reagent

In a 1 litre volumetric flask, add 150 ml of concentrated phosphoric acid [$w(H_3PO_4) = 85$ %] to 0,5 l of water. Add 0,5 g of α-naphthylethylenediamine dihydrochloride ($C_{12}H_{16}N_2Cl_2$) and swirl until dissolved. Then dissolve 10 g of sulfanilamide ($C_6H_8N_2O_2S$) in this mixture and make up to the mark with water.

6.3 Apparatus

6.3.1 Segmented flow analysis system

The system consists of a sampler, pump, dialysis unit, reduction column, nitrate unit, photometer and recorder. The flow diagram of an appropriate segmented flow analysis (SFA) system is given in annex A (figure A.1). Note that these flow diagrams may require adaptation, depending on the system used.

6.3.2 Cd/Cu reduction tube

This comprises U-shaped glass tubing, about 15 cm long and with internal diameter of 2 mm, provided with ferrules for connection to the SFA tubing. It may be purchased filled with cadmium from the SFA system manufacturer, or be home-made (see 6.2.3 and 6.2.4).

NOTE — This SFA arrangement allows determination of nitrite by simply shortcutting the reduction column (see annex A, figure A.2).

7 Determination of ammonium nitrogen

7.1 Principle

In a segmented flow analysis (SFA) system, the sample is first subjected to dialysis. The determination of ammonium is based on the Berthelot reaction [5, 6], in which a phenol derivative (here, salicylate) forms an indophenol in the presence of ammonia and hypochlorite under the catalytic action of sodium nitroferricyanide (nitroprusside). In alkaline medium, the indophenol thus formed has a green-blue colour, the absorbance of which is measured at a wavelength of 660 nm.

NOTE — Dialysis of the soil extract is necessary because turbidity may exist in the soil centrifugates.

7.2 Reagents

7.2.1 Buffer solution, pH 5,2

Dissolve, in a 1 litre volumetric flask, 24 g of sodium citrate ($Na_3C_6H_5O_7$) and 33 g of sodium potassium tartrate ($KNaC_4H_4O_6$). Make up to the mark with water. Add 1 ml of wetting agent (6.2.1).

7.2.2 Colour reagent

In a 1 litre volumetric flask containing about 800 ml of water, dissolve 80 g of sodium salicylate ($C_7H_5O_3Na$) and 25 g of sodium hydroxide (NaOH). Make up to the mark with water.

7.2.3 Nitroferricyanide (nitroprusside) solution

Dissolve 1 g of sodium nitroferricyanide dihydrate, $Na_2[Fe(CN)_5NO] \cdot 2H_2O$, in 1 litre of water.

7.2.4 Isocyanurate solution

Dissolve 2 g of sodium dichloroisocyanurate ($Cl_2C_3N_3NaO_3 \cdot 2H_2O$) and 25 g of sodium hydroxide (NaOH) in 1 litre of water.

7.3 Apparatus

7.3.1 Segmented flow analysis system

The system consists of a sampler, pump, dialysis unit, ammonium unit, photometer and recorder. The flow diagram of an appropriate segmented flow analysis system is given in annex A (figure A.3). Note that these flow diagrams may require adaptation, depending on the system used.

8 Digestion and determination of total soluble nitrogen

8.1 Principle

In a segmented flow analysis (SFA) system, the organic compounds in the extract are digested at pH 4 by potassium persulfate ($K_2S_2O_8$). Thereafter, ammonium ions originally present and those formed by the digestion are converted to nitrate by potassium persulfate oxidation, catalysed by UV radiation. The sample is subjected to dialysis and, finally, nitrate is determined colorimetrically after reduction to nitrite (for details see clause 6).

NOTES

1 Dialysis of the soil extract is necessary because turbidity may exist in the soil centrifugates.

2 Contents of organic carbon in the extracts greater than about 50 ml/l may exceed the pH buffering capacity of the system. In such cases the extracts should be diluted before the measurement.

