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Water quality — Determination of ammonium nitrogen in water — Small-scale sealed tube method

Qualité de l'eau — Détermination de l'azote ammoniacal dans l'eau — Méthode à petite échelle en tubes fermés

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Contents

Page

Foreword .... **iv**

Introduction .... **v**

1 Scope **1**

2 Normative references **1**

3 Terms and definitions **2**

4 Principle **2**

5 Interferences **2**

6 Sampling and sample preparation **3**

7 Reagents **4**

8 Apparatus **4**

9 Reaction conditions for the colour reaction **4**
- 9.1 General 4
- 9.2 Salicylate based reaction 5
- 9.3 Chlorophenol based reaction 5

10 Quality control **5**
- 10.1 Verification of the calibration and acceptability criteria 5
- 10.2 System and interferences checks 6

11 Calculation **6**

12 Expression of results **7**

13 Test report **7**

Annex A (informative) **Principle of alternative distillation** **8**

Annex B (informative) **Performance data** **11**

Bibliography **12**

Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 2, *Physical, chemical and biochemical methods*.

Any feedback or questions on this document should be directed to the user’s national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

Ammonium nitrogen is one of the possible forms of nitrogen present in water. It can come directly from industrial or public waste, from fertilizers or can be generated by the microbial breakdown of urea and proteins under anaerobic conditions. Ammonium nitrogen can also be found naturally in water in aquifers with low flow velocities, which are confined and contain organic matter.

Water quality — Determination of ammonium nitrogen in water — Small-scale sealed tube method

WARNING — Persons using this document should be familiar with normal laboratory practice. This document does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user to establish appropriate safety and health practices.

IMPORTANT — It is absolutely essential that tests conducted in accordance with this document be carried out by suitably qualified staff.

1 Scope

This document specifies a method for the determination of ammonium nitrogen (NH_4-N) in drinking water, groundwater, surface water, wastewater, bathing water and mineral water using the small-scale sealed tube method. The result can be expressed as NH_4 or NH_4-N or NH_3 or NH_3-N.

NOTE 1 In the habitual language use of sewage treatment and on the displays of automated sealed-tube test photometers or spectrophotometers, NH_4 without indication of the positive charge has become the common notation for the parameter ammonium. This notation is adopted in this document even though not being quite correct chemical nomenclature.

This method is applicable to (NH_4-N) concentration ranges from 0,01 mg/l to 1 800 mg/l of NH_4-N. The measuring ranges of concentration can vary depending on the type of small-scale sealed tube method of different manufacturers. Concentrations even slightly higher than the upper limit indicated in the manufacturers manual relating to the small-scale sealed tube method used, cannot be reported as accurate results. It is up to the user to choose the small-scale sealed tube test with the appropriate application range or to adapt samples with concentrations exceeding the measuring range of a test by preliminary dilution.

NOTE 2 The results of a small-scale sealed tube are most precise in the middle of the application range of the test.

All manufacturers' methods are based on the Berthelot reaction and its modifications to develop indophenol blue colour. Reagents mixtures can differ slightly based on manufacturers small-scale sealed tube method, see Clause 9. This method is applicable to non-preserved samples by using small-scale sealed tubes for the determination of drinking water, groundwater, surface water, wastewater and to preserved samples. The method is applicable to samples with suspended materials if these materials are removable by filtration.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 5664, *Water quality — Determination of ammonium — Distillation and titration method*

ISO 5667-1, *Water quality — Sampling — Part 1: Guidance on the design of sampling programmes and sampling techniques*

ISO 5667-3, *Water quality — Sampling — Part 3: Preservation and handling of water samples*

ISO 5667-10, *Water quality — Sampling — Part 10: Guidance on sampling of waste water*

ISO 8466-1, *Water quality — Calibration and evaluation of analytical methods — Part 1: Linear calibration function*

ISO/IEC 17025, *General requirements for the competence of testing and calibration laboratories*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminology databases for use in standardization at the following addresses:

- ISO Online browsing platform: available at https://www.iso.org/obp
- IEC Electropedia: available at https://www.electropedia.org/

3.1
small-scale sealed tube
glass tube commercially available on the market prefilled by different manufacturers with reagent(s) to develop a colour to be read by a photometer or spectrophotometer

4 Principle

This method is based on the principle of Berthelot reaction and its modification to develop indophenol blue colour. In strongly alkaline solution, ammonia reacts with dichloroisocyanurate and salicylate ions or chlorophenol ions, forming blue indophenol. The presence of sodium nitroprusside as catalyst promotes the development of the blue colour. Small-scale sealed tube is read on a photometer or spectrophotometer.

The method serves to obtain the concentration of ammonium nitrogen present in the sample.

5 Interferences

Typical interferences are due to both cations and anions. Examples for maximum tolerable concentration values are listed in Table 1. These values refer to both small-scale sealed tube methods applicable at high concentrations and to those applicable at low concentrations of NH_4-N.

Every manufacturer of small-scale sealed tubes shall provide information about interference levels above which the ion interferes. The concentration of interfering substances can depend on the ratio of sampled volume and predosed reagents, in the small-scale sealed tube.

Table 1 — Example of interfering ions

Ion	Maximum tolerable concentration mg/l
Cl^-	1 000
SO_4^{2-}	1 000
K^+	500
NO_3^-	250
Na^+	500
CO_3^{2-}	50
Ca^{2+}	500
Cu^{2+}	50
Cr^{3+}	50
Co^{2+}	50
Zn^{2+}	50

Table 1 *(continued)*

Ion	Maximum tolerable concentration mg/l
Cr(VI)	50

Primary amines can react under analysis conditions and lead to overestimated NH_4-N values. All reducing substances interfere and lead to underestimated results. The presence of urea is tolerated up to concentrations which are 10 000 times greater than those of the ammonia present in the sample to be analysed.

Turbidity, colour and high salinity levels can interfere both in the instrumental measurement and in the speed of colour development.

As specified in ISO 5667-3, preliminary filtration is necessary using filters with 0,45 µm pore width. Filtration has to be performed on-site. Filtration shall be carried out under pressure and not under vacuum, to avoid ammonia leakage. Before filtering, the filter shall be washed with water with the same pH as the sample. Generally, it can be done with the sample itself by discarding the first millilitres of sample, already filtered.

When measuring a reagent blank value, follow the normal procedure but use pure water (7.1) instead of sample.

When measuring a blank value caused by a coloured sample, add the sample to a small-scale sealed tube but do not add further reagents. Then follow the normal procedure.

In some cases, interferences can be eliminated using appropriate dilutions such that interfering ions fall below the maximum tolerable concentration and NH_4-N remains within the measurement range.

It is the laboratory's responsibility to identify interferences by, for example, spiking with reference material containing known amounts of ammonium nitrogen to the sample containing the suspected ion or to a solution of known concentration of the suspected ion.

In case of higher concentration of interfering substances, highly coloured wastewater or turbid suspension which cannot be easily filtered or diluted as described above, proceed with a distillation as described in ISO 5664. An alternative method for distillation is described in Annex A.

6 Sampling and sample preparation

For methods of sampling and storing samples before analysis, proceed according to ISO 5667-1, ISO 5667-3 and ISO 5667-10.

Persons performing the analysis shall be aware of the risks associated with the method.

It shall be noted that, in the case of acidification of unknown samples, toxic gases such as HCN and H_2S can form. Work in a fume cupboard.

Non-preserved samples shall be analysed as soon as possible, in accordance with ISO 5667-3. Longer storage periods require stabilization by acidification with sulfuric acid to pH < 2; if the sample is acidified, before proceeding with the determination, the pH shall be brought within the range indicated in the procedure for the test being used. When stabilizing samples, make sure to use ammonium-free reagents. Store samples in the refrigerator as described in ISO 5667-3.