8.2 Reagents

8.2.1 Borax buffer solution

In a 1 litre volumetric flask containing about 200 ml of water, dissolve 38 g of sodium tetraborate decahydrate ($Na_2B_4O_7 \cdot 10H_2O$). Add 4 g of sodium hydroxide (NaOH) and make up to the mark with water. **Never add wetting agent**.

8.2.2 Oxidizing agent

Dissolve, in a 1 litre volumetric flask containing about 500 ml of water, 52 g of potassium persulfate ($K_2S_2O_8$) and make up to the mark with water. Mix and leave this solution for 24 h in a refrigerator. Then decant the clear supernatant solution for use. **Never add wetting agent**.

8.2.3 Buffer solution

Dissolve, in a 1 litre volumetric flask, 25 g of ammonium chloride (NH_4Cl) in water and make up to the mark with water. Add 1 ml ammonium hydroxide solution (NH_4OH) (25 %), and 1 ml wetting agent (6.2.1) and mix well.

8.2.4 Cd/Cu reductor

See 6.2.4.

8.2.5 Colour reagent

In a 1 litre volumetric flask, dilute 150 ml of phosphoric acid [$w(H_3PO_4) = 85$ %] carefully in about 800 ml of water. Add 10 g sulfanilamide ($C_6H_8N_2O_2S$), 1 g ethylenediaminetetraacetic acid disodium salt (EDTA) ($C_{10}H_{14}N_2O_8Na_2 \cdot 2H_2O$) and 0,5 g *N*-(1-naphthyl)ethylenediamine dihydrochloride ($C_{12}H_{16}Cl_2N_2$) and dissolve. Make up to the mark with water.

8.3 Apparatus

8.3.1 Segmented flow analysis system

The system consists of a sampler, pump, dialysis unit, total soluble nitrogen unit, photometer and recorder. The flow diagram of an appropriate segmented flow analysis (SFA) system is given in annex A (figure A.4). Note that these flow diagrams may require adaptation, depending on the system used.

8.3.2 Reduction column

See 6.3.2.

9 Calibration series

9.1 Reagents

9.1.1 Calcium chloride solution, $c(CaCl_2) = 0,1$ mol/l

Dissolve 14,7 g calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$) in water and dilute with water to 1 litre.

9.1.2 Mixed stock solution, $\rho(NO_3\text{–}N) = 375$ mg/l, $\rho(NH_4\text{–}N) = 200$ mg/l

Weigh out 2,708 3 g of potassium nitrate (KNO_3) and 0,943 9 g of ammonium sulfate [$(NH_4)_2SO_4$]. Dissolve them in water in a 1 litre volumetric flask and make up to the mark with water.

The salts have to be dried to a constant mass at, for example, 150 °C before use.

NOTE — A mixed stock solution (6.2.7) is used because NO_3–N (+NO_2–N), NH_4–N and soluble organic N are determined in one run.

9.2 Procedure

Pipette 0 ml, 0,5 ml, 1,0 ml, 1,5 ml and 2,0 ml of the mixed stock solution (9.1.2) into 250 ml volumetric flasks. Add 25 ml of the calcium chloride solution (9.1.1) and make up to the mark with water. This calibration series should be prepared freshly before use. They have the concentrations given in table 1.

Table 1 — Concentrations of calibration series

N-fraction	Concentration mg/l				
NO_3–N	0	0,75	1,50	2,25	3,00
NH_4–N	0	0,40	0,80	1,20	1,60
Total soluble N	0	1,15	2,30	3,45	4,60

Use the centrifugates prepared as in clause 5. Place the soil extracts, the blank extracts and the calibration series in the automatic sampler. Operate the segmented flow system according to the flow diagrams given in annex A (or according to the manufacturer's specification).

Plot the calibration curves and record the different nitrogen concentrations.