Due to the differences in used small-scale sealed tube between all the manufacturers it is recommended to follow manufacturers' manuals.

7 Reagents

7.1 Water, the concentration of nitrogen in the used water shall be below 25 % of the lower measurement range of the used sealed tube.

7.2 Ammonium chloride, NH_4Cl, of analytical grade, previously dried at 105 °C for 2 h, to be used for preparing suitable solutions to verify the calibration in the NH_4-N concentration range. Commercially available ammonium chloride solutions, with known concentration, can also be used as reference material.

7.3 Reagents provided by the manufacturers for the small-scale sealed tube method and used to determine NH_4-N with colour reactions (see Clause 9).

7.3.1 Sodium dichloroisocyanurate, $C_3Cl_2N_3NaO_3$.

7.3.2 Sodium salicylate, $C_7H_5NaO_3$.

7.3.3 Sodium nitroprusside, $Na_2[Fe(CN)_5NO]$.

7.3.4 Chlorophenol, $(Cl)C_6H_4OH$.

7.3.5 Sodium hydroxide, $NaOH$.

7.3.6 Lithium hydroxide, $LiOH$.

8 Apparatus

Usual laboratory apparatus and, in particular, the following.

8.1 Small-scale sealed tubes, for different measuring ranges of NH_4-N concentrations: low, medium, high and very high.

8.2 Photometer or spectrophotometer, for reading small-scale sealed tubes at a wavelength according to colour reactions given in 9.2 or 9.3.

NOTE Usually, an automated instrument has calibration curves required for routine use stored in the memory and can also record new ones.

8.3 Calibrated pipettes and micropipettes of various volumes, accuracy class A.

8.4 Drying oven, to keep the temperature at (105 ± 5) °C to dry reference material.

9 Reaction conditions for the colour reaction

9.1 General

Determination is performed on non-preserved sample in a pH range specified in the following method. On preserved sample adjust pH as described in Clause 5 to reach the pH range of the following method.

Two different reactions are available to gain both blue indophenol colour (9.2 and 9.3).

9.2 Salicylate based reaction

In strongly alkaline solution, adjusted to pH > 12 with sodium hydroxide (7.3.5) or lithium hydroxide (7.3.6), ammonia reacts with dichloroisocyanurate and salicylate ions, present in the reagent, forming blue indophenol. The presence of sodium nitroprusside as catalyst promotes the development of the blue colour in the small-scale sealed tube (see Table 2 for the mass fraction percentage range).

Sample pH should be between 4 and 9.

The intensity of the colour is measured at absorption maximum. The absorption spectrum has two peaks and can be red at a wavelength of (694 ± 10) nm or (550 ± 10) nm or (585 ± 10) nm or (650 ± 10) nm depending on the concentration range (see manufacturers manuals).

Table 2 — Reagents in the small-scale sealed tubes given as mass fraction including sample, for the salicylate-based reaction

Reagent name	Mass fraction percentage range
Sodium dichloroisocyanurate (7.3.1)	0,004 to 0,05
Sodium salicylate (7.3.2)	0,1 to 5
Sodium nitroprusside (7.3.3)	0,01 to 0,2

9.3 Chlorophenol based reaction

In strongly alkaline solution, adjusted to pH > 12 with sodium hydroxide (7.3.5) or lithium hydroxide (7.3.6), ammonia reacts with dichloroisocyanurate and chlorophenol ions, present in the reagent, forming blue indophenol. The presence of sodium nitroprusside as catalyst promotes the development of the blue colour in the small-scale sealed tube (see Table 3 for the mass fraction percentage range).

Sample pH should be between 4 and 13.

The intensity of the colour is measured at absorption maximum, at a wavelength of (660 ± 10) nm or (690 ± 10) nm.