10 Calculation and expression of results

The content of the different nitrogen fractions in the soil material (w_N), expressed milligrams per kilogram, is calculated using equation (1):

$$w_N = (a - b) \times 10 \times (100 + w)/100 \quad (1)$$

where

a is the content of NO_3–N, NH_4–N and total soluble nitrogen in the soil extract, in milligrams per litre;

b is the content of NO_3–N, NH_4–N and total soluble nitrogen in the blank extract, in milligrams per litre;

w is the percentage water content (*m*/*m*) on the basis of the air-dry soil, determined in accordance with ISO 11465.

11 Test report

The test report shall contain the following information:

a) a reference to this International Standard;

b) complete identification of the sample;

c) the results of the determination;

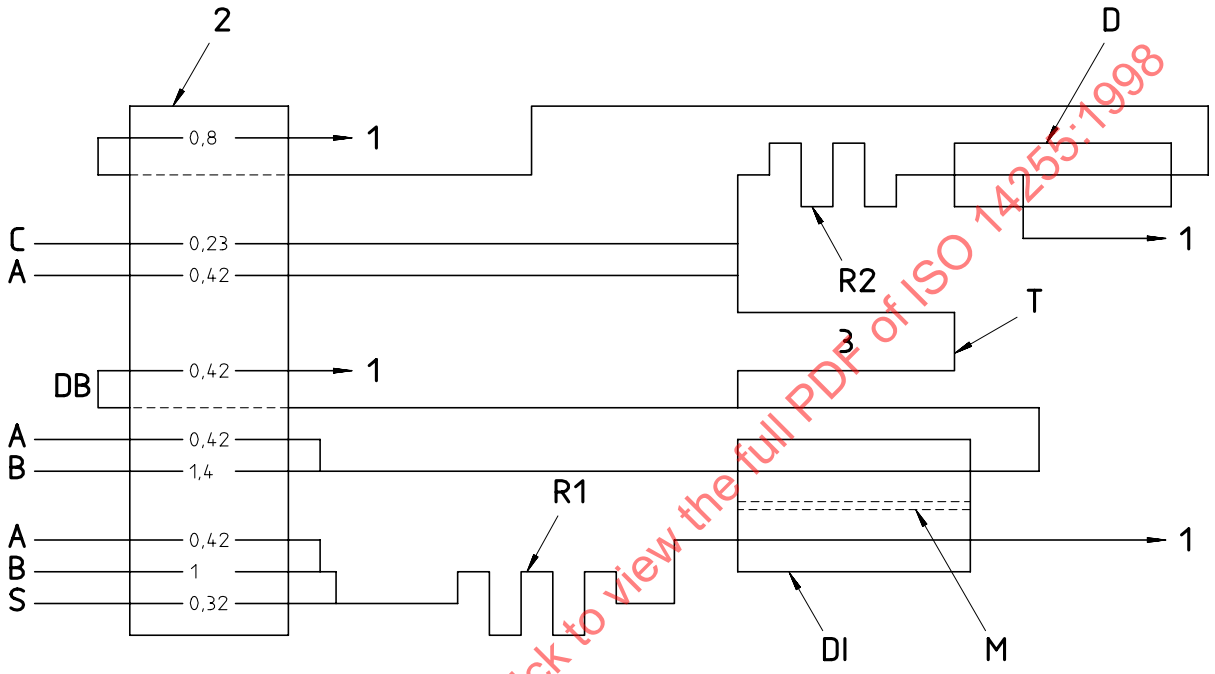
d) any details not specified in this International Standard, or which are optional, as well as any factor which may have affected the results (e.g. temperature of the extracting solution).