Table 3 — Reagents in the small-scale sealed tubes given as mass fraction including sample, for phenol substitute-based reaction

Reagent name	Mass fraction percentage range
Sodium dichloroisocyanurate (7.3.1)	0,008 to 0,05
Chlorophenol (7.3.4)	0,01 to 0,1
Sodium nitroprusside (7.3.3)	0,05 to 0,5

It is important to keep in mind that the colour development that occurs in the reactions is heavily influenced by the temperature.

In order to obtain reliable data, the sample and the small-scale sealed tubes shall be between a temperature of 15 °C and 25 °C, at the time when the reaction takes place.

10 Quality control

10.1 Verification of the calibration and acceptability criteria

In general, the photometer or spectrophotometer (8.2) for reading the small-scale sealed tubes are calibrated by the manufacturer and the corresponding curves are saved. In addition, the instrument has a program for calculating the concentration of the analyte from the absorbance measurement of the sample being analysed through the parameters of the stored calibration curve.

Verify the curve parameters when the instrumentation is set up. For this purpose, the acceptance of the calibration curve shall be evaluated using reference material as quality control solution of different concentrations within the calibration range and a blank. This shall be repeated whenever the manufacturer modifies the stored curve (information about calibration changes shall be communicated by manufacturers) and/or the analyte concentration calculation program or as described in ISO/IEC 17025.

The users establish their own reference material levels (diluted from 7.2) and acceptance criteria in accordance with the statistical variability provided by the photometer or spectrophotometer manufacturer, or in accordance with current standards or their specific requirements. If the data are acceptable, the photometer or spectrophotometer can be used. Otherwise, recalibration or appropriate maintenance and recalibration are necessary.

Each laboratory is responsible for system check including used reference material, frequency and chosen concentration levels of reference material.

It is also possible to create a calibration curve starting from a standard (7.2) and dilute it to achieve the desired calibration points (at least five shall be prescribed in accordance with ISO 8466-1), and a blank in the measuring range. Proceed to read the small-scale sealed tubes as given in 9.1. It is recommended to store the calibration curves on the photometer or spectrophotometer.

10.2 System and interferences checks

It is recommended, because of potential variability in manufacturers practices of photometer or spectrophotometer, method followed, reagents used, that the user should verify the small-scale sealed tubes before use, by using a diluted reference material (from 7.2) to accept the chosen range.

To check main potential interference substances, it is recommended to run spiked known concentration of reference material or known concentration of interference ion on sample. To check ammonia recovery, it is recommended to run a number of samples representative of laboratory activity in terms of repetitions and levels.

It is up to the laboratory to define the criteria concerning quality control frequency. The acceptance criteria selected by the laboratory shall be compatible with the declared uncertainty.

11 Calculation

The concentration of NH_4-N or NH_4 or NH_3 or NH_3-N in the test sample is calculated using Formulae (1), (2), (3) and (4):

$$C_{\mathrm{NH_4-N}} = \rho \times d \tag{1}$$

$$C_{\mathrm{NH_4}} = \rho \times d \tag{2}$$

$$C_{\mathrm{NH_3}} = \rho \times d \tag{3}$$

$$C_{\mathrm{NH_3-N}} = \rho \times d \tag{4}$$

where

$C_{\mathrm{NH4\text{-}N}}$ is the concentration of ammonium nitrogen, expressed in mg/l;

C_{NH4} is the concentration of ammonium in the sample, expressed in mg/l;

C_{NH3} is the concentration of ammonia in the sample, expressed in mg/l;

$C_{NH3\text{-}N}$ is the concentration of ammonia nitrogen in the sample, expressed in mg/l;

ρ is the concentration read on the photometer or spectrophotometer and expressed in mg/l;

d is the dilution factor, which takes into account both the need to bring the concentration within the method's scope of application and any addition for pH correction.

If the analysis is performed with a commercially available automated instrument, the concentration ρ of NH_4-N or NH_4 or NH_3 or NH_3-N is provided by the same instrument by setting the dilution factor.

Concentrations ρ even slightly higher than the upper limit of the application range indicated in the manufacturer's manual for the small-scale sealed tube applied shall not be used for calculation of results or be reported as such.

If it is necessary to convert data, use the following conversion factors of Table 4.

Table 4 — Conversion of read values

Readings	$C_{NH4\text{-}N}$ mg/l	C_{NH4} mg/l	C_{NH3} mg/l	$C_{NH3\text{-}N}$ mg/l
1 mg/l NH_4-N	1	1,288	1,216	1
1 mg/l NH_4	0,776	1	0,944	0,776
1 mg/l NH_3	0,822	1,059	1	0,822
1 mg/l NH_3-N	1	1,288	1,216	1

12 Expression of results

The analysis results obtained when applying this document are subject to a measurement uncertainty that is to be considered in the interpretation of the results.

Results shall be reported to a maximum of 3 significant figures.

EXAMPLE

$C_{NH4\text{-}N}$ = 0,125 mg/l

$C_{NH4\text{-}N}$ = 1,36 mg/l

$C_{NH4\text{-}N}$ = 12,3 mg/l

13 Test report

The test report shall contain at least the following information:

a) the test method used, together with a reference to this document, i.e. ISO 23695:2023;

b) identity of the sample;

c) expression of the results according to Clause 12;

d) description of the used colour reaction (9.2 and 9.3);

e) any deviation from this method;

f) report of all circumstances that can have affected the results;

g) date of the test.

The method performance of this document is presented in Annex B.

Annex A
(informative)

Principle of alternative distillation

A.1 General

High concentration of chloride, colour or high turbidity can interfere with the colorimetric reaction. The following method describes an alternative procedure to separate ammonia from interfering substances by distillation as described in ISO 5664.

The method involves handling concentrated sulfuric acid and distillation of the aqueous matrix. Wear protective clothing, gloves and glasses. Work in a well-ventilated area, preferably in a fume cupboard.

Inspect the distillation system before use to check for any cracks or defects.

Ammonia, a volatile weak base, can be separated from water by distillation at a pH of around 9,5. As the samples generally present different acidity and buffer properties, a buffer solution containing borate (A.2.4 or A.2.5) is added in order to maintain the required pH during the distillation process.

Distil the samples in an alkaline environment as described in A.4.1. The distilled ammonia is held in an acid solution by sulfuric acid. The determination is carried out after neutralization by measuring the colour obtained following the directions given in Clause 9.

A.2 Reagents and standard solutions

Use the reagents given in 7.1 and 7.2, and in addition:

A.2.1 **Concentrated sulfuric acid**, with a mass fraction of 96 %, ρ_{H2SO4} = 1,84 g/ml.

A.2.2 **Absorbent for ammonia diluted sulfuric acid**, c_{H2SO4} = 0,016 mol/l.

Pipette 0,9 ml of concentrated sulfuric acid (A.2.1) into a 1 000 ml graduated flask containing water (7.1) and make up to the mark.

A.2.3 **Sodium hydroxide solution**, c_{NaOH} = 0,1 mol/l.

Dissolve (4,0 ± 0,1) g of sodium hydroxide in a 1 000 ml graduated flask containing water (7.1). Mix well, leave to cool and make up to the mark.

A.2.4 **Sodium borate**, $c_{Na2B4O7}$ = 0,011 mol/l.

Dissolve (2,17 ± 0,01) g of sodium borate ($Na_2B_4O_7$) in a 1 000 ml flask containing water (7.1). Add 36 ml of sodium hydroxide solution 0,1 mol/l (A.2.3). Mix well, leave to cool and make up to the mark.

A.2.5 **Sodium borate**, $c_{Na2B4O7}$ = 0,025 mol/l.

Dissolve (4,93 ± 0,01) g of sodium borate ($Na_2B_4O_7$) in a 1 000 ml flask containing water (7.1). Add 88 ml of sodium hydroxide solution 0,1 mol/l (A.2.3). Mix well, leave to cool and make up to the mark.