Annex A
(informative)

Examples of segmented flow analysis systems

See figures A.1 to A.4

Values in millilitres per minute

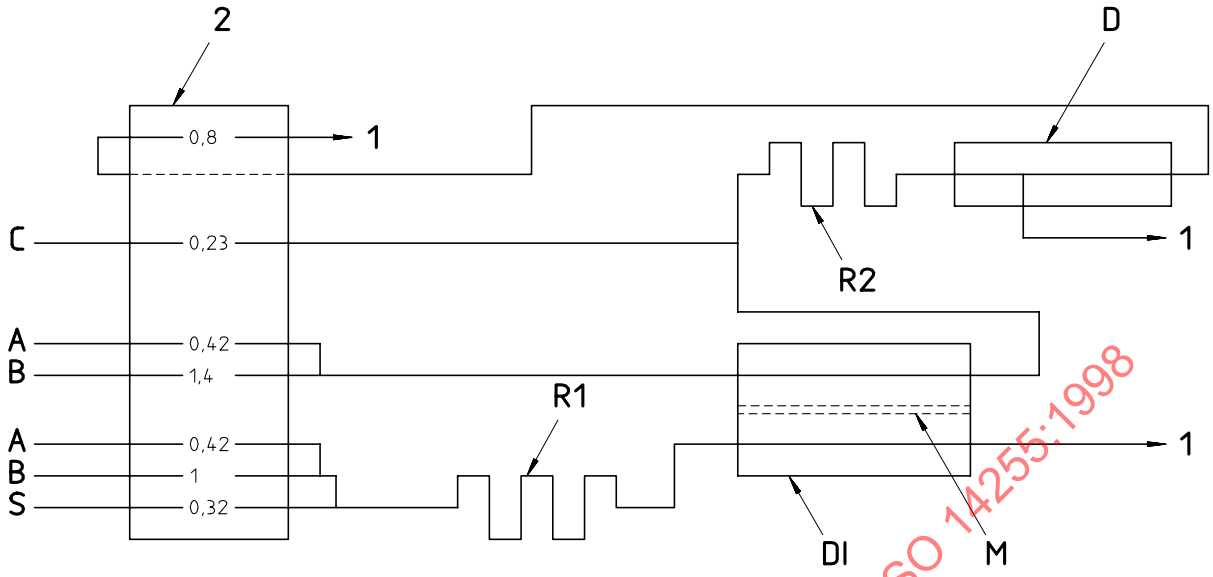


1 Waste
2 Pump
3 Nitrate reduction

S: Extract solution
B: Buffer solution (6.2.2)
A: Segmenting air
DB: Debubbling
C: Colour reagent (6.2.5)
R1: Reaction coil 1, length = 40 cm (5 turns), internal diameter = 1,5 mm
R2: Reaction coil 2, length = 150 cm (20 turns), internal diameter = 1,5 mm
DI: Dialyser, groove length = 15 cm
M: Dialysis membrane
T: Reduction tube, U-shaped, length = 15 cm, internal diameter = 2 mm, filled with copperized cadmium powder (6.2.4)
D: Detector, flow cell 50 mm, filter 540 nm

Figure A.1 — Example of a segmented flow analysis system for the determination of the sum of nitrate and nitrite nitrogens (clause 6)