A.3 Apparatus

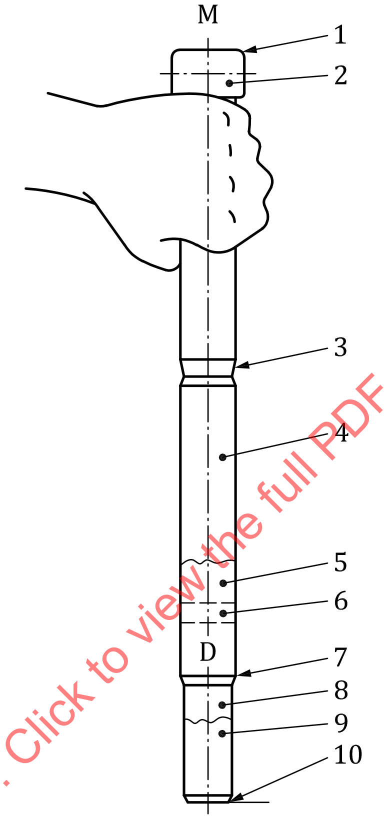
Use the apparatus given in Clause 8 and in addition:

A.3.1 Distillation system

Figure A.1 shows an example of a commercial device for performing distillation as per this method.

It consists of a heating block (not shown in figure) which can maintain a temperature of (125 ± 5) °C and has multiple positions to handle multiple samples for each work cycle.

The part related to distillation and distillate collection, shown in Figure A.1, consists of a polyethylene sample tube, a collector tube D and a measurement end M.



Key

1	membrane	7	stop ring
2	cap	8	sample tube
3	breakaway	9	sample
4	collector tube	10	distillation end
5	distillate	D	collector tube
6	membrane	M	measurement end

Figure A.1 — Distillation and collection apparatus

A.4 Method

A.4.1 Distillation

Follow the manufacturer's instructions and recommendations in the instrumentation operating manual.

If the device in Figure A.1 is used, proceed as follows.

Set the temperature to (125 ± 5) °C on the heating block (A.3.1) and leave it to heat to this temperature (about 20 min). Place the distillation and collection device on the appropriate holder with section M facing upwards. From the open end of section M, add 1,0 ml of sulfuric acid solution 0,016 mol/l (A.2.2) and position a suitable gas permeable filter at the centre of the opening. Seal the device with a suitable cap applying gentle and continuous pressure. If mounted correctly, the filter should fold sideways.

Add 6,0 ml of the sample to the sample tube and add 1,00 ml of borate buffer 0,025 mol/l (A.2.5) if the sample has been treated with sulfuric acid or 0,75 ml of sodium borate 0,011 mol/l (A.2.4) if the sample has not been acidified.

Immediately and rapidly insert the sample tube into the distillation and collection device. The seal is secured with a stop ring.

Insert the joined parts – the sample tube and the distillation and collection device – into the device heating block (A.3.1) firmly and using the appropriate methods, and leave for 30 min.

Remove from the heating block and immediately separate by pulling down and turning backwards and forwards. Use thermal insulation gloves when performing this operation. The joined parts should be separated within a few seconds otherwise the sulfuric acid solution contained in the distillation and collection device will be sucked into the test tube due to the fall in pressure resulting from the sample cooling.

Place the distillation and collection device in a suitable holder with section D facing upwards and leave to cool for at least 10 min.

Collect the condensation from the walls of the distillation and collection device by rotating it horizontally and slowly bringing it back to a vertical position with part D facing upwards. Holding it in this position, break it in half by applying pressure at the pre-scored point ("breakaway" in Figure A.1). Discard part D and keep part M containing the sulfuric acid solution.

Top up with water to 6,0 ml. The solution obtained will be used for colorimetry with the most suitable small-scale sealed tubes depending on the required concentration.

A.4.2 Colorimetry

Proceed as described in Clause 9.