Values in millilitres per minute

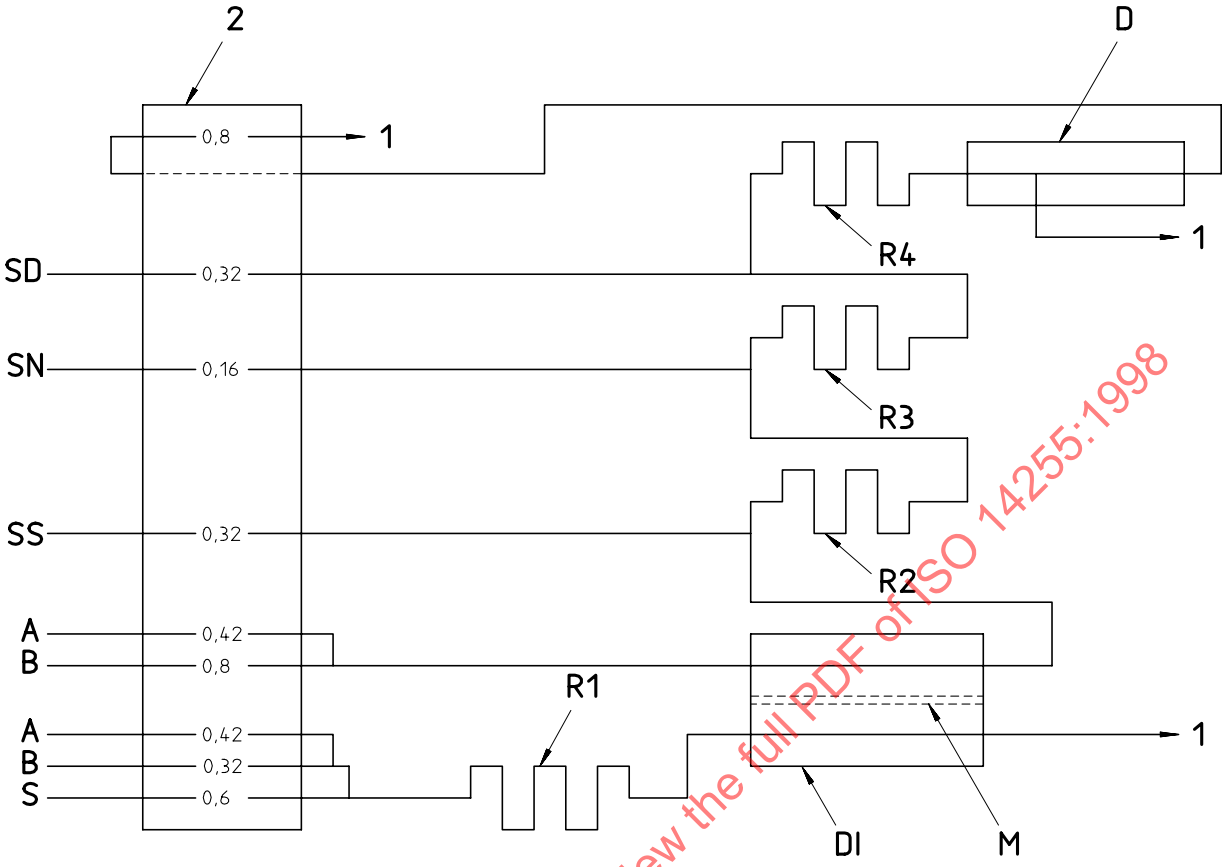


1 Waste
2 Pump

S: Extract solution
B: Buffer (6.2.2)
A: Segmenting air
C: Colour reagent (6.2.5)
R1: Reaction coil 1, length = 40 cm (5 turns), internal diameter = 1,5 mm
R2: Reaction coil 2, length = 150 cm (20 turns), internal diameter = 1,5 mm
DI: Dialyser, groove length = 15 cm
M: Dialysis membrane
D: Detector, flow cell 50 mm, filter 540 nm

Figure A.2 — Example of a segmented flow analysis system for the determination of nitrite nitrogen
(see NOTE in 7.1)

Values in millilitres per minute



1 Waste
2 Pump

S: Extract solution
B: Buffer solution (7.2.1)
A: Segmenting air
SS: Colour reagent, sodium salicylate solution (7.2.2)
SN: Sodium nitroprusside solution (7.2.3)
SD: Sodium dichloroisocyanurate solution (7.2.3)
R1, R2, R3: Reaction coils, length = 40 cm (5 turns), internal diameter = 1,5 mm
R4: Reaction coil 4, length = 370 cm (50 turns), internal diameter = 1,5 mm, with a constant temperature of 40 °C
DI: Dialyser, groove length = 30 cm
M: Dialysis membrane
D: Detector, flow cell 50 mm, filter 660 nm

Figure A.3 — Example of a segmented flow analysis system for the determination of ammonium nitrogen
(clause 7)